HIV-Associated *Pneumocystis* Pneumonia

Laurence Huang^{1,2}, Adithya Cattamanchi¹, J. Lucian Davis¹, Saskia den Boon³, Joseph Kovacs⁴, Steven Meshnick⁵, Robert F. Miller^{6,7}, Peter D. Walzer⁸, William Worodria⁹, and Henry Masur⁴, on behalf of the International HIV-associated Opportunistic Pneumonias (IHOP) Study and the Lung HIV Study

¹Division of Pulmonary and Critical Care Medicine and ²HIV/AIDS Division, Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California; ³Makerere University - University of California San Francisco Research Collaboration, Kampala, Uganda; ⁴Critical Care Medicine Department, National Institutes of Health, Bethesda, Maryland; ⁵Department of Epidemiology and Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; ⁶Centre for Sexual Health and HIV Research, Research Department of Infection and Population Health, Division of Population Health, University College London, London, United Kingdom; ⁷Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; ⁸Veterans Affairs Medical Center and Division of Infectious Diseases, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio; ⁹Department of Medicine, Mulago Hospital and Makerere University and Makerere University - University of California San Francisco Research Collaboration, Kampala, Uganda

During the past 30 years, major advances have been made in our understanding of HIV/AIDS and *Pneumocystis* pneumonia (PCP), but significant gaps remain. *Pneumocystis* is classified as a fungus and is host-species specific, but an understanding of its reservoir, mode of transmission, and pathogenesis is incomplete. PCP remains a frequent AIDS-defining diagnosis and is a frequent opportunistic pneumonia in the United States and in Europe, but comparable epidemiologic data from other areas of the world that are burdened with HIV/AIDS are limited. *Pneumocystis* cannot be cultured, and bronchoscopy with bronchoalveolar lavage is the gold standard procedure to diagnose PCP, but noninvasive diagnostic tests and biomarkers show promise that must be validated. Trimethoprim-sulfamethoxazole is the recommended first-line treatment and prophylaxis regimen, but putative trimethoprim-sulfamethoxazole drug resistance is an emerging concern. The International HIV-associated Opportunistic Pneumonias (IHOP) study was established to address these knowledge gaps. This review describes recent advances in the pathogenesis, epidemiology, diagnosis, and management of HIV-associated PCP and ongoing areas of clinical and translational research that are part of the IHOP study and the Longitudinal Studies of HIV-associated Lung Infections and Complications (Lung HIV).

Keywords: acquired immune deficiency syndrome; HIV; *Pneumocystis*; *Pneumocystis* pneumonia; dihydropteroate synthase

The pre-eminence of *Pneumocystis* pneumonia (PCP) as a herald of the HIV/AIDS epidemic and as a major cause of HIV-associated morbidity and mortality has focused considerable attention and resources on this previously uncommon opportunistic pneumonia. Over the past 30 years, major advances have been made in our understanding of HIV/AIDS and PCP, but significant gaps remain. This review describes recent advances in the pathogenesis, epidemiology, diagnosis, and management of HIV-associated PCP and ongoing areas of clinical and translational research that are part of the International HIV-associated Opportunistic Pneumonias (IHOP) study and the Longitudinal Studies of HIV-associated Lung Infections and Complications (Lung HIV).

(Received in original form September 30, 2010; accepted in final form November 26, 2010)
Supported by National Heart Lung and Blood Institute grants HL087713, HL090335, and HL090335-02S1.

Correspondence and requests for reprints should be addressed to Laurence Huang, M.D., HIV/AIDS Division, Ward 84, San Francisco General Hospital, 995 Potrero Avenue, San Francisco, CA 94110. E-mail: lhuang@php.ucsf.edu

Proc Am Thorac Soc Vol 8. pp 294-300, 2011

DOI: 10.1513/pats.201009-062WR

Internet address: www.atsjournals.org

BACKGROUND AND PATHOGENESIS

Pneumocystis is an opportunistic eukaryote that is classified as a fungus (1). The genus *Pneumocystis* infects mammalian species and is host-species specific. Infection in humans is caused by *Pneumocystis jirovecii*; *Pneumocystis carinii* currently refers to one of the *Pneumocystis* species that infects rats. Humans are a reservoir of *P. jirovecii*, although the exact relationship is incompletely understood, and environmental reservoirs have also been suggested. Primary infection occurs early in childhood, likely manifesting as a self-limited upper respiratory tract illness (2, 3), and the majority of children throughout the world have detectable antibodies by 2 to 4 years of age (4–6). Studies in animals demonstrate that *Pneumocystis* is transmitted from animal to animal via an airborne route. Animal studies also demonstrate that animals carrying *Pneumocystis* develop PCP after being immunosuppressed (reactivation of latent infection) and that immunocompromised *Pneumocystis*-free animals develop PCP after exposure to immunocompromised animals infected with *Pneumocystis* (new exogenous infection) and to immunocompetent animals that are colonized with *Pneumocystis*. Numerous reports of cluster outbreaks of PCP among different immunocompromised populations support person-to-person transmission and recent acquisition of infection in the pathogenesis of PCP in humans. In addition, molecular typing of *P. jirovecii* genetic loci from persons with PCP has demonstrated the diversity of *P. jirovecii* infecting humans and has provided molecular evidence in support of interhuman transmission and recent infection (7–9).

EPIDEMIOLOGY

Before the HIV/AIDS epidemic, PCP was uncommon. From November 1967 to December 1970, a total of 194 patients were diagnosed with PCP and reported to the Centers for Disease Control, which was the sole supplier of pentamidine isethionate, the only treatment for PCP at that time (10). In 1981, two reports of PCP in 15 previously healthy men who had sex with other men and/or who were injection drug users heralded the onset of the HIV/AIDS pandemic (11, 12) that currently affects an estimated 33.4 million people worldwide and has caused an estimated 25 million deaths (13).

PCP is a frequent AIDS-defining diagnosis in the United States and in Europe. At its peak in the United States, PCP was the leading AIDS-defining diagnosis and was responsible for more than 20,000 new AIDS cases per year from 1990 to 1993 (14–17). In Europe, PCP was the leading AIDS-indicator disease in the World Health Organization 2008 HIV/AIDS Surveillance in Europe Report, accounting for 16.4% of the AIDS

cases diagnosed in adults and adolescents that year (18). PCP remains a leading cause of AIDS in North American and European HIV cohorts. In the Antiretroviral Therapy Cohort Collaboration, a network comprised of 15 North American and European cohorts established in 2000, PCP was the second most frequent AIDS-defining event after esophageal candidiasis (19).

PCP remains an important cause of HIV-associated pneumonia, but rates of PCP have decreased. At San Francisco General Hospital, nearly 1,000 cases of HIV-associated PCP were microscopically diagnosed from 1990 to 1993 (average, 250 cases per year) (Figure 1) (20). This number has decreased to 20 to 30 cases per year. Most of these cases occurred in persons who were not receiving antiretroviral therapy or PCP prophylaxis, and many were unaware of their HIV infection at the time of presentation (21, 22). This experience is similar at other institutions, where 23 to 31% of reported PCP cases occurred in patients who were newly diagnosed with HIV infection at the time of PCP (21, 23, 24).

HIV-associated PCP is reported at varying rates throughout the world (25, 26). Clinical studies from Africa that performed bronchoscopy with bronchoalveolar lavage (BAL) in HIV-infected patients with pneumonia report that PCP accounted for 0.8 to 38.6% of cases (26–28). At Mulago Hospital in Kampala, Uganda, the frequency of PCP among HIV-infected patients hospitalized with suspected pneumonia who had negative sputum acid-fast bacilli smears and underwent bronchoscopy has decreased from nearly 40% of bronchoscopies to less than 10% (28, 29). However, the mortality associated with PCP remains high. Although its current incidence is low in Uganda, patients with PCP had a higher mortality (75%, 3/4) than did those with culture-positive pulmonary TB (31%, 59/190) or cryptococcal pneumonia (10%, 1/10) (30).

PRESENTATION AND DIAGNOSIS

Classically, HIV-associated PCP presents with fevers, nonproductive cough, and dyspnea. Symptoms may be subtle at first but gradually progress and may be present for several weeks before diagnosis. This presentation differs from that typically seen in non-HIV immunocompromised patients with PCP in whom the duration of symptoms is often much shorter (31). The lung examination is often normal, but, when abnormal, inspiratory crackles are the most common finding. The chest radiograph is

the cornerstone of the diagnostic evaluation and demonstrates bilateral, symmetric, reticular (interstitial), or granular opacities (Figure 2) (32, 33). PCP may also present with a pneumothorax or bilateral pneumothoraces. Although relatively uncommon, pneumothorax presents a difficult problem, often requiring prolonged chest tube management. Occasionally, PCP presents with a normal chest radiograph. In these cases, chest high-resolution computed tomography (HRCT) may be useful. Chest HRCT demonstrates patchy areas of ground glass opacity (Figure 3) (34). Although the presence of ground glass opacities is non-specific for PCP, their absence strongly argues against the diagnosis of PCP, and no further diagnostic testing for PCP or PCP treatment is generally warranted in these cases (34). There is no universal approach to the management of suspected PCP. Some institutions empirically treat these individuals, whereas others pursue a definitive diagnosis. Regardless of the approach selected, close follow-up is recommended because the presentation of PCP can overlap with those of other HIV-associated pneumonias, and HIV-infected patients can have more than one concurrent pneumonia.

Pneumocystis cannot be cultured, and the diagnosis of PCP relies on microscopic visualization of characteristic cystic or trophic forms in respiratory specimens obtained most often from sputum induction or bronchoscopy. Bronchoscopy with BAL is regarded as the gold standard procedure to diagnose PCP in HIV-infected patients and has a reported sensitivity of 98% or greater (20). However, bronchoscopy requires specialized personnel, rooms, and equipment, and it is also expensive and carries an associated risk of complications. Thus, bronchoscopy is limited in its availability throughout many areas of the world that are burdened with HIV/AIDS, and an accurate noninvasive procedure to diagnose PCP would be a significant clinical advance.

The development of specific PCR assays has revolutionized the diagnosis of many infectious diseases, and PCR assays for *P. jirovecii* have been developed. *P. jirovecii* PCR assays combined with BAL specimens have been shown to be sensitive for the diagnosis of PCP. The availability of sensitive PCR-based assays led to studies that examined whether these assays could be combined with a noninvasive pulmonary procedure to effectively diagnose PCP. Two studies from San Francisco General Hospital examined oropharyngeal wash (OPW; i.e., gargling) specimens and tested three different PCR-based assays, comparing results

Figure 1. Annual number of microscopically confirmed cases of *Pneumocystis pneumonia* (PCP) diagnosed at San Francisco General Hospital, 1990–2009. ART = antiretroviral therapy.

Figure 2. Chest radiograph demonstrating the characteristic bilateral, symmetric granular opacities in an HIV-infected patient with *Pneumocystis* pneumonia (courtesy of L. Huang, used with permission).

with induced sputum or BAL specimens and microscopic examination after Diff-Quik staining as the gold standard. These studies found that OPW-PCR had a diagnostic sensitivity of up to 88% and a specificity of up to 90% for PCP (35, 36). Procedural factors, such as collecting the OPW specimen before PCP treatment initiation or within 1 day of initiation and having the patient cough vigorously before specimen collection, increased the sensitivity of the test. Although the sensitivity of OPW-PCR for PCP approaches that of BAL-microscopy and may exceed that of induced sputum-microscopy, OPW-PCR can detect *P. jirovecii* DNA in the absence of PCP, resulting in false-positive PCR results. The imperfect specificity of PCR for PCP likely relates to the highly sensitive nature of these assays and the fact that HIV-infected patients and other patients can be colonized with *Pneumocystis* (i.e., *Pneumocystis* DNA is detected by PCR in the absence of PCP) (37, 38). Further study is needed to determine whether the application of a “cut-off” in quantitative PCR assays can be used to distinguish between PCP and *Pneumocystis* colonization.

Plasma and serum assays have been studied for the diagnosis of PCP. One assay examined plasma S-adenosylmethionine (SAM or AdoMet) as a potential biomarker for PCP. SAM is an important biochemical intermediate involved in methylation reactions and polyamine synthesis (39, 40). The original rationale for developing a SAM assay was that *Pneumocystis* lacks a SAM synthetase and therefore is unable to synthesize its own SAM and must scavenge this intermediate from its host (a subsequent study has demonstrated that *Pneumocystis* has a functional SAM synthetase) (41). Thus, patients with PCP might be expected to have low SAM levels. A series of studies from New York found that plasma AdoMet levels could be used to distinguish between HIV-infected patients with PCP and those with non-PCP pneumonia and healthy control subjects (39, 40). In one study, patients with PCP had significantly lower plasma AdoMet levels compared with patients with non-PCP pneumonia (bacterial pneumonia or TB), and there was no overlap in AdoMet levels between these two groups of patients (40). A subsequent study that measured serum SAM found overlapping levels between HIV-infected patients with PCP and those with non-PCP pneumonia (42). Whether the differing results from these studies relates to differences between plasma and serum SAM levels, as has been hypothesized, or to other factors, is unclear, and further studies are needed. More re-

cently, serum (1–3)- β -D-glucan, a cell wall component of all fungi including *Pneumocystis*, has been investigated as a biomarker for PCP because patients with PCP might be expected to have high levels (43, 44). One report found that patients with PCP with and without underlying HIV infection had significantly higher serum (1–3)- β -D-glucan levels compared with patients without PCP (43). Using a 100 pg/ml cutoff, another study reported a diagnostic sensitivity of 100% and a specificity of 96.4% (44). (1–3)- β -D-glucan is elevated in a number of fungal pneumonias, and this test cannot distinguish among fungal etiologies (e.g., PCP and *Aspergillus* species). Thus, although results from these noninvasive diagnostic or biomarker tests are promising, additional validation is needed, and bronchoscopy with BAL remains the gold standard diagnostic test for PCP.

TREATMENT

Trimethoprim-sulfamethoxazole is the recommended first-line treatment for PCP in HIV-infected patients with mild, moderate, and severe PCP, with intravenous therapy generally recommended for inpatients with moderate to severe disease and oral therapy used for outpatients with milder disease (45). Alternative regimens include intravenous pentamidine, clindamycin plus primaquine, trimethoprim plus dapsone, and atovaquone suspension. Adjunctive corticosteroids are recommended for patients with moderate to severe PCP as demonstrated by a PaO_2 less than 70 mm Hg or an alveolar-arterial oxygen gradient greater than 35 mm Hg (45). Patients should be started on adjunctive corticosteroids at the same time that PCP therapy is initiated. The recommended duration of treatment is 21 days (45). However, a substantial proportion of individuals cannot complete a full course of trimethoprim-sulfamethoxazole due to treatment-limiting toxicity or are switched to an alternate treatment regimen due to perceived treatment failure (46). Although there are only limited data from prospective, randomized clinical trials comparing second-line PCP treatments, a tri-center observational study, and a systematic review suggest that the combination of clindamycin plus primaquine is an effective alternative to intravenous pentamidine as second-line PCP treatment (47, 48).

PROPHYLAXIS

Trimethoprim-sulfamethoxazole is also the recommended first-line regimen for primary and secondary prophylaxis against PCP (45). Alternative regimens include dapsone with or without pyrimethamine and leucovorin, atovaquone suspension, and aerosolized pentamidine. HIV-infected adolescents and adults, including pregnant women, should receive PCP prophylaxis if their CD4+ cell count is below 200 cells/ μl or if they have a history of oral candidiasis (primary prophylaxis) and after an episode of PCP (secondary prophylaxis) (45). Persons with a CD4+ cell count below 14% and those with a history of an AIDS-defining illness should also be considered candidates for PCP prophylaxis (45).

Once initiated, PCP prophylaxis is recommended for life, but it can be discontinued in HIV-infected adolescents and adults who are receiving combination antiretroviral therapy and have responded with an increase in their CD4+ cell count from below 200 cells/ μl to above 200 cells/ μl for at least 3 months (45). One potential exception is patients who developed PCP when their CD4+ cell count was above 200 cells/ μl ; these individuals should probably remain on PCP prophylaxis regardless of their CD4+ cell count (45). After discontinuation of PCP prophylaxis, the risk of subsequent PCP on combination anti-

Figure 3. Chest high-resolution computed tomograph demonstrating the characteristic ground glass opacities in an HIV-infected patient with *Pneumocystis pneumonia* who had a normal chest radiograph (courtesy of L. Huang, used with permission).

retroviral therapy with a sustained CD4+ cell count above 200 cells/ μ l (and generally accompanied by a sustained suppression of plasma HIV RNA below the limits of detection) has been shown to be extremely low, but rare cases have been described (49). Prophylaxis should be resumed if the CD4+ cell count declines below 200 cells/ μ l (45). Recent data from a 12-cohort collaboration suggest that PCP incidence is low in HIV-infected persons with CD4+ cell counts of 100 to 200 cells/ μ l and HIV RNA levels less than 400 copies/ml irrespective of PCP prophylaxis use, suggesting that it may be safe to stop prophylaxis earlier, though additional data are needed (50).

PUTATIVE TRIMETHOPRIM-SULFAMETHOXAZOLE DRUG RESISTANCE IN *P. JIROVECI*

The widespread use of trimethoprim-sulfamethoxazole for PCP prophylaxis has been associated with increases in trimethoprim-sulfamethoxazole-resistant bacteria (51) and has raised concerns over potential trimethoprim-sulfamethoxazole drug resistance in *P. jirovecii* (52). Similar concerns have been raised related to the use of atovaquone and potential atovaquone drug resistance (53). Trimethoprim-sulfamethoxazole drug resistance might also result in resistance to trimethoprim plus dapsone (a sulfone), thereby further limiting the therapeutic options available to treat (and prevent) PCP. The inability to culture *P. jirovecii* has hindered efforts to document drug resistance in *Pneumocystis*, but researchers have explored this important question by examining genetic mutations within the dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) genes, the enzymatic targets of trimethoprim and sulfa (sulfamethoxazole and dapsone) medications, respectively, and by correlating the observed genetic mutations with clinical outcomes (52). This approach was chosen because DHFR and DHPS genetic mutations have been shown to cause drug resistance as demonstrated in other microorganisms, such as *Plasmodium falciparum* (54).

Six studies have examined *P. jirovecii* DHFR mutations from patients with PCP with and without underlying HIV infection in the United States, Japan, Europe, South Africa, and Thailand (55–60). The first two studies reported that DHFR mutations were uncommon and unrelated to trimethoprim use as part of PCP prophylaxis (i.e., trimethoprim-sulfamethoxazole) (55, 56). In these studies, nonsynonymous DHFR mutations, resulting in amino acid substitution(s), were found in 0% (0/37) and 7% (2/27) of PCP samples. A similar proportion (4%, 5/128 samples) was found in the largest study

to date, which also failed to find an association between trimethoprim use and the presence of nonsynonymous DHFR mutations (59). In contrast, a European study reported nonsynonymous DHFR mutations in 33% (11/33) of PCP samples (57). This study found that use of DHFR inhibitors (trimethoprim or pyrimethamine) for PCP prophylaxis was associated with the presence of DHFR mutations ($P = 0.008$) and that most of the patients with DHFR mutations were receiving pyrimethamine ($n = 7$) rather than trimethoprim ($n = 2$) as part of their prophylaxis regimen. This study raises the possibility that different DHFR inhibitors may select for different DHFR mutations or may select nonsynonymous DHFR mutations at different frequencies. Because no outcomes were reported in this study, it is unknown whether the presence of DHFR gene mutations is associated with increased morbidity, mortality, or PCP treatment failure in persons receiving trimethoprim-sulfamethoxazole or trimethoprim plus dapsone.

Compared with six DHFR studies, more than 20 studies have examined *P. jirovecii* DHPS mutations from patients with PCP with and without underlying HIV infection in North America, Europe, Asia, Africa, South America, and Australia. The preponderance of studies on DHPS compared with DHFR relates to the fact the sulfamethoxazole is more active against *Pneumocystis* compared with trimethoprim in animal models of PCP, and therefore DHPS mutations would be expected to be more important than DHFR mutations to the development of potential trimethoprim-sulfamethoxazole drug resistance. These studies report a wide range in the frequency of DHPS mutations (from 3.7 to 81%) (58, 61, 62). In general, these studies also reveal a geographic variation in the proportions of DHPS mutations observed, with the highest proportions reported in the United States (San Francisco) and the lowest proportions reported in Spain and South Africa. In addition, some studies report an increase in the proportion of DHPS mutations over time (63). Specifically, two nonsynonymous mutations that result in amino acid substitutions at amino acid position 55 (Thr→Ala) and/or position 57 (Pro→Ser) are almost exclusively reported (64, 65). Overall, these studies have generally demonstrated a significant association between sulfa (sulfamethoxazole or dapsone) use as part of PCP prophylaxis and the presence of nonsynonymous DHPS mutations (52). This finding is noteworthy because the DHPS locus is well conserved in *Pneumocystis* obtained from other mammals and because DHPS mutations are rarely encountered in nonhuman primates (66), suggesting that use of sulfa drugs by humans has selected for *P. jirovecii* DHPS mutations.

In several studies, the presence of DHPS mutations has been associated with poor outcomes in HIV-infected persons with PCP. One study reported that the presence of DHPS mutations was an independent predictor associated with an increased 3-month mortality (adjusted hazard ratio, 3.10; 95% confidence interval, 1.19–8.06; $P = 0.01$) (67). Another study noted that the presence of DHPS mutations was associated with an increased risk of PCP treatment failure with trimethoprim-sulfamethoxazole or trimethoprim plus dapsone (RR = 2.1; $P = 0.01$) (68). Finally, a small study reported that all four patients with DHPS mutations who were treated with trimethoprim-sulfamethoxazole failed PCP therapy (69). In contrast, other studies have failed to demonstrate these associations and instead have reported that risk factors such as low serum albumin and early ICU admission were stronger predictors of PCP mortality than the presence of DHPS mutations (62).

Thus, a seeming paradox exists regarding the clinical significance of DHPS mutations and inferences related to putative trimethoprim-sulfamethoxazole drug resistance. Studies consistently report that the majority of patients with PCP and DHPS

mutations who are treated with trimethoprim-sulfamethoxazole respond to this treatment (62, 67, 68, 70). However, patients with DHPS mutations who are treated with trimethoprim-sulfamethoxazole tend to have worse outcomes compared with those with wild-type DHPS who are treated with trimethoprim-sulfamethoxazole and compared with those with DHPS mutations who are treated with a non-sulfa-based regimen (62). The precise explanation for these observations is unclear, but concurrent DHFR mutations, low serum trimethoprim-sulfamethoxazole levels, and host factors have been postulated as potential cofactors for trimethoprim-sulfamethoxazole treatment failure in patients with PCP and DHPS mutations. No study has examined all of these postulated factors at the same time in patients with PCP. Until the clinical significance of DHPS and possibly DHFR mutations can be further defined, clinicians treating patients with PCP should use trimethoprim-sulfamethoxazole as first-line therapy in all patients unless contraindicated by allergic reaction or adverse effects.

THE LUNG HIV AND IHOP STUDIES

The Longitudinal Studies of HIV-associated Lung Infections and Complications (Lung HIV) Study is a novel, collaborative, multi-R01 consortium of research projects established by the National Heart, Lung, and Blood Institute (NHLBI) to examine a broad range of infectious and noninfectious pulmonary diseases that affect people living with HIV/AIDS. The Lung HIV Study's specific aims, study design, and study protocols are described in the online supplement to this issue. Within the Lung HIV Study, eight clinical centers conduct their own separate research studies but also join under the stewardship of the NHLBI and a data coordinating center to conduct multisite and group level collaborative studies. Each clinical site has its own research focus. The IHOP Study focuses on opportunistic pneumonias, primarily PCP, but includes the establishment of a clinical database and specimen bank that seamlessly enables research on tuberculosis, bacterial pneumonia, and other opportunistic pneumonias. For example, studies on tuberculosis, the dominant opportunistic pneumonia in sub-Saharan Africa, are incorporated within the IHOP infrastructure. The IHOP Study's specific aims include (1) to determine the frequency and mortality of HIV-associated opportunistic pneumonias in an international, longitudinal cohort and to test the hypothesis that PCP is associated with increased mortality; (2) to estimate the sensitivity and specificity of molecular tools for PCP and TB diagnosis and to test the hypotheses that OPW specimens combined with PCR assays are sensitive tests to diagnose PCP and TB; and (3) to test the hypothesis that DHPS gene mutations are associated with an increased morbidity and mortality and to explore potential mechanisms for these outcomes. IHOP and Lung HIV have established specimen banks linked to clinical data, and investigators interested in studying HIV-associated opportunistic pneumonias are encouraged to contact the authors of this review.

CONCLUSIONS

The HIV/AIDS pandemic has witnessed significant advances in our understanding of HIV/AIDS and PCP, one of the prominent diseases associated with the pandemic. This review describes recent advances in the pathogenesis, epidemiology, diagnosis, and management of HIV-associated PCP and ongoing areas of clinical and translational research that are part of the IHOP and the Lung HIV Studies. The IHOP and Lung HIV Studies have established a clinical specimen bank accompanied by clinical data for future studies. Given the decline in the incidence of

PCP but its enduring importance as a cause of morbidity and mortality in HIV-infected and other immunosuppressed patients, this specimen bank may accelerate and further our understanding of *P. jirovecii* and PCP.

Author Disclosure: L.H. received grant support from the Foundation for Innovative New Diagnostics (FIND). A.C. and J.L.D. received grant support from the WHO and the FIND. S.d.B. and J.K. do not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.M. received grant support from Abbott and the Gates Foundation. R.F.M. received lecture fees from Gilead and Merck. P.D.W., W.W., and H.M. do not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

- Kovacs JA, Masur H. Evolving health effects of *Pneumocystis*: one hundred years of progress in diagnosis and treatment. *JAMA* 2009; 301:2578–2585.
- Vargas SL, Hughes WT, Santolaya ME, Ulloa AV, Ponce CA, Cabrera CE, Cumsille F, Gigliotti F. Search for primary infection by *Pneumocystis carinii* in a cohort of normal, healthy infants. *Clin Infect Dis* 2001;32:855–861.
- Larsen HH, von Linstow ML, Lundgren B, Hogh B, Westh H, Lundgren JD. Primary *Pneumocystis* infection in infants hospitalized with acute respiratory tract infection. *Emerg Infect Dis* 2007;13:66–72.
- Meuwissen JH. Infections with *Pneumocystis carinii*. *Natl Cancer Inst Monogr* 1976;43:133–136.
- Pifer LL, Hughes WT, Stagno S, Woods D. *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. *Pediatrics* 1978;61:35–41.
- Peglow SL, Smulian AG, Linke MJ, Pogue CL, Nurre S, Crisler J, Phair J, Gold JW, Armstrong D, Walzer PD. Serologic responses to *Pneumocystis carinii* antigens in health and disease. *J Infect Dis* 1990;161:296–306.
- Huang L, Beard CB, Creasman J, Levy D, Duchin JS, Lee S, Pieniazek N, Carter JL, del Rio C, Rimland D, et al. Sulfa or sulfone prophylaxis and geographic region predict mutations in the *Pneumocystis carinii* dihydropteroate synthase gene. *J Infect Dis* 2000;182:1192–1198.
- Ripamonti C, Orenstein A, Kutty G, Huang L, Schuegger R, Sing A, Fantoni G, Atzori C, Vinton C, Huber C, et al. Restriction fragment length polymorphism typing demonstrates substantial diversity among *Pneumocystis jirovecii* isolates. *J Infect Dis* 2009;200:1616–1622.
- Gianella S, Haeberli L, Joos B, Ledergerber B, Wuthrich RP, Weber R, Kuster H, Hauser PM, Fehr T, Mueller NJ. Molecular evidence of interhuman transmission in an outbreak of *Pneumocystis jirovecii* pneumonia among renal transplant recipients. *Transpl Infect Dis* 2010;12:1–10.
- Walzer PD, Perl DP, Krogstad DJ, Rawson PG, Schultz MG. *Pneumocystis carinii* pneumonia in the United States. Epidemiologic, diagnostic, and clinical features. *Ann Intern Med* 1974;80:83–93.
- Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* 1981;305:1425–1431.
- Masur H, Michelis MA, Greene JB, Onorato I, Stouwe RA, Holzman RS, Wormser G, Brettman L, Lange M, Murray HW, et al. An outbreak of community-acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med* 1981;305:1431–1438.
- WHO/UNAIDS. AIDS epidemic update [accessed September 30, 2010]. Available from: http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_enpdf 2009.
- Centers for Disease Control and Prevention. HIV/AIDS surveillance report. 1990.
- Centers for Disease Control and Prevention. HIV/AIDS surveillance report. 1991.
- Centers for Disease Control and Prevention. HIV/AIDS surveillance report. 1992.
- Centers for Disease Control and Prevention. HIV/AIDS surveillance report. 1993.
- Regional Office for Europe. HIV/AIDS surveillance in Europe 2008. Stockholm, Sweden: Stockholm European Centre for Disease Prevention and Control; 2009.
- Mocroft A, Sterne JA, Egger M, May M, Grabar S, Furrer H, Sabin C, Fatkenheuer G, Justice A, Reiss P, et al. Variable impact on mortality

- of AIDS-defining events diagnosed during combination antiretroviral therapy: not all AIDS-defining conditions are created equal. *Clin Infect Dis* 2009;48:1138–1151.
20. Huang L, Hecht FM, Stansell JD, Montanti R, Hadley WK, Hopewell PC. Suspected *Pneumocystis carinii* pneumonia with a negative induced sputum examination: is early bronchoscopy useful? *Am J Respir Crit Care Med* 1995;151:1866–1871.
 21. Fei MW, Sant CA, Kim EJ, Swartzman A, Davis JL, Jarlsberg LG, Huang L. Severity and outcomes of *Pneumocystis* pneumonia in patients newly diagnosed with HIV infection: an observational cohort study. *Scand J Infect Dis* 2009;41:672–678.
 22. Fei MW, Kim EJ, Sant CA, Jarlsberg LG, Davis JL, Swartzman A, Huang L. Predicting mortality from HIV-associated *Pneumocystis* pneumonia at illness presentation: an observational cohort study. *Thorax* 2009;64:1070–1076.
 23. Radhi S, Alexander T, Ukwu M, Saleh S, Morris A. Outcome of HIV-associated *Pneumocystis* pneumonia in hospitalized patients from 2000 through 2003. *BMC Infect Dis* 2008;8:118.
 24. Walzer PD, Evans HE, Copas AJ, Edwards SG, Grant AD, Miller RF. Early predictors of mortality from *Pneumocystis jirovecii* pneumonia in HIV-infected patients: 1985–2006. *Clin Infect Dis* 2008;46:625–633.
 25. Fisk DT, Meshnick S, Kazanjian PH. *Pneumocystis carinii* pneumonia in patients in the developing world who have acquired immunodeficiency syndrome. *Clin Infect Dis* 2003;36:70–78.
 26. Davis JL, Huang L. *Pneumocystis* pneumonia. In: Volberding PA, Sande MA, Lange J, Greene WC, editors. Global HIV/AIDS Medicine, First ed. Philadelphia: Saunders Elsevier, Inc.; 2007. pp. 309–321.
 27. Daley CL, Mugisi F, Chen LL, Schmidt DM, Small PM, Bearer E, Aris E, Mtoni IM, Cegielski JP, Lallinger G, et al. Pulmonary complications of HIV infection in Dar es Salaam, Tanzania: role of bronchoscopy and bronchoalveolar lavage. *Am J Respir Crit Care Med* 1996;154:105–110.
 28. Worodria W, Okot-Nwang M, Yoo SD, Aisu T. Causes of lower respiratory infection in HIV-infected Ugandan adults who are sputum AFB smear-negative. *Int J Tuberc Lung Dis* 2003;7:117–123.
 29. Worodria W, Davis JL, Cattamanchi A, Andama A, den Boon S, Yoo SD, Hopewell PC, Huang L. Bronchoscopy is useful for diagnosing smear-negative tuberculosis in HIV-infected patients. *Eur Respir J* 2010;36:446–448.
 30. Keyune R, Den Boon S, Cattamanchi A, Davis JL, Worodria W, Yoo SD, Huang L. Causes of early mortality in HIV-infected TB suspects in an East African referral hospital. *J Acquir Immune Defic Syndr* 2010;2010:55:446–350.
 31. Kovacs JA, Hiemenz JW, Macher AM, Stover D, Murray HW, Shelhamer J, Lane HC, Urmacher C, Honig C, Longo DL, et al. *Pneumocystis carinii* pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. *Ann Intern Med* 1984;100:663–671.
 32. DeLorenzo LJ, Huang CT, Maguire GP, Stone DJ. Roentgenographic patterns of *Pneumocystis carinii* pneumonia in 104 patients with AIDS. *Chest* 1987;91:323–327.
 33. Huang L, Stansell J, Osmond D, Turner J, Shafer KP, Fulkerson W, Kvale P, Wallace J, Rosen M, Glassroth J, et al. Pulmonary Complications of HIV Infection Study Group. Performance of an algorithm to detect *Pneumocystis carinii* pneumonia in symptomatic HIV-infected persons. *Chest* 1999;115:1025–1032.
 34. Gruden JF, Huang L, Turner J, Webb WR, Merrifield C, Stansell JD, Gamsu G, Hopewell PC. High-resolution CT in the evaluation of clinically suspected *Pneumocystis carinii* pneumonia in AIDS patients with normal, equivocal, or nonspecific radiographic findings. *AJR Am J Roentgenol* 1997;169:967–975.
 35. Larsen HH, Huang L, Kovacs JA, Crothers K, Silcott VA, Morris A, Turner JR, Beard CB, Masur H, Fischer SH. A prospective, blinded study of quantitative touch-down polymerase chain reaction using oral-wash samples for diagnosis of *Pneumocystis* pneumonia in HIV-infected patients. *J Infect Dis* 2004;189:1679–1683.
 36. de Oliveira A, Unnasch TR, Crothers K, Eiser S, Zucchi P, Moir J, Beard CB, Lawrence GG, Huang L. Performance of a molecular viability assay for the diagnosis of *Pneumocystis* pneumonia in HIV-infected patients. *Diagn Microbiol Infect Dis* 2007;57:169–176.
 37. Davis JL, Welsh DA, Beard CB, Jones JL, Lawrence GG, Fox MR, Crothers K, Morris A, Charbonnet D, Swartzman A, et al. *Pneumocystis* colonisation is common among hospitalised HIV infected patients with non-*Pneumocystis* pneumonia. *Thorax* 2008;63:329–334.
 38. Morris A, Wei K, Afshar K, Huang L. Epidemiology and clinical significance of *Pneumocystis* colonization. *J Infect Dis* 2008;197:10–17.
 39. Skelly M, Hoffman J, Fabbri M, Holzman RS, Clarkson AB Jr, Merali S. S-adenosylmethionine concentrations in diagnosis of *Pneumocystis carinii* pneumonia. *Lancet* 2003;361:1267–1268.
 40. Skelly MJ, Holzman RS, Merali S. S-adenosylmethionine levels in the diagnosis of *Pneumocystis carinii* pneumonia in patients with HIV infection. *Clin Infect Dis* 2008;46:467–471.
 41. Kuty G, Hernandez-Novoa B, Czapiga M, Kovacs JA. *Pneumocystis* encodes a functional S-adenosylmethionine synthetase gene. *Eukaryot Cell* 2008;7:258–267.
 42. Wang P, Huang L, Davis JL, Swartzman A, Roth B, Stone J, Wu AH. A hydrophilic-interaction chromatography tandem mass spectrometry method for quantitation of serum s-adenosylmethionine in patients infected with human immunodeficiency virus. *Clin Chim Acta* 2008;396:86–88.
 43. Tasaka S, Hasegawa N, Kobayashi S, Yamada W, Nishimura T, Takeuchi T, Ishizaka A. Serum indicators for the diagnosis of *Pneumocystis* pneumonia. *Chest* 2007;131:1173–1180.
 44. Desmet S, Van Wijngaerden E, Maertens J, Verhaegen J, Verbeken E, De Munter P, Meersseman W, Van Meensel B, Van Eldere J, Lagrou K. Serum (1–3)-beta-D-glucan as a tool for diagnosis of *Pneumocystis jirovecii* pneumonia in patients with human immunodeficiency virus infection or hematological malignancy. *J Clin Microbiol* 2009;47:3871–3874.
 45. Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009;58:1–207.
 46. Fisk M, Sage EK, Edwards SG, Cartledge JD, Miller RF. Outcome from treatment of *Pneumocystis jirovecii* pneumonia with co-trimoxazole. *Int J STD AIDS* 2009;20:652–653.
 47. Benfield T, Atzori C, Miller RF, Helweg-Larsen J. Second-line salvage treatment of AIDS-associated *Pneumocystis jirovecii* pneumonia: a case series and systematic review. *J Acquir Immune Defic Syndr* 2008;48:63–67.
 48. Helweg-Larsen J, Benfield T, Atzori C, Miller RF. Clinical efficacy of first- and second-line treatments for HIV-associated *Pneumocystis jirovecii* pneumonia: a tri-centre cohort study. *J Antimicrob Chemother* 2009;64:1282–1290.
 49. Crothers K, Huang L. Recurrence of *Pneumocystis carinii* pneumonia in an HIV-infected patient: apparent selective immune reconstitution after initiation of antiretroviral therapy. *HIV Med* 2003;4:346–349.
 50. Opportunistic Infections Project Team of the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE), Mocroft A, Reiss P, Kirk O, Mussini C, Girardi E, Morlat P, Stephan C, De Wit S, Doerholt K, Ghosn J, et al. Is it safe to discontinue primary *Pneumocystis jirovecii* pneumonia prophylaxis in patients with virologically suppressed HIV infection and a CD4 cell count <200 cells/microL? *Clin Infect Dis* 2010;51:611–619.
 51. Martin JN, Rose DA, Hadley WK, Perdreau-Remington F, Lam PK, Gerberding JL. Emergence of trimethoprim-sulfamethoxazole resistance in the AIDS era. *J Infect Dis* 1999;180:1809–1818.
 52. Huang L, Crothers K, Atzori C, Benfield T, Miller R, Rabodonirina M, Helweg-Larsen J. Dihydropteroate synthase gene mutations in *Pneumocystis* and sulfa resistance. *Emerg Infect Dis* 2004;10:1721–1728.
 53. Kazanjian P, Armstrong W, Hossler PA, Lee CH, Huang L, Beard CB, Carter J, Crane L, Duchin J, Burman W, et al. *Pneumocystis carinii* cytochrome b mutations are associated with atovaquone exposure in patients with AIDS. *J Infect Dis* 2001;183:819–822.
 54. Khalil I, Ronn AM, Alifrangis M, Gabar HA, Satti GM, Bygbjerg IC. Dihydrofolate reductase and dihydropteroate synthase genotypes associated with in vitro resistance of *Plasmodium falciparum* to pyrimethamine, trimethoprim, sulfadoxine, and sulfamethoxazole. *Am J Trop Med Hyg* 2003;68:586–589.
 55. Ma L, Borio L, Masur H, Kovacs JA. *Pneumocystis carinii* dihydropteroate synthase but not dihydrofolate reductase gene mutations correlate with prior trimethoprim-sulfamethoxazole or dapsone use. *J Infect Dis* 1999;180:1969–1978.
 56. Takahashi T, Endo T, Nakamura T, Sakashita H, Kimurat K, Ohnishi K, Kitamura Y, Iwamoto A. Dihydrofolate reductase gene polymorphisms in *Pneumocystis carinii* f. sp. *hominis* in Japan. *J Med Microbiol* 2002;51:510–515.
 57. Nahimana A, Rabodonirina M, Bille J, Francioli P, Hauser PM. Mutations of *Pneumocystis jirovecii* dihydrofolate reductase associated with failure of prophylaxis. *Antimicrob Agents Chemother* 2004;48:4301–4305.

58. Robberts FJ, Chalkley LJ, Weyer K, Goussard P, Liebowitz LD. Dihydropteroate synthase and novel dihydrofolate reductase gene mutations in strains of *Pneumocystis jirovecii* from South Africa. *J Clin Microbiol* 2005;43:1443–1444.
59. Costa MC, Esteves F, Antunes F, Matos O. Genetic characterization of the dihydrofolate reductase gene of *Pneumocystis jirovecii* isolates from Portugal. *J Antimicrob Chemother* 2006;58:1246–1249.
60. Siripattanapipong S, Leelayoova S, Mungthin M, Worapong J, Tan-Ariya P. Study of DHPS and DHFR genes of *Pneumocystis jirovecii* in Thai HIV-infected patients. *Med Mycol* 2008;46:389–392.
61. Alvarez-Martinez MJ, Moreno A, Miro JM, Valls ME, Rivas PV, de Lazzari E, Sued O, Benito N, Domingo P, Ribera E, et al. *Pneumocystis jirovecii* pneumonia in Spanish HIV-infected patients in the combined antiretroviral therapy era: prevalence of dihydropteroate synthase mutations and prognostic factors of mortality. *Diagn Microbiol Infect Dis* 2008;62:34–43.
62. Crothers K, Beard CB, Turner J, Groner G, Fox M, Morris A, Eiser S, Huang L. Severity and outcome of HIV-associated *Pneumocystis* pneumonia containing *Pneumocystis jirovecii* dihydropteroate synthase gene mutations. *AIDS* 2005;19:801–805.
63. Kazanjian PH, Fisk D, Armstrong W, Shulin Q, Liwei H, Ke Z, Meshnick S. Increase in prevalence of *Pneumocystis carinii* mutations in patients with AIDS and *P. carinii* pneumonia, in the United States and China. *J Infect Dis* 2004;189:1684–1687.
64. Lane BR, Ast JC, Hossler PA, Mindell DP, Bartlett MS, Smith JW, Meshnick SR. Dihydropteroate synthase polymorphisms in *Pneumocystis carinii*. *J Infect Dis* 1997;175:482–485.
65. Beard CB, Carter JL, Keely SP, Huang L, Pieniazek NJ, Moura IN, Roberts JM, Hightower AW, Bens MS, Freeman AR, et al. Genetic variation in *Pneumocystis carinii* isolates from different geographic regions: implications for transmission. *Emerg Infect Dis* 2000;6:265–272.
66. Demanche C, Guillot J, Berthelemy M, Petitt T, Roux P, Wakefield AE. Absence of mutations associated with sulfa resistance in *Pneumocystis carinii* dihydropteroate synthase gene from non-human primates. *Med Mycol* 2002;40:315–318.
67. Helweg-Larsen J, Benfield TL, Eugen-Olsen J, Lundgren JD, Lundgren B. Effects of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of AIDS-associated *P. carinii* pneumonia. *Lancet* 1999;354:1347–1351.
68. Kazanjian P, Armstrong W, Hossler PA, Burman W, Richardson J, Lee CH, Crane L, Katz J, Meshnick SR. *Pneumocystis carinii* mutations are associated with duration of sulfa or sulfone prophylaxis exposure in AIDS patients. *J Infect Dis* 2000;182:551–557.
69. Takahashi T, Hosoya N, Endo T, Nakamura T, Sakashita H, Kimura K, Ohnishi K, Nakamura Y, Iwamoto A. Relationship between mutations in dihydropteroate synthase of *Pneumocystis carinii* f. sp. *hominis* isolates in Japan and resistance to sulfonamide therapy. *J Clin Microbiol* 2000;38:3161–3164.
70. Navin TR, Beard CB, Huang L, del Rio C, Lee S, Pieniazek NJ, Carter JL, Le T, Hightower A, Rimland D. Effect of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of *P. carinii* pneumonia in patients with HIV-1: a prospective study. *Lancet* 2001;358:545–549.