GAFFI Fact Sheet

Pneumocystis pneumonia - Introduction

Pneumocystis pneumonia (PCP) is a life-threatening illness of largely immunosuppressed patients such as those with HIV/AIDS. However, when diagnosed rapidly and treated, survival rates are high. The etiologic agent of PCP is *Pneumocystis jirovecii*, a human only fungus that has co-evolved with humans and could be considered a commensal. Other mammals have their own *Pneumocystis* species. Primary infection occurs early in life as suggested by the high rate of antibody formation in children younger than 2 years old. Immunocompetent individuals are the reservoir of this organism with constant transmission throughout life. Some individuals likely clear the fungus rapidly, while others remain carriers of variable intensity[1]. In about 10-20% of adults *P. jirovecii* can be detected but higher carriage rates occur in children and immunosuppressed adults; ethnicity and genetic associations with colonization are poorly understood. Co-occurrence of other respiratory infections may increase transmission in most instances. Patients with *Pneumocystis* pneumonia (PCP) and carriers can transmit *P. jirovecii* to other patients. In tropical countries, the prevalence of pneumocystosis correlated positively with the income level[2]. Prophylaxis with oral co-trimoxazole is usually effective in preventing infection [2].

Pneumocystis pneumonia - Epidemiology

The occurrence of fatal *Pneumocystis* pneumonia in homosexual men in the U.S. provided one of earliest signals of the impending AIDS epidemic in the 1980s. Profound immunosuppression, especially T-cell depletion and dysfunction, is the primary risk group for PCP. Early in the AIDS epidemic, PCP was the AIDS-defining diagnosis in ~60% of individuals. This frequency has fallen in the western world, but infection is poorly documented in most low-income countries because of the lack of diagnostic capability. Rates in HIV-infected individuals in various countries (using different diagnostic techniques) vary substantially (2000 onwards):

Table 1: Incidence of PCP in HIV patients by country or region, 2000 onwards

Country/region	Sample Size	Adult/Child	Incidence	Specimen studied	Method of Detection	Reference
South Africa	151	C	10%	Induced sputum; nasopharyngeal aspirate	GMS; IF	Zar et al., 2000[3]
India	143	A	5%	Autopsy study	GMS	Lanjewar, 2001[4]
Malawi	352	A	9%	BAL	IF, PCP	Hargreaves, 2001[5]
Poland	141	A	21%*	N/A	N/A	Janowska, 2001[6]
Tunisia	27	A	33%	BAL	Giemsa, GMS	Ennalfer-Jerbl, 2002[7]
Australia	4351	A	26-30%	N/A	N/A	Dore, 2002[8]
South Africa	105	C	49%	Induced sputum; nasopharyngeal aspirate	IF	Ruffini and Madhi, 2002[9]
Zambia	180	C	29%	Post mortem lung biopsy	GMS	Chintu et al., 2002[10]
Botswana	47	C	31%	Autopsy	GMS	Ansari, 2003[11]
Europe	181,296	A	18%*	N/A, clinical	N/A	Serraino, 2003[12]

Kenya	51	A	37%	BAL	Toluidine Blue O, IF	Chakaya, 2003[13]
India	135	A	7.4%	Induced sputum, clinical	N/A	Sharma, 2004[14]
Spain	7.3M	A+C	3.4/100,000	N/A	N/A	Calderon, 2004[15]
India	300	A	13%	BAL + sputum	N/A	Udwadia, 2005[16]
Poland	868	A	12%*	N/A	N/A	Podlasin, 2006[17]
Kenya	30	C	16%	Induced sputum	IF	Bii, 2006[18]
Malawi	660	A	11%	Induced sputum	IF, PCR	Van Oosterhout, 2007[19]
Vietnam	1500	A	3%	N/A	N/A	Klotz, 2007[20]
France	560	A	19%[a]	N/A	N/A	Grabar, 2008[21]
Ethiopia	131	A	30%	BAL, sputum	Toluidine Blue O	Aderaye et al, 2008[22]
Brazil	8601	A	4.7%[b]	N/A	ICD10 coding	Saraceni, 2008[23]
Malaysia	107	A	60%	Clinical	None	Asmal, 2009[24]
Uganda	226	A	4%	BAL	Modified Giemsa	Kyeyune et al., 2010[25]
Tanzania	65	A	1.5%	Oral wash	PCR	Jensen et al, 2010[26]
India	147	A	11%	BAL, sputum, tracheal aspirates	IF, GMS, PCR	Tyagi, 2010[27]
South Africa	124	C	35%	N/A	N/A	Morrow, 2010[28]
Ukraine	245	C	6%	N/A	N/A	Mahdavi, 2010[29]
Malawi	327	C	5%	Lung aspirate	PCR	Graham, 2011[30]
Namibia	475	A	5%	Sputum	GMS, PCR	Nowaseb, 2012[31]
Tanzania	125	A	10%	Induced sputum	Toluidine Blue O, PCR	Mwita, 2012[32]
Uganda	129	A	11%*	BAL	Giemsa	Taylor, 2012[33]
Uganda	178	A	6.7%	Induced sputum	PCR	Okwera, 2013[34]
Bangladesh	24	C	16.7%	N/A	N/A	Sharhin, 2013[35]
China	834	A	22.4%	Induced sputum, BAL	GMS	Xiao, 2013[36]
Japan	225	A+C	29.3%	Autopsy	GMS	Katano, 2014[37]
France	35,876	A+C	26.1%	N/A	N/A	Bitar , 2014[38]
India	55	A	1.81%	Sputum	GMS	Shahapour, 2014[39]
US/Puerto Rico/South Africa	282	A	64%	N/A	N/A	Grant , 2014[40]
China	297	A	22.1%	N/A	N/A	Cao, 2014[41]
India	232	A	4.7%	N/A	N/A	Sanadhya, 2014[42]
UK + France	437	A	37%	N/A	N/A	Deconinck, 2014[43]
Bahrain	194	A	5.1%	BAL	IF	Saeed, 2015[44]
Brazil	700	A+C	20%	N/A	N/A	Galisteu, 2015[45]
India	164	A	16%	N/A	N/A	Ramesh, 2015[46]
Libya	227	A	8.8%	N/A	N/A	Shalaka, 2015[47]
Namibia	177	A	11.3%	N/A	N/A	Mgori , 2015[48]
Mozambique	834	C	6.8%	Nasopharyngeal aspirates	PCR	Lanaspa, 2015[49]
Korea	1,086	A	11.0%	N/A	GMS, Clinical, Radiology, PCR	Kim, 2016[50]
India	111	A+C	5.8%	N/A	N/A	Patil, 2016[51]

China	920	A	42.1%[b]	N/A	N/A	Luo, 2016[52]
South Africa	34	A	6%	Autopsy (minimally invasive)	GMS	Karat, 2016[53]
Portugal	107	A	55.1%[a]	N/A	N/A	Grilo, 2016[54]
Iran	160	A	77.5%	Serum	Indirect fluorescent antibody test	Homayouni, 2017[55]

a = AIDS-defining; b = cause of death; * in newly diagnosed HIV
Abbreviations: GMS = Gomori Methenamine Silver; BAL – bronchoalveolar lavage; IF = immunofluorescence; PCR = polymerase chain reaction

PCP in non-HIV infected people

Multiple groups are at risk for PCP, including transplant recipients and patients treated with corticosteroid and immunomodulating agents. Non-HIV patients with PCP have more symptoms and signs, worse radiology and 50% mortality with treatment[56,57]. Amongst children, malnourished children, those with inherited immunodeficiency syndromes and cancer are particularly susceptible.

P. jirovecii has been detected in the lungs of infant with sudden death syndrome, but a causative link between them is not established[58]. The role of *P. jirovecii* in COPD exacerbations or progression of lung disease is also not clear but *P. jirovecii* is commonly found - 42.1% of explanted lungs in advanced COPD carry the organism[59].

Diagnosis of PCP

Microscopy has been the cornerstone of diagnosis for detection of *P. jirovecii*, as the organism does not grow in culture. In recent years, molecular biomarkers have been developed that provide an alternative for rapid and sensitive detection.

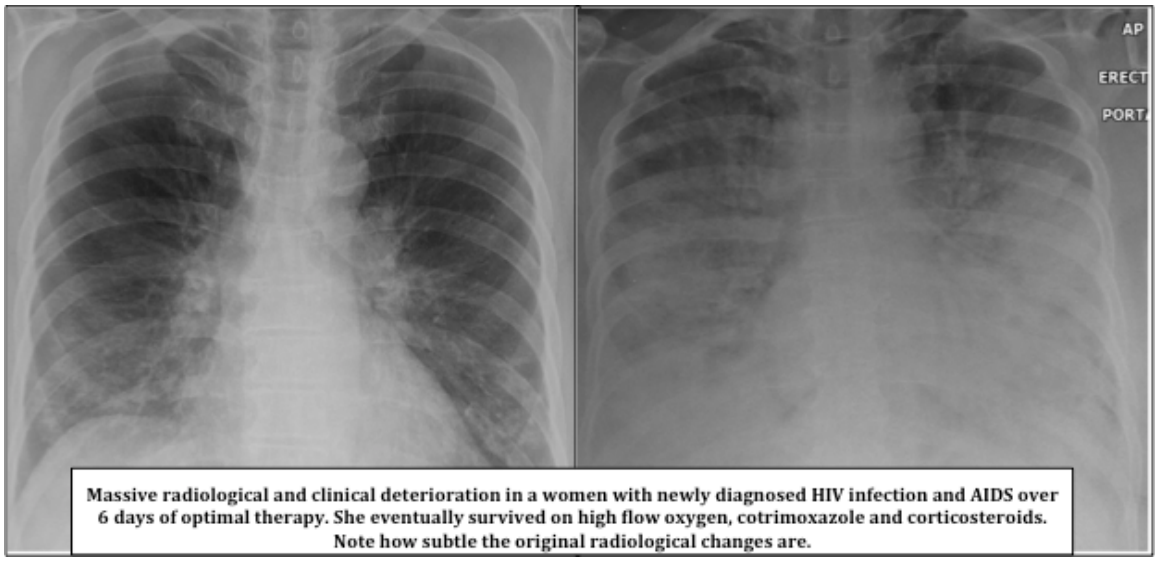
Table 2: Comparison of microscopy and PCR techniques for detection of *P. jirovecii* [60]

	Real-time PCR	IF	GMS	Toluidine blue-O	Gram-Weigert	Giemsa	Diff-Quick
Target	Cyst forms Trophic forms	Cyst forms	Cyst forms	Cyst forms	Cyst forms trophic forms	Cyst forms trophic forms	Trophic forms
Sensitivity	+++++	++++	+++	+++	++	++	++
Specificity	+++++	++++	++++	++++	++	++	++++
Total procedure time in minutes	180	180	100	50	60	80	30

IF = immunofluorescence; GMS = Gomori Methenamine Silver; TBO = Toluidine Blue-O; *cyst form = asci; trophic forms similar a cell wall deficient cell
+ = 0-25%; ++ = 26-50%; +++ = 51-75%; ++++ = 76-85%; +++++ 86-95%

Of these methods, real-time PCR, and IF are the best, with the sensitivity of PCR about 15-20% better than classical staining (GMS, TBO, Giemsa)[61]. Real-time PCR has the advantage of not requiring much microscopy training, but the disadvantage of requiring specialized equipment. It is more precise than immunofluorescence to detect the fungal load with large overlaps of quantification detected between IF+ and IF- samples[6263]. Real-time microscopy allows determination of the fungal load, although no universal thresholds have been defined so far to separate carriage from active infection[61]. PCR also identifies carrier patients because of

increased sensitivity, which microscopy does not. More sensitive and earlier diagnosis appears to result in lower mortality[64]. However, microscopy misses ~25% of pneumocystosis and more are missed if microscopists are improperly trained. Detection of beta-D-glucan in blood is very sensitive, but not specific[65]; the test is not suitable for most laboratories, even in highly developed countries, and is not useful for assessing response to therapy[66]. The level of beta-glucan correlated with the fungal load detected in respiratory samples[67].

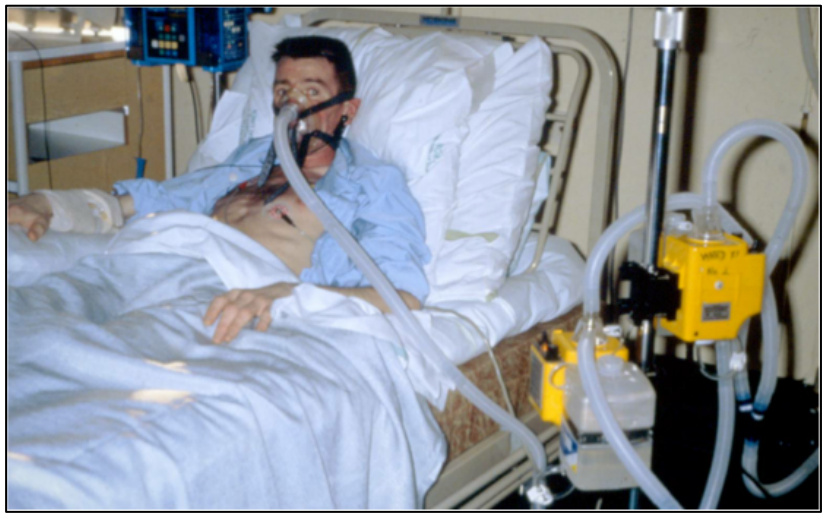


Massive radiological and clinical deterioration in a women with newly diagnosed HIV infection and AIDS over 6 days of optimal therapy. She eventually survived on high flow oxygen, cotrimoxazole and corticosteroids. Note how subtle the original radiological changes are.

All respiratory samples can be used for microscopy and PCR, but bronchoalveolar lavage has been preferred in developed countries as the most sensitive specimen – *Pneumocystis* is proliferating at the surface of type I pneumocytes in the lung alveoli[65]. Induced and expectorated sputum are both adequate (assuming a good sample is obtained). Oral mouthwash has also been valuable in some studies. A positive result in upper respiratory samples (expectoration, oral wash, nasopharyngeal aspirate, nasal swab) reflects a higher fungal load in the alveoli[64,68]. Detection of circulating DNA in serum is a promising tool[69]. Co-infection with other pathogens is common.

Treatment and outcome from PCP

High dose co-trimoxazole (sulphamethoxazole/trimethoprim) is the standard therapy for PCP, best given IV initially for very ill patients. A switch to oral is usual after 3-10 days depending on progress and severity. Moderate and severe PCP (judged by hypoxaemia) responds better if corticosteroids are used concurrently, for 10 days to 3 weeks, in AIDS patients[70,71]. This adjuvant steroid therapy has not been yet evaluated properly in non-HIV infected patients.



Resistance to co-trimoxazole only affects prophylaxis, not full dose treatment. Complications of therapy are common including nausea, vomiting, skin rash, neutropenia and abnormal liver function tests. Mild cases can be treated with all oral therapy, using co-trimoxazole, dapsone, or

atovaquone. Second line therapy is preferably a combination of clindamycin and primaquine; IV pentamidine, can be used for cotrimoxazole intolerance.

Untreated PCP is fatal. Management by highly experienced clinicians leads to 85-90% survival in AIDS patients and ~50% in non-AIDS patients. Once improved, AIDS patients can be started on antiretroviral agents (ARVs) and most make an excellent clinical recovery, without residual lung disease, and immunological recovery.

Transmission of *P. jirovecii* - outbreaks and carriage

P. jirovecii circulates in immunocompetent hosts through air continuously in the human population[1]. It is present in exhaled air close to patients with PCP, amounts decreasing the further the sampling moved away from patients[72]. Direct person-to-person transmission has now been documented[73], and some genotypes may be more transmissible or virulent than others [72,74,75]. All hospitalized patients with PCP should be isolated in single rooms and that vulnerable patient groups should be kept away from them. Transmission has also occurred from carrier patients, making more complicated the prevention of transmission[76].

Prevention

Primary and secondary prophylaxis with oral co-trimoxazole at regular doses is highly effective, if taken regularly, and provides some cross protection against toxoplasmosis and some bacterial infections. It is routinely given to HIV positive patients with CD4 cell counts <200 x10^6/ml and transplant recipients. Some alternatives are available for patients with intolerance or allergy. Non-compliance is common in some communities and patient groups.

Observations and research needs for *Pneumocystis* pneumonia:

- A low cost quantitative molecular detection method including automated extraction should be developed for rapid and sensitive diagnosis of PCP.
- Alternative diagnostic tools are required for patients who do not produce sputum and in whom bronchoscopy is not possible that are applicable in low and middle-income countries.
- The relative proportion of PCP cases versus other community acquired pneumonias in both adults and children, using the most sensitive diagnostic method should be ascertained for countries with high HIV caseloads.
- The impact of corticosteroid adjunctive therapy should be studied in countries with a high TB burden, to ascertain if there are any negative consequences of corticosteroids and whether the criteria used for defining moderate and severe PCP apply in children and adults equally.
- Co-trimoxazole prophylaxis breakthroughs need to be evaluated for co-trimoxazole resistance or are a function of non-adherence.

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