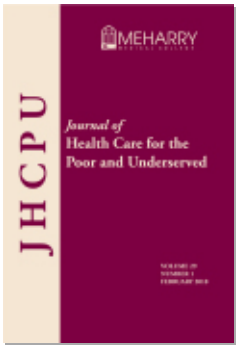
Diagnosis and Management of Pneumocystis Pneumonia in Resource-poor Settings



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Diagnosis and Management of Pneumocystis Pneumonia in Resource-poor Settings

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Abstract: Globally, *Pneumocystis* pneumonia (PCP) remains a common and lethal infection in both HIV-positive and HIV-negative patients, particularly in developing countries where rates of PCP increases with rising GDP. *Pneumocystis jirovecii* cannot be cultured in routine clinical laboratories; thus diagnosis relies on microscopy, histology, serology and/or polymerase chain reaction (PCR) of the *Pneumocystis* DNA. Most of these methods are expensive and require training. Accessing lower respiratory tract specimens in young children is often challenging and only PCR testing of nasopharyngeal aspirates is useful. Early treatment with high-dose co-trimoxazole is effective therapy; however, adverse reactions are common along with reports of emerging resistance. Improved outcomes are associated with adding corticosteroid to treatment in those with moderate/severe PCP, although this has not been studied in resource-poor settings. This review compares the available diagnostic techniques in relation to their suitability for use in resource-poor settings. We also addressed the non-availability of the alternative medications in these regions.

Key words: Pneumonia, *Pneumocystis*, HIV, diagnostic tests, children, Africa, low-middle income countries.

Among people with AIDS worldwide, *Pneumocystis* pneumonia (PCP) remains a common and life-threatening opportunistic infection.[1] With the increased use of chemotherapeutic agents and immunosuppressants, the incidence of PCP among patients without HIV infection has progressively increased, and is associated with mortality rates of 35–55% compared with 10–20% among HIV-infected patients.[2] A rough estimate of the annual incidence of PCP among people with AIDS is more than 400,000 adults and

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children worldwide (~14.7% of 1.76 million with CD4 cell count <100).[3] *Pneumocystis* pneumonia has increased as gross domestic product (GDP) increases globally.[3]

Pneumocystis jirovecii (formerly *carinii*) is a ubiquitous organism and it is estimated to infect as high as 95% of the worldwide population during the first two years of life with asymptomatic carriage in healthy people.[4] *P. jirovecii* is specific to humans with multiple genotypes.[5] Early diagnosis and treatment with high dose co-trimoxazole is effective therapy; however, adverse reactions are very common, notably: nausea (>90%) and vomiting, rash 35%, neutropenia (55%), 25% fall in haemoglobin (20%), and abnormal liver function tests (2–5x rise in enzymes) (35%).[6] A meta-analysis revealed that improved outcomes are associated with adding corticosteroid to treatment regimens in those with moderate or severe PCP.[7] Resistance is rare but has been reported and is associated with dihydropteroate synthase polymorphisms.[8,9] In resource-poor settings where empirical therapy is advocated (due to lack of diagnostics) many patients who do not have PCP are unnecessarily exposed to high dose co-trimoxazole. *Pneumocystis* pneumonia morbidity and mortality remains high in HIV-positive individuals who do not have access to antiretroviral therapy (ART) and in whom ART tolerance is an issue or the drugs are ineffective. It is also high in HIV-positive people who are ignorant of their HIV status and those who, due to fear of stigmatisation, choose not to seek medical care.[10]

A multicenter, 8-year prospective study to determine the impact of HIV-associated conditions on mortality showed an overall mortality rate of 5.41 deaths per 100 person-years, and PCP was associated with a doubling of mortality.[11] Presently, *P. jirovecii* cannot be cultured in routine clinical laboratories so diagnosis rests on microscopy of respiratory fluids, histology of lung tissue, serological biomarkers and/or polymerase chain reaction (PCR) detection of *Pneumocystis* DNA. (See Figure 1.) More sensitive than microscopy (~98% versus 75% for silver stains), PCR has the advantage in resource-poor settings of requiring minimal training; however it is expensive.[12] Only moderate to severe cases are readily diagnosed based on clinical and radiological findings, thereby missing mild cases. A high performing diagnostic assay would allow earlier treatment of mild cases and discontinuation of therapy in those without PCP. The cost-effectiveness of diagnostic assays improves in places where there is a high disease prevalence.

This review focuses on the epidemiology, challenges with laboratory diagnosis and therapeutic management of PCP in relation to resource-poor settings.

Search strategy and selection criteria. The literature search for publications on diagnosis and management of PCP preceding 30 March 2016, was performed using PubMed (accessed MEDLINE), Web of Science, Google Scholar, Cochrane Library, African Journals Online (AJOL), Africa-Wide: NiPAD, CINAHL (accessed via EBSCO Host) databases and grey literature to identify all published papers regarding the topic. The references were reviewed for additional publications that may not have been cited elsewhere ("snowballing"). Articles published in other languages (e.g., French and Portuguese) were considered if they were cited in any of the databases searched. The main search comprised individual searches using detailed medical subject heading (MeSH) terms for *pneumocystis* pneumonia, community-acquired pneumonia and HIV/AIDS combined with terms relevant to PCP diagnosis and management. The Boolean operators 'AND' and 'OR' were used to combine and narrow the searches.

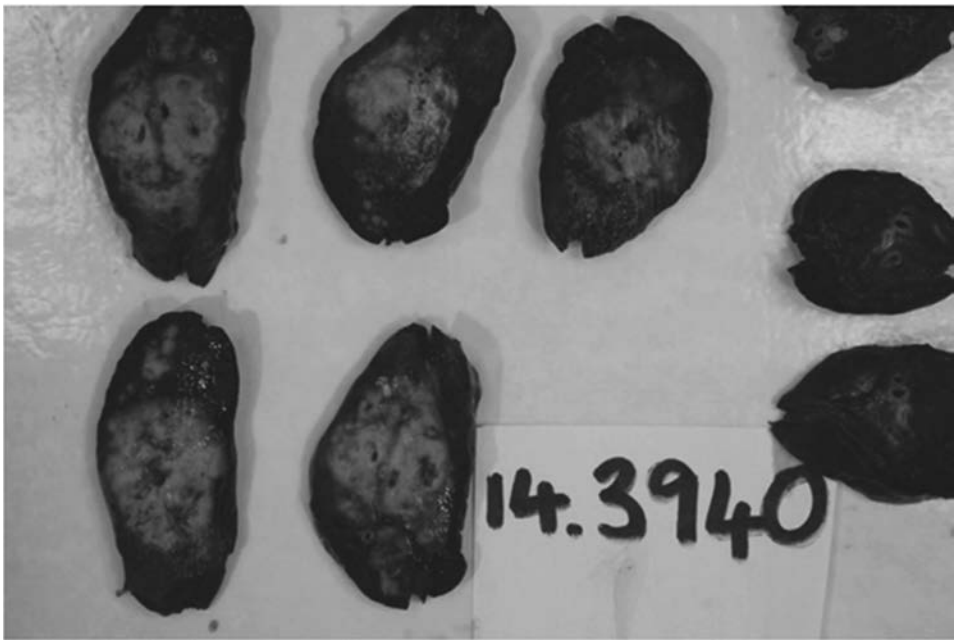


Figure 1: Lung (right upper lobe), wedge excision biopsy of an HIV-infected male patient on ART, CD4+ count 240cells/ml. Had cutaneous Kaposi sarcoma and presented with clinical features of atypical pneumonia. Diagnosis of granulomatous PCP was made. Images courtesy of Anshuman Chaturvedi, Manchester University NHS Foundation Trust.

Epidemiology. In 1981, two case reports of PCP in five previously healthy homosexual males who were injection drug users announced the beginning of the HIV/AIDS pandemic.[13,14] *Pneumocystis* pneumonia is the commonest AIDS defining opportunistic infections in HIV infected people in the U.S. and Europe,[15,16] but it was initially assumed to be rare in low and middle-income countries (LMIC), such as African countries.[17,18] However, more recent studies have reported contrary findings.[19–21] There are plausible reasons for the low PCP rates previously reported in LMICs. One is the widespread poverty combined with low quality of health care that may result in most HIV infected patients dying from infection before they can develop PCP; another is the lack of diagnostic facilities and trained personnel to identify *Pneumocystis* in most of these countries.

Studies from Asia reveal varying rates of PCP in the last two decades, ranging from 18.7–25.4% in HIV infected patients in Thailand, with attendant high mortality,[22–26] and 16.7% in Bangladesh,[27] 8.4% in Cambodia[28] and 5% in Vietnam.[29] Earlier data from India demonstrated rates of 5–6.1% of PCP in HIV-infected individuals.[30–32] However, with better molecular detection techniques, higher PCP rates of 12.2–26.5% are being reported in India.[33–35] A recent study from India reported an incidence of 14% in 94 immunocompromised children of whom 14 were HIV-infected.[36]

In South America, the picture is practically the same, although there is paucity of data. Studies on PCP in HIV-infected people there revealed a 24% incidence rate in Mexico,[37] 48% in Panama,[38] 27% in Guatemala,[39] 32% in Cubans,[40] and 35% in Haiti.[41]

Other South American reports demonstrated 36.6% from Venezuela,[42] Peru 12.5%,[43] 38% in Chile,[44] and 27% in Brazil.[45]

Burden in Africa. In Africa, PCP was previously assumed to be uncommon among the HIV population.[18,46–49] Early studies from Uganda and Zambia reported no cases of PCP among HIV-infected patients.[17,49] A South African report revealed similar findings of one (0.6%) positive sample out of 181 patients tested for PCP.[50] However, in the same period an incidence of 3.6–11% was documented among HIV-infected people in Tanzania, Congo and Ivory Coast.[18,51–53] Additionally, in a setting that had better diagnostic facilities and increased access to ART, a PCP prevalence of 33% from 64 smear negative tuberculosis (TB) patients in Zimbabwe using methenamine silver staining on bronchoalveolar lavage (BAL) samples was reported.[54] Another report from Kenya, using immunofluorescence (IF) and toluidine blue staining identified *Pneumocystis* in 37.2% and 27.4% respectively of 51 HIV/AIDS infected patients.[55] In an Ethiopian report, *P. jivovecii* was detected by PCR in 42.7% of 131 BAL samples from HIV-infected patients with atypical radiological reports who were acid fast bacilli (AFB) smear negative[56] and 29.7% by IF.[57] In Nigeria, 12.6% was reported positive using *Pneumocystis* PCR.[58]

Studies in children showed similar results and PCP appears to occur early among HIV-infected infants (median age: approximately 13 months), suggesting that exposure to *Pneumocystis* is relatively extensive. A recent study from Mozambique demonstrated a 6.8% prevalence of PCP with 14.3% in HIV-infected children and 3.3% in non HIV-infected children.[59] At the start of the HIV/AIDS pandemic, the incidence of PCP was 1.3 cases per 100 child-years from early childhood to adolescence and went up to 9.5 cases per 100 child-years in infancy.[60,61] Postmortem studies of lung tissues from children with AIDS revealed an incidence of 67% in Zimbabwe;[21] 31% in children younger than 15 months old in Ivory Coast[62] and 48% in HIV infected children under 12 months in Botswana.[63] One of the challenges with diagnosis in infancy is that age three-six months has been shown to be a period of high incidence of PCP.[4] However, the child's HIV status is usually undetermined at that period in most resource-poor settings because these patients are not routinely presented for care.[64] Anti-*Pneumocystis* antibodies were demonstrated in HIV-negative children in early years of life (aged 1.9–19 months; mean, 7.1 months; median, 5 months; SD, 4.9)[65] and as early as two-six months in African children, often with it being the first presentation of HIV related disease.[66] Following improvement of prenatal HIV testing and introduction of ART to prevent vertical spread, there has been a significant decrease in paediatric HIV infections. The incidence of PCP also reduced substantially in children from 1992 to 1997, with a sharp decline from 1995 and this was attributed to improving ART administration in labour.[67] Despite this, a study from Mozambique among children younger than five years of age reported a prevalence of 6.8% in newly presenting children with severe pneumonia, of whom 25.7% had HIV infection and 59% of the PCP cases were in those with HIV infection.[59] Table 1 shows the distribution across resource limited countries.

Outbreaks. Outbreaks of PCP suggestive of human transmission were first documented among hospitalized oncology and transplant patients in United States and Europe.[68–71] These were followed by reports of outbreaks among AIDS patients and

Table 1.

COHORT INCIDENCE OF *PNEUMOCYSTIS* PNEUMONIA (PCP) IN LMICS

Country	Studied population	PCP rates (%)	Method of diagnosis	Mortality rates (%)	HIV rates in studied population (%)	References, year of publication
Africa						
Mozambique	Paediatric	6.8	PCR	20.8	25.7	Lanaspa et al. 2015[59]
South Africa	Paediatric	54.0	IF, PCR	32	45.9	Morrow et al. 2014[265]
South Africa	Paediatric	72.0	IF, PCR	0	64	Djawe et al. 2013[263]
South Africa	Adults (93%)	24.0	IF, PCR	NA	100	Dini et al. 2010[264]
South Africa	Paediatric	21.3	IF	39.5	61.4	Morrow et al. 2010[265]
South Africa	Paediatric	48.6	IF	27.8	100	Ruffini & Madhi 2002[266]
Namibia	Adult	5.3	PCR, GMS	NA	36.8	Nowaseb et al. 2014[163]
Cameroun	Adult	36.1	PCR, GMS, Giemsa	NA	53.2	Riebold et al. 2014[267]
Cameroun	Adult	82*	Elisa	NA	50.1	Nkinin et al. 2009[268]
Tanzania	Adult	10.4	ToB, PCR	NA	100	Mwita et al. 2012[269]
Tanzania	Adult*	0.3	PCR	53	100	Jensen et al. 2010[170]
Tanzania	Adult	7.5	IF, GMS, PCR	0	100	Kibiki et al. 2007[270]
Uganda	Adult	1.1	PCR	11.5	1.3	Blount et al. 2012[271]
Uganda	Adult	3.9	Giemsa PCR	60	73	Taylor et al. 2012[272]
Uganda	Paediatric	16.5	IF	40	35.5	Bakeera-Kitaka et al. 2004[273]
Malawi	Paediatric	4.9	PCR	57	51	Graham et al. 2011[274]
Malawi	Paediatric	10.7	PCR	20.1	62	Graham et al. 2000[275]
Malawi	Paediatric	8.3	IF	80	31.7	Kamiya et al. 1997[276]
Malawi	Adult	9.0	PCR	33.3	89.0	Hargreaves et al. 2001[277]
Ethiopia	Adult	30.3	IF,PCR	NA	38.5	Aderaye et al. 2003[278]
Ethiopia	Adult	29.7	IF	NA	91	Aderaye et al. 2007[56]
Kenya	Adult	37.2	IF, ToB	PM	31.4	Chakaya et al. 2003[279]
Kenya	Paediatric	13	IF	NA	50	Bii et al. 2006[280]
Senegal	Mixed (98.4% >13years)	9	IF, Giemsa	NS	41	Dieng et al. 2016[281]
Senegal	Adult	22.2	ToB	NA	100	Sow et al. 1992[282]
Zambia	Adult	0.0	IF, ToB	NA	100	Elvin et al. 1989[283]
Zimbabwe	Paediatric	67.0	ToB	PM	100	Nathoo et al. 2001[284]
Zimbabwe	Adult	21.0	GMS, ToB, DQ	9.5	100	Malin et al. 1995[285]
Tunisia	Adult	33.3	GMS, Giemsa	NA	100	Ennaifer et al. 2002[286]
Botswana	Paediatric	31.0	GMS	PM	68	Ansari et al. 2003[63]
Ivory coast	Paediatric	31.0	GMS	PM	50	Lucas et al. 1996[287]
Nigeria	Adults	7.4	GMS	NA	100	Ogba et al. 2014[288]
Nigeria	Combined	12.6	PCR	NA	17.4	Alli et al. 2012[58]
Asia						
India	Adult	27.2	IF, GMS, ToB	NA	100	Kaur et al. 2015[289]
India	Paediatric	43	GMS, PCR	21.4	14.9	Das et al. 2014[36]
India	Adult	30.7	IF, PCR	NA	50	Revathy et al. 2014[290]
India	Adult	13.0	IF	15.8	100	Udwadia et al. 2005[291]
India	Adult	11.0	IF, GMS, PCR	18.7	100	Tyagi et al. 2010[292]
India	Adult	6.0	IF	58.3	100	Kumarasamy et al. 2003[293]

(*continued on p. 112*)

Table 1. (*continued*)

Country	Studied population	PCP rates (%)	Method of diagnosis	Mortality rates (%)	HIV rates in studied population (%)	References, year of publication
India	Paediatric	3.9	Clinical	18.2	100	Merchant et al. 2001[294]
Bangladesh	Paediatric	8.0	Clinical	6	52	Shahrin et al. 2014[27]
Taiwan	Adult	14.8	Giemsa	37.7	100	Wang et al. 2011[295]
Thailand	Adult	25.4	Clinical	6.7	100	Tansuphasawadikul et al. 2005[296]
Thailand	Paediatric	35.0	Giemsa, GMS, IF	44.4	100	Chokephaibulkit et al. 1999[297]
Malaysia	Adult	60.0	Clinical	NA	100	Asmal et al. 2009[298]
Malaysia	Adult	12.2	Clinical	NA	100	Nissapatorn et al. 2004[299]
Malaysia	Combined	32.9	NS	12.5	100	Ismail et al. 1995[300]
Philippines	Adult	30.4	IF	71.4	100	Manaloto et al. 1994[301]
Cambodia	Adult	8.4	Clinical	NA	28.3	Senya et al. 2003[28]
Central/South America						
Panama	Adult	45.5	GMS, Giemsa	NA	100	Rodriguez et al. 1996[38]
Venezuela	Adult	23.3	IF	NS	36.6	Panizo et al. 2008[302]
Brazil	Adult	17.5	NS	PM	6.2	Cury et al. 2003[303]
Mexico	Adult	24	GMS	PM	100	Mohar et al. 1992[37]
Brazil	Adult	55.0	GMS, Giemsa	26.7	100	Weinberg and Duarte1993[304]
Brazil	Adult	10.9	Clinical	33.3	100	Lambertucci et al. 1999[305]
Brazil	Adults	22.2	clinical	33.5	199	Soares et al. 2008[306]
Brazil	Adult	35.0	Clincal	13.9	100	Santos et al. 1994[307]
Brazil	>13years	23.6	NS	NA	100	Galisteu et al. 2015[308]
Argentina	Paediatric	35.9	clinical	21.6	100	Fallo et al. 2002[309]
Chile	Adult	37.7	PCR	47.4	100	Chernilo et al. 2005[44]
Chile	Mixed	23.3	GMS	42.9	12.2	Cruz et al. 2012[310]
Colombia	Adult	32.6	GMS, PCR	NA	60.2	Munoz et al. 2012[311]
Peru	Adult	12.5	GMS	PM(n-16)	100	Eza et al. 2006[43]

Note:
IF:Immunofluorescence; DQ:Diff-Quick; GMS:Gomori methenamine-silver; ToB:toluidine *O* blue; PCR:Polymerase chain reaction; NS:not stated; PM: post mortem; NA: not available/applicable; * colonisation;

immunosuppressed rheumatoid arthritis patients.[72–75] The possibility of transmission of *P. jirovecii* to and by health care workers (HCWs) has also been investigated with some studies reporting substantial differences in antibody titer levels in HCWs exposed to PCP.[76] Another study demonstrated a significant increase in those that been exposed to PCP, keeping in mind that this is an aerosol transmitted disease.[76,77] A study measuring levels of antibodies to the major surface glycoprotein (Msg) of *Pneumocystis* demonstrated higher levels in HCWs exposed to PCP than in non-HCWs that were not exposed to the infection[78] implying that HCWs can serve as a reservoir for *P. jirovecii*. These reports pose a challenge in the management of PCP patients considering that current international guidelines do not advocate respiratory isolation for these patients. Single room isolation for PCP to minimize transmission is desirable for the first week of therapy but not realistic in most LMICs.

***Pneumocystis*, the organism**. *Pneumocystis* is an ubiquitous, obligate, biotrophic, extracellular eukaryotic organism that exists in trophic and cystic forms.[79] It has unique mechanisms of adaptation to life exclusively in mammalian species and is known to be host-species specific.[80] Ma and colleagues in 2016 reported successful genome analysis of three *Pneumocystis* species (human, rat and mice) which demonstrated that adaptation mechanisms occurs exclusively in mammalian hosts lungs.[81] Humans act as a reservoir for *P. jirovecii*; however, the precise association is not fully understood and environmental reservoirs have also been documented outside the lungs.[82–84] *Pneumocystis* was first grouped with protozoans, but in 1988 it was reclassified as a fungus because its ribosomal RNA was most similar to that found in fungi.[79,85,86]

Pneumocystis spp. does not appear to grow in standard fungal culture, although it can be detected in the environment by molecular methods.[87] However, a recent study from Germany described an innovative method to culture *P. jirovecii* using differentiated pseudostratified CuFi-8 cells that were inoculated with BAL fluid (confirmed positive by PCR for *P. jirovecii*).[88] Although the efficacy of such a culture system for propagating the organism and/or directed therapy selection is yet to be determined, it is nevertheless an innovation that will affect the diagnosis and management of PCP.

Transformation from cyst to trophozoite. *Pneumocystis* appears to have a bi-phasic life cycle within the alveolar lumen, consisting of an asexual phase characterized by binary fission of trophic forms and a sexual cycle resulting in formation of cysts.[89] The trophic form possesses a single nucleus and has a flexible cell wall that is often tightly attached to type I pneumocytes in lung alveoli. The cyst can contain up to eight 'sporozoites' and is protected by a distinctive thick cyst wall. When the wall ruptures (encystment), these 'sporozoites' are released and then develop into new trophic forms.[90–92] The cell wall of the organism, both cystic and trophic forms, contains melanin-like compounds which protects it from environmental stressors.[93] The cyst wall is maintained by a unique system of building and breaking down which sustains its rigidity and viability, may reduce immune recognition and also ensures the organism completes its life cycle. β-glucan synthetases are the enzymes involved in production of β-1,3-glucan homopolymers that make up the cyst wall; β-glucanases and other enzymes drive the active process of encystment.[94–98] These enzymes are noteworthy as targets for the development of new therapeutic molecules.

HIV and other risk factors. A number of factors are associated with the development of PCP but impaired T-cell immunity is the pivotal risk factor for PCP.[10,92,99] Sustained defective immunity from past immunosuppressive therapy, a number of immunosuppressive conditions such as haematological malignancies especially leukemia and lymphomas, solid organ cancers and transplants are known risk factors, as well as a wide variety of specific immunosuppressive medications.[100] Other patient groups at risk include post-transplant patients, those with autoimmune and inflammatory conditions such as rheumatologic and other anti-inflammatory processes, particularly when they are exposed to prolonged high dose corticosteroid therapy.[92,101] Cytotoxic drugs such as methotrexate, cyclosporine and cyclophosphamide have been implicated in the development of PCP.[102–104] Additionally, newer immunomodulating agents such as TNF-a inhibitors have also been associated with this disease.[105,106]

The most substantial risk factor for PCP in HIV infected patients is a CD4+ cell count

below 200 cells/ml.[10,99,107–109] CD4+ cells are necessary for the clearing of *Pneumocystis*. This has been revealed both in experimental models, where a direct relationship between CD4+ cell count below 200 cells/ml and the development of PCP infection was shown.[108] Fortunately, the advent of ART, which boosts recovery of CD4+ cell count levels, has led to significant reduction in rates of PCP in HIV-infected patients.[87] This seems to be the case in industrialized countries where the majority of HIV positive patients have access to ART. However, the contrary is the case in resource-poor countries.

A recent systematic review showed that the most significant predictor of PCP was per capita GDP, which demonstrated strong linear association with odds of PCP diagnosis ($p < .0001$).[3] It is likely that poverty exposes HIV-infected patients to a variety of pathogenic organisms with *P. jirovecii* being just one of many in LMICs. However, as the economics of the LMICs improve, it is plausible that many virulent bacterial infections circulate less frequently and in HIV-infected people PCP may assume a greater role. The surrounding indoor air is also important in PCP transmission. A study of hospitalized PCP patients in France demonstrated *P. jirovecii* DNA in 80% of air samples in the patients' immediate surroundings, with progressive reduction with increasing distance from patient's bedside.[110] Other researchers have reported the detection of DNA sequences identical to *P. carinii* in samples of ambient air.[111–113] Yet another study highlighted *P. jirovecii* exhalation from colonized patients and emphasized the risk of nosocomial transmission of the disease.[114] Morris and colleagues[113] found that geographical location is another factor associated with PCP and other researchers have confirmed this finding.[19,115–117] Several studies have also assessed the role of seasonal variation in PCP with conflicting results, with some peaks of the infection in summer or winter seasons or no seasonal variation at all.[116–118] It will be interesting to see if studies from tropical regions such as Africa will reflect seasonal variations.

Impact of prophylaxis on incidence. The Centers for Disease Control and Prevention (CDC) HIV/AIDS surveillance reports of 1990–1993 in pre-ART and prophylaxis era showed that PCP accounted for over 20,000 new AIDS cases yearly in the United States.[119] With the institution of ART and PCP prophylaxis, the rates of infection dropped from 31% to 9% in the U.S.[120] In Europe, PCP accounted for 16.4% of AIDS cases,[121] though most of the cases were in ART naïve patients. In a EuroSIDA study, 7333 HIV-infected people were recruited from 52 centres across Europe and Israel, and the PCP rates dropped from 4.9 cases per 100 person-years to 0.3 cases per 100 person-years after the use of ART was consolidated.[122] Most recent data from these countries suggest that PCP appears mainly among HIV-infected patients that do not know their HIV serostatus, or who have challenges with ART or PCP prophylaxis compliance.[67,123–125]

Clinical presentation. In the HIV-infected population, the diagnosis of PCP presents a clinical dilemma because there are no specific signs and symptoms of the disease. There is also the challenge of co-existing morbidities with other pathogens and the use of prophylactic drugs in managing these patients.[12,126,127] These patients usually present with sub-acute onset of gradual dyspnoea, nonproductive or minimally productive cough, low grade fever and malaise. Early in the course of infection patients may be asymptomatic. Acute dyspnoea with associated pleuritic chest pain is likely indicative of pneumothorax complicating PCP. In contrast, non-HIV immunosuppressed patients tend to present more acutely, with significant dyspnoea, high fever, chills and

in some cases with respiratory failure which could result in up to 40% mortality.[129] In children, in addition to dyspnoea and fever, they may have cyanosis, nasal flaring, and intercostal retractions. Physical examination in both adults and children tends to reveal tachycardia, tachypnoea and a 'clear chest' on auscultation, but sometimes inspiratory crackles are heard.[99] *Pneumocystis* pneumonia is graded based on clinical features into mild, moderate and severe to aid in management of patients (see Table 2; see Table 3 for comparison of diagnostic methods).

HIV-infected PCP patients generally have more *Pneumocystis* organisms with fewer neutrophils in their bronchoalveolar lavage (BAL) specimen than non-HIV immunosuppressed patients.[99] This greater burden of infecting organism correlates with a significantly higher diagnostic yield.[99] However, the smaller number of inflammatory cells does not seem to result in worsening oxygenation or impact on survival. In fact, the opposite tends to occur. HIV infected PCP patients also appear to have higher arterial oxygen tension and a lower alveolar-arterial oxygen gradient than non-HIV immunosuppressed PCP patients.[129] This probably explains why non-HIV immunosuppressed PCP patients are more likely to develop respiratory failure than HIV infected PCP patients.[129]

Radiological features. Chest radiographic findings in PCP are nonspecific, and as many as one third of infected patients may have normal radiographic findings.[130] High-resolution computed tomography (HRCT), which is more sensitive than chest radiography, is used when the chest radiograph is appears normal but there is a high index of suspicion or when the diagnosis is unclear. The HRCT may reveal extensive 'ground-glass' appearance or cystic lesions reflecting accumulation of intra-alveolar fibrin, debris, and organisms in PCP.[130] High-resolution computed tomography is expensive and not cost-effective in LMICs.

Granulomatous reactions (see Figure 2) are estimated to occur in 5% of HIV-infected patients with PCP and usually occur early in the course of the infection when immunodeficiency is more reduced; they may become evident on HRCT as a single

Table 2.

GRADING OF SEVERITY OF *PNEUMOCYSTIS* PNEUMONIA (PCP) IN HIV AND NON-HIV PATIENTS

Clinical features	Mild PCP	Moderate	Severe
Dyspnoea	Dyspnoea on mild exercise $_{+/-}$ cough and sweats	Dyspnoea on mild exercise, fever (+/= sweats)	Breathlessness at rest, persistent fever and cough.
Arterial blood gases and oxygen saturation at rest, on air	PaO_2 >11 kPa; SaO_2 >96%.	PaO_2 8–11 kPa; SaO_2 91–96%.	PaO_2 <8 kPa; SaO_2 <91%.
Radiological findings	Normal or minor perihilar infiltrates	Diffuse interstitial shadowing.	Extensive interstitial shadowing, with or without alveolar shadowing

Table 3.

COMPARISON OF THE DIFFERENT DIAGNOSTIC METHODS FOR *PNEUMOCYSTIS* DETECTION CURRENTLY USED

	Real time PCR	IFA	GMS	Toludine Blue O	Gram Weigert	Giemsa	Diff Quik	BG
Target	Cyst forms Trophic foms	Cyst forms	Cyst forms	Cyst forms	Cyst forms trophic forms	Trophic forms	Trophic forms	Cyst form
Sensitivity	+++++	+++++	++++	++++	++++	+++	++	+++++
Specificity	++++	++++	++++	++++	++	+++	++++	++++
Total time (duration) of procedure	50–150[a]	200	100	50	60	80	33	>200
Affordable	Expensive	Expensive	Affordable	Affordable	Affordable	Affordable	Affordable	Expensive
Skills/ Expertise required	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Note:
IFA = immunofluorescence; GMS = Gomori Methenamine Silver; *cyst form = asci; trophic forms similar a cell wall deficient yeast cell; new nomenclature for cysts, trophozoites and sporozoites. + = 0-25%; ++ = 26-50%; +++ = 51-75%; ++++ = 76-85%; +++++ 86-95%
Adapted and modified from Procop, G.W. et al., 2004.[178]

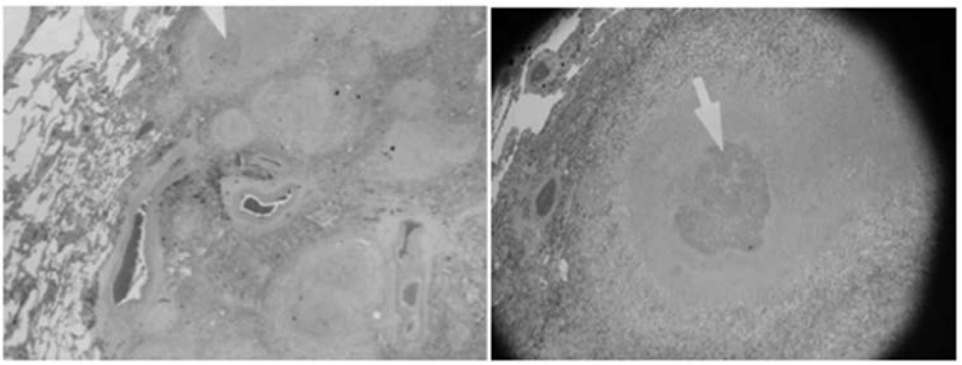


Figure 2: Granulomatous inflammation with presence of fungal yeast forms. Arrow indicating granulomatous area.
Images courtesy of Anshuman Chaturvedi, University Hospital of South Manchester.

nodule or mass mimicking lung carcinoma or as multiple nodules ranging from a few millimeters to more than 1 cm.[131] Cystic lesions may also be seen in PCP in AIDS; Tokuda and colleagues found that the presence of AIDS was found to be a risk factor for the formation of pulmonary cystic lesions using multivariate analysis.[132] Cysts are associated with an increased frequency of spontaneous pneumothorax, however, spontaneous pneumothorax can also occur in the absence of definable lung cysts.[133] These cysts may resolve after drug therapy and resolution of infection.[131]

Other radiological features include diffuse bilateral interstitial infiltrates, solitary or multiple nodules, upper-lobe infiltrates in patients receiving aerosolized pentamidine, pneumatoceles, pneumothorax and patchy asymmetric infiltrates.[64,134–136] In more advanced disease, septal lines with or without intralobular lines superimposed on ground-glass findings ('crazy paving')[137] and consolidation may develop.[130] A recent case report showed symmetric bi-apical cystic spaces in chest radiographs.[138] These air spaces may subsequently get infected with *Aspergillus* spp and an aspergilloma may form.[139–142] Patients recovering from PCP may have residual interstitial fibrosis.[143] In addition, although rare, interstitial fibrosis can occur in AIDS patients with low-grade chronic PCP, a condition termed chronic *Pneumocystis* pneumonia.[144]

Extrapulmonary PCP does occur rarely in patients with advanced AIDS.[145–150]. Some of these manifestations include: pneumocystic lesions of bone, brain, kidney, liver, spleen, eye, thyroid and the gastrointestinal tract.[145–147,149,151–153]

Diagnosis. While severe and typical PCP can be diagnosed clinically, survival is better with earlier diagnosis.[154] Perhaps the greatest benefit of laboratory testing for PCP is to rule out the diagnosis of PCP thereby avoiding unnecessary exposure to toxic doses of cotrimoxazole. Early diagnosis may also prevent hospital admission in mild/moderate cases.

Sample types. A variety of specimens have been used for diagnosis, including lung biopsy, bronchoalveolar lavage (BAL), induced and expectorated sputum, naso-pharyngeal aspirates and oral washings. The high morbidity associated with biopsy specimens has limited their clinical utility so BAL has largely been the sample of choice.[155,156] Bronchoscopy is required for lung biopsy and BAL; its unpleasantness, cost, invasiveness and expertise required renders it impractical in resource-poor settings and so induced sputum has been preferred.[157–161] Expectorated sputum is also a useful diagnostic specimen, and obviates the need for sputum induction.[55,162,163] One study reported a 55% detection sensitivity for *P. jirovecii* in expectorated sputum,[162] which was similar to the sensitivity with induced sputum.[159,161] A retrospective review of PCP cases in the United States also found no significant difference in *P. jirovecii* yield between induced or expectorated sputum.[164] Moreover, comparative evaluations of the general specimen quality of induced and expectorated sputum concluded that sputum induction did not improve the specimen quality substantially.[165,166] In a recent study on expectorated sputum from HIV-infected and smear-negative TB patients in Namibia; of 475 samples analysed, 5.3% samples were positive for *P. jirovecii*, (13.6% using both qPCR and GMS staining and 1.7% using qPCR only).[163] The study demonstrated that both standard microscopy with silver staining and real-time PCR were frequently positive and approximately concordant, indicating that expectorated sputum is probably a good specimen for PCP diagnosis.[163]

Physiotherapeutic interventions can safely and effectively procure a greater yield of sputum from patients that have difficulty producing sputum normally.[167] However, sputum induction may be unsafe in some patients, particularly infants and weaker AIDS patients, because of the risk of haemoptysis in patients with tuberculosis or chronic pulmonary aspergillosis and the health care worker is potentially exposed to *M. tuberculosis*.[167] It cannot be performed in children less than about 4 years old, because they don't understand or follow instructions, and usually swallow anything they

cough up. Therefore, correct detection of *Pneumocystis* presents a myriad of challenges especially in resource-poor settings. Though the procedures to obtain oral washes,[168–170] nasopharyngeal aspirates[171] and sputa[160,172] are relatively less invasive compared with bronchoalveolar lavage, and are suitable for diagnosis with molecular methods but not so well with microscopy

Non-specific markers of PCP—lactate dehydrogenase (LDH). Increased serum lactate dehydrogenase levels have been documented in patients with PCP in some studies but it is most probably due to the underlying lung inflammatory responses and damage rather than a precise biomarker for PCP.[173,176] Lactate dehydrogenase has a high sensitivity for PCP but it is not specific.[173]

Microscopy. Direct microscopy of respiratory samples has been the gold standard for diagnosis of PCP. However it has limitations as inspection of stained slides is subjective and may be non-specific; sensitivity relies heavily on the organism load in the specimen, the type of sample collected and the expertise of the microscopist visualizing the slide. These challenges have led clinicians managing at risk patients to rely on radiological findings and clinical evaluations for diagnosis of PCP.

There are several useful methods for *Pneumocystis* detection on all sample types including immunofluorescence microscopy utilizing monoclonal antibodies; cyst wall stains (toluidine blue O, cresyl echt violet and calcofluor white), and trophic form stains (modified Papanicolaou, GramWeigert, Grocott's methenamine silver stain, Diff-Quick, Wright or Giemsa). These different methods have their advantages and challenges (see Table 4). A comparison of three stains (IFA, Diff-Quik and Toludine blue O) demonstrated a sensitivity of 92% for IFA, 76% for Diff-Quik and 80% for toluidine blue O with no false positives for IFA.[177] Another study by Procop and colleagues documented that "the sensitivity and specificity of calcofluor white stain (CW) were 73.8 and 99.6%, respectively; that of Grocott-Gomori methenamine silver stain (GMS) were 79.4 and 99.2%, respectively; Diff-Quik stain (DQ) were 49.2 and 99.6%, respectively while that of the Merifluor *Pneumocystis* stain were 90.8 and 81.9%, respectively.[178] Only CW and GMS had positive and negative predictive values of >90%.[178] Calcofluor white is quick, convenient, and can detect simultaneously the presence of other fungi in samples but expertise is required for identification of cysts of *Pneumocystis* so this stain may miss severely infected patients. All of these microscopy methods can have false-negative results, especially in samples from non-HIV immunosuppressed patients who have fewer organisms present in the specimens.[129] Figure 3 demonstrates *Pneumocystis* using special stains.

Molecular diagnosis. Over 75 studies, most using in-house *Pneumocystis* assays, attest to the superiority of PCR amplification assays over microscopy both in sensitivity and in being able to analyse all sample types for the presence of *P. jirovecii* DNA. The advent of real-time quantitative PCR (qPCR) has enabled the rapid and contamination-free molecular diagnosis of PCP and several companies have commercialized tests. However, PCR is not technically and financially viable for resource-poor settings where the burden of this disease is high. A reliable electricity supply is often problematic, as is shipping reagents in dry ice through customs. A cost-effectiveness analysis by the Center for Disease Control and Prevention concluded that for the detection of

Table 4.

RECOMMENDATIONS FOR PROPHYLAXIS AND TREATMENT

Prophylaxis	Treatment of PCP
Preferred Therapy • Cotrimoxazole, 1 DS PO daily (AI) or • Cotrimoxazole , 1 SS PO daily (AI). **Indications for prophylaxis**: CD4 count <200 cells/mm^3 (AI) or • Oropharyngeal candidiasis (AII) or • CD4% <14% (BII) or • History of AIDS-defining illness (BII) or • CD4 count >200 but <250 cells/mm^3 and if CD4 cell count monitoring (e.g., every 3 months) is not possible (BII). Note—Patients who are receiving pyrimethamine/sulfadiazine for treatment or suppression of toxoplasmosis do not require additional prophylaxis for PCP (AII).	**Preferred Therapy** Moderate to Severe PCP—Total Duration = 21 Days (AII): • Cotrimoxazole: (TMP 15–20 mg and SMX 75–100 mg)/kg/day IV given q6h or q8h (AI), may switch to PO after clinical improvement (AI). For Mild to Moderate PCP—Total Duration = 21 days (AII): • Cotrimoxazole: (TMP 15–20 mg/kg/day and SMX 75–100 mg/kg/day), given PO in 3 divided doses (AI) or • Cotrimoxazole DS - 2 tablets TID (AI).
Alternative Therapy: • Cotrimoxazole 1 DS PO TIW (BI) or • Dapsone 100 mg PO daily or 50 mg PO BID (BI) or • Dapsone 50 mg PO daily + pyrimethamine 50 mg + leucovorin 25 mg PO weekly (BI) or • Dapsone 200 mg + pyrimethamine 75 mg + leucovorin 25 mg) PO weekly (BI) or • Aerosolized pentamidine 300 mg via Respigard II™ nebulizer every month (BI) or • Atovaquone 1500 mg PO daily with food (BI) or • Atovaquone 1500 mg + pyrimethamine 25 mg + leucovorin 10 mg PO daily with food (CIII).	**Alternative Therapy** Moderate to Severe PCP (AII): • Primaquine 30 mg (base) PO once daily + clindamycin [IV 600 q6h or 900 mg q8h] or [PO 300 mg q6h or 450 mg q8h] (AI). Or Pentamidine 4 mg/kg IV once daily infused over at least 60 minutes (AI); may reduce the dose to 3 mg/kg IV once daily because of toxicities (BI) For Mild to Moderate PCP: • Dapsone 100 mg PO daily + TMP 15 mg/kg/day PO (3 divided doses) (BI) or • Primaquine 30 mg (base) PO daily + clindamycin PO (300 mg q6h or 450 mg q8h) (BI) or • Atovaquone 750 mg PO BID with food (BI)

Notes:
Key to Abbreviations: BID = twice daily; DS = double strength; IV = intravenously;; PO = orally; q "n"h = every "n" hour; SS = single strength; TID = three times daily; TIW = thrice weekly. A: Strong recommendation for the statement. B: Moderate recommendation for the statement. C: Optional recommendation for the statement. I: One or more randomized trials with clinical outcomes and/or validated laboratory endpoints. II: One or more well-designed, non-randomized trials or observational cohort studies with long-term clinical outcomes. III: Expert opinion
*IDSA guidelines[10]

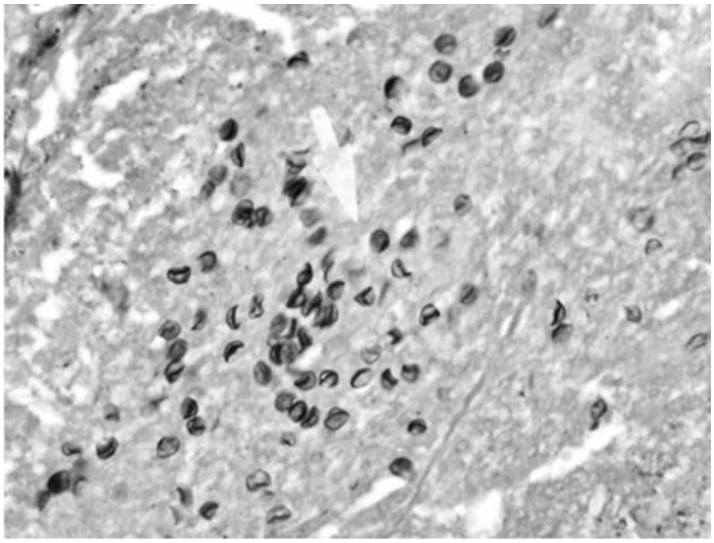


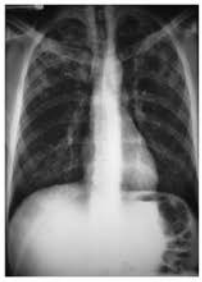
Figure 3: Granulomatous inflammation with *Pneumocystis*. Grocott stain. Arrow pointing to cyst of Pneumocystis jirovecii.
Images courtesy of Anshuman Chaturvedi, University Hospital of South Manchester.

Pneumocystis, use of PCR assays, combined with less-invasive patient specimens such as nasopharygeal aspirate (NPA), oral washings, expectorated or induced sputum, represent more cost-effective alternatives than diagnostic techniques using BAL, or radiological findings alone.[12]

Polymerase chain reaction assays have also been used to analyze oral wash samples in the search for more non-invasive techniques.[168,179,180] The generally higher sensitivity of these assays when compared to microscopy could be of greater value among non-AIDS patients in whom the fungal load is generally lower.[181–184] These more sensitive methods have also demonstrated the presence of a new clinical form of PCP, termed *colonization*, which corresponds to the detection of *P. jirovecii* DNA in respiratory samples in the absence of clinical and radiological features of PCP (see Figure 4). Incidences of colonization have ranged between 9% and 69%, depending on the patient populations studied.[86,185] False negatives are however seen when fewer copies are present than the lower limit of detection for a given assay.[92] So despite increasing specificity and sensitivity of *Pneumocystis* detection, PCR interpretation still has challenges in differentiating between active infection and colonization. (See Figure 5 for PCR tracings for *Pneumocystis*.)

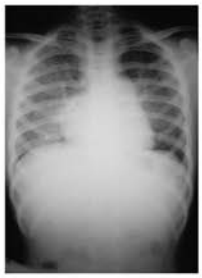
One of the other advantages of qPCR is that it allows for the possibility of quantifying the fungal burden in the respiratory samples.[182] Precise cutoffs to distinguish between colonization and infection have not been defined since different techniques and different genes have been targeted in reported studies.[186,187] There is also the challenge of a different burden: differences in HIV positive and negative patients. A recent report by Louis and colleagues demonstrated the need for lower cutoffs for HIV negative patients because of lower inoculum size and concluded that different cutoffs must be

a



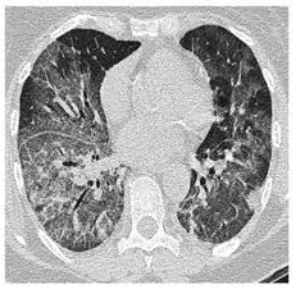
A patient with advanced HIV infection presented with weight loss, fever and marked fatigue. He had a cough, but did not report breathlessness because he was chair and bed bound. His chest Xray shows bilateral, mostly upper lobe cavitary infiltrates most consistent with pulmonary tuberculosis, histoplasmosis, coccidioidomycosis or aspergillosis, but bronchoscopy yielded only *P. jirovecii* on immunofluorescence microscopy. He was treated for PCP but gradually deteriorated and died and was found to have disseminated *P. jirovecii* infection in many organs, including the lungs and brain.

b



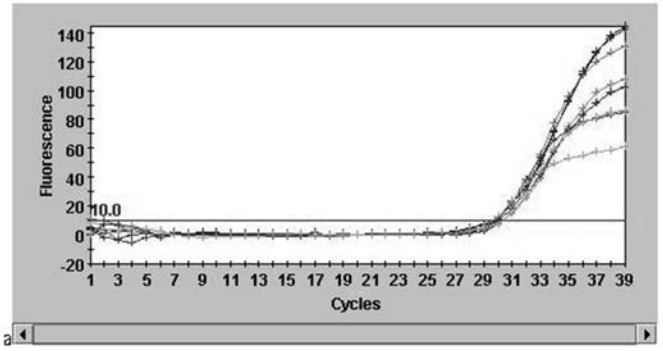
This child with acute lymphoblastic leukaemia was unable to take cotrimoxazole because of some nausea and low blood counts. Trimethoprim alone was substituted. Some weeks later he presented with a cough, fever and dyspnea, and the chest Xray shows bilateral lower and mid zone lobe infiltrates, with some right upper lobe involvement. Bronchoalveolar lavage revealed *P. jirovecii* on GMS stain.

c

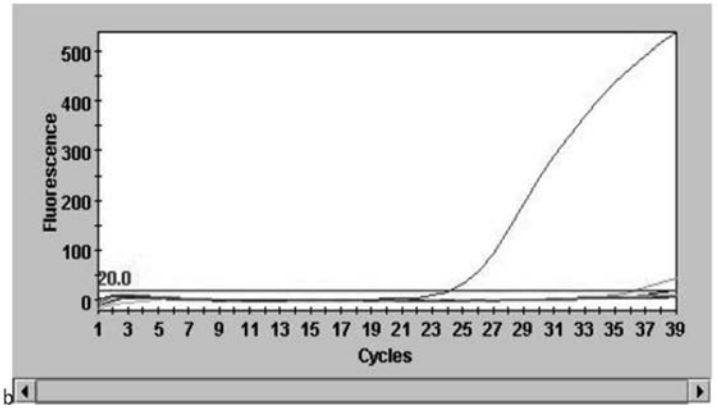


A married man who presented in respiratory failure with bilateral soft infiltrates was suspected of having PCP. His HIV antibody test was positive, his CD4 count was $85x10^6$/L and once intubated, his bronchial aspirate was found to be PCR positive for *P. jirovecii*. Despite high dose intravenous contrimoxazole and corticosteroids given within 48 hours of admission, he deteriorated and died after ~ 3 weeks.

Figure 4: Radiological features of PCP
Images courtesy of LIFEWorldwide (Leading International Fungal Education), http://life-worldwide.org.



A real time PCR tracing, fluorescence channel (Cy3) internal amplification control which was addec to the assay to show no inhibition of reaction.



The FAM channel for pneumocysitis detection with a 20.0 threshold and positive control below CT 25.0. Pneumocystis DNA detected seen by a positive Ct value above threshold of 20.0 using FAM channel.

Figure 5: PCR Tracings for *Pneumocystis*
Images courtesy of Mycology Reference Centre, Manchester University NHS Foundation Trust.

used in relation to HIV status to differentiate between colonized and infected patients using quantitative real time PCR.[182] They used receiver operating characteristic curves (ROC) curve analysis to determine the cutoffs in order to distinguish between colonization and infection, according to the patient's HIV status. It was also observed that prior anti-*Pneumocystis* therapy to sample collection affected the sensitivity of the test.

β-1-3-D-glucan (BDG). β-1-3-D-glucan is a polysaccharide that is present in the cell wall of most fungi including the *Pneumocystis* cyst wall.[188,189] It has been demonstrated to trigger an intrinsic immune reaction that can be detected in patients' BAL and serum specimens infected with *Pneumocystis*.[189,190] Though BDG specificity for *Pneumocystis* is relatively poor, it is however highly sensitive for PCP.[137,174,191,192] Due to the fact that BDG levels are also increased in other mycotic infections such as candidaemia and aspergillosis, it is better used as an adjuvant test in patients with a high clinical index

for PCP. While doing this is feasible in resource rich countries, it is not cost-effective in resource-poor settings.

In a retrospective study of specimens from 295 HIV infected patients suspected of having PCP, BDG was compared with microscopy with a BDG cut-off level of 31.1 pg/ml. The sensitivity and specificity of the BDG assay were 92.3% and 86.1%, respectively with positive and negative predictive values of 0.610 and 0.980, respectively.[174] In this same study, comparison was made with the three other serum markers (CRP, LDH and Krebs von den Lungen-6 antigen (KL-6)) and the ROC suggest that BDG is the most reliable indicator. Another study of 35 PCP patients indicated that, though BDG levels seem a dependable assay for PCP diagnosis, the sensitivity and specificity in patients who were not infected with HIV was lower than in those with HIV infection.[193] This could be attributed to the greater burden of *Pneumocystis* in HIV-infected patients samples compared to that of non-HIV patients. A more recent comparison of the four serological biomarkers (BDG, KL-6, lactate dehydrogenase (LDH) and S-adenosyl methionine (SAM/Adomet)) demonstrated that BDG was the most reliable serologic biomarker for PCP diagnosis, and that the BDG/KL-6 combination test was the most precise serologic assay for PCP diagnosis, with 94.3% sensitivity and 89.6% specificity.[194] The BDG/KL-6 combo may provide a less difficult procedure for PCP diagnosis, as it uses blood, which may be also be a significant advantage especially for pediatric cases thus avoiding the associated risk of complications of bronchoscopy. Another study involving 28 HIV-infected patients and 28 control patients with a BGD cutoff value of 100pg/ml revealed a sensitivity of and specificity of 100 and 96.4% respectively.[195] More recent studies have revealed that BDG is a useful marker to differentiate between PCP and *Pneumocystis* colonization. A positive BDG assay result might be a good indication to begin anti-PCP treatment.[196–198]

S-Adenosylmethionine. S-Adenosyl-L-methionine (AdoMet) plays a vital role in the physiology of all cells, both as a methyl group donor in countless numbers of metabolic processes and as a precursor of polyamines.[199,201] It was assumed that *P. carinii* lacks SAM synthetase so the organism must source this intermediate compound from its mammalian host. However, another study has shown that *Pneumocystis* possesses a working SAM synthetase.[202] *Pneumocystis* does not synthesize AdoMet, so it scavenges it from the infected host thus suggesting that low plasma AdoMet levels might be a useful marker for PCP.[200,201] Several studies from the United States demonstrated that low AdoMet levels could be used to differentiate between PCP and non-PCP pneumonia in HIV-infected patients and healthy control subjects and, additionally, that increasing levels correlated with clinical improvement.[200,201,203] One of these studies revealed that *Pneumocystis* infected patients had considerably lower plasma AdoMet levels when compared with those with non-PCP pneumonia, and no overlap in AdoMet levels were observed between the two groups.[201] These researchers found that reduced AdoMet levels were indicative of a PCP infection and when these levels increased it correlated with clinical improvement.[201,204] Contrarily, another study measuring serum AdoMet levels found overlaps in levels between PCP and non-PCP pneumonia in HIV infected patients.[205] A similar study by another group of researchers did not find any association between AdoMet levels and PCP.[206] Whether these differing results relate to variances between plasma and serum AdoMet levels or to other issues is uncertain. Therefore,

more studies are needed to further elucidate the association between Ado Met levels and PCP. Presently, the test is not recommended for clinical use.

Treatment. A number of factors such as late presentation, missed opportunities for giving prophylaxis (i.e., cough attributed to TB or chest infection, poor compliance and/or side effects), failing ARV therapy[207,209] and probably cotrimoxazole resistance in *P. jirovecii* are all responsible for prophylaxis failures.[210,212] Cotrimoxazole, the first line drug for PCP, is widely available and relatively cheap. However, in patients who fail treatment or develop hypersensitivity, drug intolerance or overt toxicity, the alternative therapeutic agents are pentamidine, atovaquone, trimethoprim plus dapsone and clindamycin plus primaquine. All these choices are more expensive and not readily available in resource-poor settings. *Prophylaxis*. Primary prophylaxis is pertinent for groups such as adolescents, adults and pregnant people with HIV and low CD4 counts or a history of oral candidiasis.[10] Guidelines of the World Health Organization (WHO) recommend prophylaxis for HIV-infected people with CD4 counts lower than 350 cells/μL and to consider discontinuation when CD4 counts rise above 350 cells/μL. These guidelines go on to recommend prophylaxis in settings with a high burden of infectious disease or with limited laboratory infrastructure. This position is supported by a recent systematic review and meta-analysis that report benefits of Cotrimoxazole prophylaxis use in HIV positive people (irrespective of CD4 count) in settings with a high burden of infectious diseases.[213] The Infectious Diseases Society of America (IDSA) guidelines advocate the use of prophylaxis among human immunodeficiency virus (HIV) infected adults with CD4+ counts less than 200 cells/μl with discontinuation at CD4 counts above 200 cells/μl (Table 4).[214] A slightly divergent view supports the discontinuation of prophylaxis earlier among HIV-infected people with CD4 count between 100–200 cells/μl.[214] Secondary prophylaxis needs to be initiated in people who have previously had PCP, with continuation until the CD4 count has been above 200 cells/μl for a sustained period. Data from a 12-cohort collaboration suggests that, in HIV-infected people with CD4+ counts of 100–200 cells/mm3 and HIV-RNA levels less than 400 copies/mL, where PCP incidence is low irrespective of PCP prophylaxis use, suggests that it may be safe to stop prophylaxis earlier, however additional data are needed.[215]

For primary and secondary prophylaxis against PCP, cotrimoxazole , a synergistic combination of the dihydrofolate-reductase inhibitor trimethoprim (TMP) and sulfamethoxazole (SMX) is the recommended first-line drug.[10] TMP/SMX also has the added benefits of being very effective in preventing malaria and malaria-related complications, toxoplasmosis, non-typhoid salmonellosis and many respiratory bacterial infections, and this is particularly beneficial in the tropics that are resource-poor settings.[216] A double-strength tablet daily is the preferred regimen but a double-strength tablet given thrice weekly is also effective.[10]

The frequency of adverse events with use is related to prolonged use of the higher doses.[214] Fever, skin rash (Figure 6), nausea, headache and bone marrow suppression are some of the adverse events more commonly documented with cotrimoxazole use. Nephritis and liver function abnormalities are rare events but occur. If adverse reactions are mild, symptomatic management is advocated whilst the patient is encouraged to continue with the drug.[10] However, for more serious adverse reactions, it is necessary to change therapy (Table 4). Studies have shown that approximately 25% of

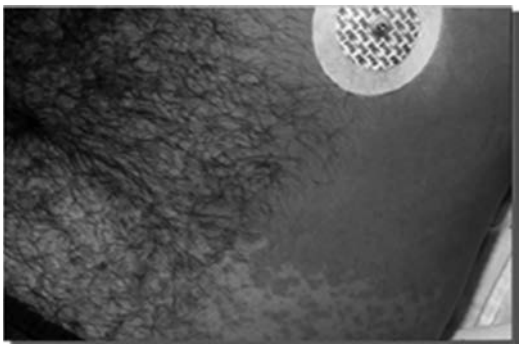


Figure 6: Typical florid maculopapular rash attributable to cotrimoxazole in a patient with moderate PCP as his first presentation of AIDs.
Image courtesy of David Denning, National Aspergillosis Centre, Manchester University NHS Foundation Trust.

HIV infected patients are unable to endure a full course (21 days) of cotrimoxazole.[10] Alternative prophylactic regimens for those who are unable to tolerate cotrimoxazole are dapsone, dapsone plus pyrimethamine plus leucovorin, aerosolized pentamidine and atovaquone.[217,218]

Definitive therapy. Factors which influence the outcome of treatment of PCP include the degree of hypoxia at the onset of therapy, the degree of immunosuppression, co-morbid conditions, and tolerability of the most effective agents.[219,220] Intravenous formulations are preferred for hospitalized patients, if available. Milder forms of disease are treated with oral therapy from the outset. Treatment is usually limited due to the toxicity of the various agents available.[221] For mild to moderate cases of PCP, cotrimoxazole remains the gold standard and first line therapy. The recommended dose is TMP 15–20 mg/kg/day and SMX 75–100 mg/kg/day, given per oral in 3 divided doses[10], a total of 120mg/Kg/day initially, given 6 hourly. Cotrimoxazole should be dose adjusted for abnormal renal function and laboratory monitoring of renal function and electrolytes is vital. In pregnancy, cotrimoxazole is the preferred initial therapy in spite of the association of trimethoprim use in the first trimester with an increased risk of neural tube defects and cardiovascular, urinary tract, and multiple anomalies.[222,223]

The second line drugs include intravenous pentamidine 4 mg/kg IV once daily or primaquine 30 mg (base) per oral once daily plus clindamycin administered either intravenously or orally (Table 5).[214] All oral alternatives including trimethoprim plus dapsone, and atovaquone are reserved for mild cases.[214] Intravenous pentamidine is the most studied drug as an alternative to cotrimoxazole. Pentamidine was thought to be as active as cotrimoxazole; however, the incidence of adverse events, such as nephrotoxicity and dysglycemia, during treatment with pentamidine is higher.[133] Pentamidine use appears to be linked to significantly worse outcomes when compared with other treatment regimes. This is reflected in the findings of a recent study involving 1,188 episodes of HIV-associated PCP cases in Copenhagen (Denmark), London (UK) and Milan (Italy).[7] Inferior efficacy was identified as the cause of the increased risk of death associated with pentamidine in this study. This increased risk of death was recorded among the patients switched to pentamidine either because of suspected treatment

Table 5.

ANTI-*PNEUMOCYSTIS* PNEUMONIA (PCP) DRUGS AND THEIR TOXIC EFFECTS

Drug	Toxic effect
Cotrimoxazole	Neutropenia and anaemia (40%), fever (25%), skin rash (19%), nausea, headache, bone marrow suppression, thrombocytopenia (5%), interstitial nephritis, liver function abnormalities (10%), aseptic meningitis, distributive shock syndrome and Stevens-Johnson syndrome or toxic epidermal necrolysis (TEN).
Pentamidine	Renal dysfunction (60%), leucopenia (50%), hypotension (50%), nausea and vomiting (25%) dysglycemia, pancreatitis, hypoglycaemia (20%), hyperglycaemia, electrolyte dysfunction, bone marrow suppression, cardiac dysrhythmia such as torsades de pointes.
Dapsone	Rash, fever, methemoglobinemia and hemolytic anaemia.
Primaquine	Rash, fever, diarrhoea, methemoglobinemia and hemolytic anaemia.
Clindamycin	Fever, rash, anaemia and diarrhoea. Long term use predisposes to *Clostridium difficile* infection.
Atovaquone	Nausea, diarrhoea, headache, rash, and liver function abnormalities.

failure or because of toxicity.[7] The aerosolized form of pentamidine has been found to have limited efficacy and is associated with more frequent relapse.[10,224]

The combination of clindamycin and primaquine appears to be more effective than intravenous pentamidine for the treatment of PCP where there is clinical failure to cotrimoxazole.[225,226] Additionally, oral atovaquone has been demonstrated as being as effective as intravenous pentamidine in the treatment of mild to moderate PCP and has notably fewer treatment-limiting adverse events.[217,227] Atovaquone, though less effective than cotrimoxazole is better tolerated and is a useful alternative for mild to moderate PCP.[228]

Clinical parameters to be monitored to assess response to therapy include respiratory rate, arterial oxygenation, and temperature. The median time to initial clinical response to therapy is 4 to 10 days, although deterioration prior to recovery is common.[132] Excess IV fluids should be avoided and fluid overload can mimic failure of therapy.

A number of hospitalized PCP patients require mechanical ventilation with an antecedent high in-hospital mortality.[229] Data from a 10 year retrospective study in France revealed that PCP accounted for 35.4% of respiratory failure cases among HIV-infected patients admitted to the intensive care unit (ICU).[230] In a more recent cohort study by same researchers, the overall prevalence of PCP reduced significantly to 8.6% at ICU admission and of AIDS and this decrease was progressive over a ten-year period.[231] Evidence from a case-control study suggests that the use of noninvasive positive pressure ventilation (NPPV) should be considered as a first-line therapeutic

choice for respiratory failure in AIDS patients with severe PCP to reduce the incidence of pneumothorax and improve survival outcome.[232] More recent reports support the use of extracorporeal membrane oxygenation (ECMO) for salvage therapy for respiratory and/or circulatory failure refractory to optimal medical treatment.[233,234]

Challenge of emerging resistance. Recent data has raised concerns about potential anti-pneumocystis drug resistance due to selective pressure.[8,235,236] An appreciation of the magnitude of this problem has been limited somewhat by the lack of *in vitro* culture systems to facilitate direct testing of the organism. Studies have shown a direct relationship between contact with sulfa containing agents and transmutations of the DHPS gene of *P. jirovecii*, however the association between these alterations and therapeutic failure is yet to be proven.[48,237,238] This information is of utmost importance in development of guidelines for clinicians managing PCP patients. Researchers have focused on direct sequencing of genes that code for enzymes which are targeted by anti-*Pneumocystis* drugs.[239]

DHFR and DHPS are the enzymatic targets of cotrimoxazole and dapsone.[236] Associations between exposure to sulphur drugs and genetic mutations to the DHFR and DHPS genes have been established.[8,239] Mutations at amino acids 55 (Thr3Ala) and 57 (Pro3Ser) in the *P. jirovecii* DHPS gene have been linked to prior use of sulphur drugs.[240–242] However, the role of these mutations in promoting prophylaxis or treatment failure is yet still unclear. Several studies have shown an increased risk of treatment failure with cotrimoxazole in patients with DHPS mutations.[242,243] HIV-infected patients with *P. jirovecii* which have DHFR genetic mutations have been reported to have worse clinical outcomes when compared with patients infected with *P. jirovecii* strains containing wild-type DHPS.[240] The contribution of DPHS mutations and drug resistance to worse clinical outcomes remains uncertain as other investigators have failed to show that DHFR mutations are predictors of PCP mortality; instead they highlight low serum albumin and early intensive care admission as stronger predictors of mortality.[244]

Availability of first line drugs. The use of cotrimoxazole prophylaxis especially among HIV-infected people with severe immune suppression who are antiretroviral therapy (ART) naïve has been associated with reduction in overall mortality of 19–46% in LMICs.[245] In spite of the clear benefits of cotrimoxazole prophylaxis and its availability and affordability, the use of cotrimoxazole among HIV-infected people remains suboptimal worldwide.[245] A 2010 WHO survey of 38 countries whose national policies was to provide cotrimoxazole to HIV-infected people revealed that only 25 of the 38 had fully implemented this policy.[246] A recent study of 23,816 HIV-infected patients in China reports that 12,047 (51%) had never taken cotrimoxazole, whereas 11,769 (49%) had taken the drug within 6 months of antiretroviral (ART) initiation. Of those who reported cotrimoxazole use, 2,252 (19%) did not begin taking the drug at ART initiation.[245] Erratic drug supply, drug stock outs and poor knowledge of cotrimoxazole among both health workers and patients have been identified as root causes of the low utilization.[247,248] In many LMICs, intravenous forms of cotrimoxazole are not readily available for the treatment of PCP.

The availability of the other agents for prophylaxis and treatment of PCP is poor when compared to cotrimoxazole. This limits the therapeutic options available to physicians

especially among patients who cannot tolerate cotrimoxazole or a lack of response to treatment with cotrimoxazole. The cost implications of providing alternative therapies is considerably greater than that required to provide cotrimoxazole.

The challenge of toxicity. In spite of the overwhelming benefits of cotrimoxazole use for PCP prophylaxis and treatment, the toxicity associated with its use continues to hinder its use. These toxicities have been found to occur more commonly among HIV-positive people when compared to HIV-negative people.[249] The toxicities which limit the use of TMP-SMX tends to occur between day 6 and 10 of therapy.[250] The toxic effects include headache, nausea, vomiting, fever, rash, pancytopenia, aseptic meningitis, hepatitis, hyperkalemia and renal dysfunction as seen in Table 5. The life threatening toxicities include a distributive shock syndrome and Stevens-Johnson syndrome.[250] On re-introduction of cotrimoxazole following discontinuation due to adverse events such as fever and vomiting, it is better tolerated if the dose is gradually increased (desensitization) according to published regimens.[251] It has been reported that up to 70% of patients tolerate such re-institution of therapy.[252]

Corticosteroids. Patients on treatment for PCP typically worsen clinically after 2–3 days of starting therapy and this is presumably due to increased inflammation in the lungs as organisms are killed. This worsening is also reflected in an increase in the alveolar-arterial oxygen gradient and can be prevented or blunted with the concomitant administration of corticosteroids at the initiation of therapy. The administration of corticosteroids in conjunction with anti-*Pneumocystis* therapy has been clearly shown to reduce the incidence of mortality and respiratory failure associated with severe PCP.[253] Despite the clear benefits of corticosteroid use in conjunction with cotrimoxazole for moderate and severe cases of PCP in AIDS patients, there are concerns that corticosteroid use increases the risk of opportunistic disease such as cytomegalovirus (CMV), herpes simplex virus (HSV) infections, mycobacterial and fungal diseases, and Kaposi's sarcoma.[254,255] Additionally, there is no consensus on the ideal dose and duration of corticosteroid use in PCP treatment. Currently, the WHO does not recommend corticosteroids for PCP in AIDS.

Immune reconstitution inflammatory syndrome and PCP. Despite the undeniable benefits of ART in the setting of HIV, the danger of immune reconstitution inflammatory syndrome (IRIS) in relation to PCP antigens needs to be borne in mind. IRIS can be as a result of immune recovery following ART use thereby unmasking an underlying infection, tumour or disease.[256] The two forms of PCP-IRIS are unmasking PCP which occurs within weeks of commencing ART or paradoxical worsening of PCP following stoppage of anti-PCP treatment and initiation of ART.[257–260] Cotrimoxazole-resistant PCP remains a strong differential diagnosis of PCP-IRIS and should always be borne in mind. Immune system recovery following ART use in HIV-infected people with PCP favours an effective but exaggerated inflammatory response which is CD4–cell driven.[256] This exaggerated response paradoxically leads to severe lung injury and severe PCP. The opposite of this is a CD8+ driven response which occurs in advanced HIV. This CD8+ driven response results in lung damage, a failure to clear the organism and prolonged inflammation which may ultimately be fatal if untreated.[256] The optimal timing of introduction of ART in patients with PCP is still open to debate. However, caution is required when introducing ART early in patients with PCP, especially when

adjunctive steroids are used. A case series has described acute respiratory failure occurring after early introduction of ART in patients treated for severe PCP.[258] In this study, ART was introduced 1 to 16 days after PCP diagnosis and steroids were stopped on day 15. These patients developed a second episode of severe acute respiratory failure 7 to 17 days after commencement of ART. They all recovered after discontinuation of ART or steroid reintroduction. The marked reduction in plasma viral load recorded in these patients was not matched by an increase in circulating CD4+ cell counts.[258]

Another case series described the development of a pneumonic illness in people who started ART shortly after PCP had been effectively treated.[259] Interestingly, their CD4+ counts had all risen and there was no evidence of PCP, mycobacteria and viruses in the BAL preparations thereby supporting an immune reconstitution illness. This illness could be temporally related to the recovery of their immune system. Therefore, the decision of when to commence ART in HIV-infected people with PCP is further compounded by the fact that clearance of *P. jirovecii* can be prolonged in HIV-infected people despite clinical improvement.[260] A study using direct fluorescent antigen testing demonstrated *P. jirovecii* cysts in 24% of HIV-infected people 3 weeks after receiving standard PCP treatment.[261]

Conclusions. *Pneumocystis* pneumonia still poses a major challenge in managing HIV-infected patients and a high index of suspicion is necessary when caring for this group of patients. Unfortunately, the facilities to diagnose this disease are lacking in many resource-poor settings that are also afflicted by a high burden of HIV cases. Though PCR-based detection techniques are now readily available in many institutions in resource rich countries, they are expensive, require reliable electricity and frozen reagents and thus are not feasible for resource-poor settings. Getting an appropriate sample for testing is challenging especially in children and the lack of bronchoscopy in resource-poor settings remains a limiting factor.

Cotrimoxazole remains the drug of choice both for prophylaxis and treatment though alternative medications are available for patients who are intolerant to cotrimoxazole or in cases of treatment failure. However, there is a dearth of these alternative medications in many resource-poor countries and this might be related to their comparatively higher cost. There is a dire need for cheaper generics and for further drug development to expand the existing armamentarium.

Colonization is a major challenge which needs to be further explored to determine if there is need for therapeutic intervention or if there is a place for respiratory isolation in critically ill AIDS patient.

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