Summary:

Pneumocystis pneumonia (PcP) remains a significant cause of morbidity and mortality in immunocompromised persons, especially those with human immunodeficiency virus (HIV) infection. Pneumocystis colonization is described increasingly in a wide range of immunocompromised and immunocompetent populations and associations between Pneumocystis colonization and significant pulmonary diseases such as chronic obstructive pulmonary disease (COPD) have emerged. This mini-review summarizes recent advances in our clinical understanding of Pneumocystis and PcP, describes ongoing areas of clinical and translational research, and offers recommendations for future clinical research from researchers participating in the “First centenary of the Pneumocystis discovery”.

KEY WORDS: Pneumocystis, colonization, Pneumocystis pneumonia (PcP), human immunodeficiency virus (HIV), acquired immune deficiency syndrome (AIDS).

INTRODUCTION

The year 2009 marked the 100th anniversary of the first description of Pneumocystis by Carlos Chagas. Over the past 100 years, significant advances have been made in our understanding of both Pneumocystis and Pneumocystis pneumonia (PcP). The preeminence of PcP as a herald of the human immunodeficiency virus (HIV) / acquired immune deficiency syndrome (AIDS) epidemic and as a major cause of HIV-associated morbidity and mortality focused attention and resources on this previously uncommon opportunistic pneumonia. With the use of combination antiretroviral therapy to treat underlying HIV infection, the incidence of PcP has declined, but an appreciation of Pneumocystis colonization in both immunocompromised and immunocompetent populations, and associations between Pneumocystis colonization and significant pulmonary diseases such as chronic obstructive pulmonary disease (COPD), have emerged. This mini-review summarizes recent advances in our clinical understanding of Pneumocystis and PcP, describes ongoing areas of clinical and translational research, and offers recommendations for future clinical research from researchers participating in the “First centenary of the Pneumocystis discovery” held in Brussels, Belgium on November 5-6, 2009.

EPIDEMIOLOGY OF PcP

PcP is a frequent AIDS-defining diagnosis. At its peak, PcP was an AIDS-defining diagnosis for greater than 20,000 new AIDS cases per year in the US (Centers for Diseases Control and Prevention, 1990-1993). Although the incidence of PcP has decreased in the current era of combination antiretroviral therapy, PcP remains a leading cause of AIDS in North American and European cohorts (Mocroft et
In the multinational Antiretroviral Therapy Cohort Collaboration (ART-CC) established in 2000, PCP was the second most frequent AIDS-defining event after esophageal candidiasis (Mocroft et al., 2009). Therefore, continued efforts to improve our understanding of both Pneumocystis and PCP are warranted (Table I).

PCP is an important cause of HIV-associated pneumonia but rates of PCP have decreased. At San Francisco General Hospital, the Division of Pulmonary and Critical Care Medicine has tracked confirmed cases of PCP—diagnosed by microscopic visualization of characteristic Pneumocystis cysts and/or trophic forms obtained from sputum induction or bronchoscopy—since 1990 (Fig. 1). At its peak in 1992, nearly 300 cases of HIV-associated PCP were diagnosed at this institution (Huang et al., 1995). Today, this number has decreased to 20-30 cases per year. Most of these cases occurred in persons who were not receiving antiretroviral therapy or PCP prophylaxis and many were actually unaware of their HIV infection at the time of presentation (Fei et al., 2009). This experience is similar at other institutions, where 23-31% of reported PCP cases occurred in persons who were newly diagnosed with HIV infection at the time of presentation (Fei et al., 2009). Thus, efforts to test persons at risk of HIV, to engage HIV-infected persons in regular medical care, and to initiate and adhere to combination antiretroviral therapy and PCP prophylaxis are important strategies to decrease the incidence of the disease.

Comprehensive reviews document that HIV-associated PCP is reported throughout the world, in varying rates (Davis et al., 2007; Fisk et al., 2003). However, data on the current rates of PCP in regions of the world that bear the greatest burden of HIV are limited. The scarcity of diagnostic and microbiologic tools to diagnose the disease is an important reason for the limited data on PCP rates. Thus, those institutions that possess these tools offer valuable windows into the epidemiology of PCP in low- and middle-income settings. At Mulago Hospital in Kampala, Uganda, the frequency of PCP among HIV-infected persons hospitalized with suspected pneumonia who have negative sputum acid-fast bacilli (AFB) smears and undergo bronchoscopy has decreased from nearly 40% of bronchoscopies to less than 10% (Worodria et al., 2003; Worodria et al., 2010). Yet, the mortality associated with PCP remains high. Thus, efforts to improve both diagnostic and microbiologic capacity in low- and middle-income settings and to establish surveillance networks to track PCP cases are important clinical care and epidemiologic resources that should be developed.

Although the populations of non-HIV immunosuppressed are rising with the increased use of immunosuppressive or immunomodulating therapies to treat a wide spectrum of medical illnesses, data on the frequency of PCP among these populations are limited. As such, consensus on optimal diagnostic, therapeutic, and preventative strategies lag behind those for HIV-infected populations. Similar to recommendations for tracking PCP in low- and middle-income settings, collaborative networks of institutions that care for substantial numbers of these non-HIV individuals (similar to those established for HIV-infected persons) should be created.
Epidemiology

1. What is the current incidence of PcP in HIV-infected populations?
   a. What is the incidence in high-income countries, including the US and Western Europe, where access to combination anti-retroviral therapy is generally widely available?
      i. In these countries, which populations continue to develop PcP?
      ii. Strategies to identify persons at risk for HIV and PcP need to be refined and preventative measures need to be implemented.
   b. What is the incidence in low- and middle-income countries, where access to combination antiretroviral therapy is generally more limited?
      i. In these countries, which populations develop PcP?
      ii. Strategies to identify persons at risk for HIV and PcP need to be refined and preventative measures need to be implemented.
      iii. Efforts to improve diagnostic and microbiologic capacity are needed.
      iv. Surveillance networks to track PcP cases should be developed.

2. What is the current incidence of PcP in non-HIV, immunocompromised populations?
   a. What is the incidence in populations immunocompromised from “traditional” immunosuppressive agents (e.g., glucocorticoid medications) and disease therapies (e.g., therapies for hematologic malignancy and cancer and after hematopoietic stem cell transplantation)?
   b. What is the incidence in populations immunocompromised from “newer” biologic, immunomodulating agents (e.g., tumor necrosis factor-alpha inhibitors)?
   c. Surveillance networks to track PcP cases should be developed.

Diagnosis

3. What is the optimum approach to the diagnosis of PcP?
   a. Bronchoscopy with bronchoalveolar lavage (BAL)?
   b. Sputum induction (SI), followed by BAL, if SI is negative?

4. Is there a role for non-invasive tests in PcP diagnosis?
   a. Oropharyngeal washing (OPW): Which specimen? Which assay? Which protocol?
   b. Plasma s-adenosylmethionine?
   c. (1-3)-beta-D-glucan?
   d. Is the diagnostic accuracy different in non-HIV populations (lower Pneumocystis burden)?

Treatment

5. New drugs to treat and prevent PcP are needed.
6. What is the optimum second-line treatment for PcP?
   a. Prospective, randomized clinical trials are needed.
7. Does TMP-SMX drug resistance exist?
   a. What is (are) the mechanisms?
      i. Dihydropteroate synthase (DHPS) gene mutations?
      ii. Dihydrofolate reductase (DHFR) gene mutations?
      iii. Sub-therapeutic trimethoprim or sulfamethoxazole drug levels?
      iv. Host factors?

Outcome and Intensive Care

8. What is the optimal timing for initiation of ART in HIV-associated PcP?
   a. In Intensive care unit (ICU)?
   b. Outside of the ICU?
9. What is the incidence of PcP-IRIS?
   a. What are the risk factors?
   b. Can PcP-IRIS be prevented (prophylaxis)?

Pneumocystis colonization

10. What are the implications of Pneumocystis colonization for the colonized individual?
    a. COPD?
    b. Other pulmonary diseases?
11. Can persons colonized with Pneumocystis transmit infection to others?
    a. Risk factors for transmission – source, environment, contact.
PcP DIAGNOSIS

Classically, PcP presents with fevers, non-productive cough, and progressive shortness of breath. Chest radiograph demonstrates bilateral, symmetric reticular (interstitial) or granular opacities. Traditionally, bronchoscopy with bronchoalveolar lavage (BAL) is regarded as the gold standard procedure to diagnose PcP in HIV-infected persons with diagnostic sensitivity ≥ 98% reported (Huang et al., 1995). An early study reported a lower number of *Pneumocystis* organisms and a lower sensitivity of BAL for PcP in non-HIV immunocompromised persons compared to HIV-infected persons (Limper et al., 1989). More recently, BAL combined with sensitive laboratory techniques (e.g., immunofluorescence testing, polymerase chain reaction, PCR) has been reported as sensitive to diagnose PcP in these non-HIV immunocompromised populations (Azoulay et al., 2009). Since *Pneumocystis* DNA can be detected by PCR assay in the absence of clinical or microbiological pneumonia (Davis et al., 2007), the increased sensitivity of PCR-based assays may be offset by a decreased specificity for PcP.

*Pneumocystis* cannot be cultured. Historically, the diagnosis of PcP has involved an invasive pulmonary procedure (i.e., bronchoscopy) to obtain specimens combined with a basic laboratory test (i.e., microscopic examination of stained respiratory specimens) to visualize the cysts and/or trophic forms. 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.

Laboratory advances (e.g., PCR), however, have revolutionized the diagnosis of many infectious diseases and PCR assays for *P. jirovecii* have been developed. These factors led researchers to examine whether the use of an advanced laboratory test (e.g., PCR) could be combined with a non-invasive pulmonary procedure to effectively diagnose PcP.

Oral or oropharyngeal (i.e., gargle) wash specimens combined with PCR assays have been examined as non-invasive tests to diagnose PcP. Two studies from San Francisco General Hospital that tested three different PCR-based assays found a diagnostic sensitivity of OPW up to 88% and a specificity up to 90% (de Oliveira et al., 2007; Larsen et al., 2004). Procedural factors such as collecting the OPW specimen within one day of PcP treatment initiation and having the patient cough vigorously prior to specimen collection may increase the sensitivity of the procedure. Studies are being conducted to validate these findings in both high- and low-income settings.

Blood-based assays have also been studied for diagnosis of PcP. A series of studies from New York indicate that plasma S-adenosylmethionine (SAM) levels could be used to distinguish between persons with PcP and those with non-PcP pneumonia and healthy controls (Skelly et al., 2003; Skelly et al., 2008). More recently, serum (1-3)-beta-D-glucan has shown promise as a test for PcP. Studies from 2009 reported that serum (1-3)-beta-D-glucan had a sensitivity of 100% and a specificity of 96.4% (using a cutoff of 100 pg/mL), that the assay may be more useful for PcP diagnosis than for monitoring response to treatment, that levels differ between patients with PcP and those who are PcP-negative but colonized with *Pneumocystis jirovecii*, and that the detection rate is lower in non-HIV PcP patients than in HIV-associated PcP patients (Desmet et al., 2009; Nakamura et al., 2009; Shimizu et al., 2009; Watanabe et al., 2009). Studies to determine which non-invasive test has the best performance characteristics in different immunocompromised populations at risk for PcP should be done.

PcP TREATMENT AND PREVENTION AND PUTATIVE TRIMETHOPRIM-SULFAMETHOXAZOLE DRUG RESISTANCE

Trimethoprim-sulfamethoxazole is the recommended first-line treatment for PcP in HIV-infected patients with mild, moderate, and severe PcP and also in non-HIV patients. 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 an arterial oxygen tension (PaO2) less than 70 mm Hg or an alveolar-arterial oxygen gradient (A-a O2) greater than 35 mm Hg. In HIV-infected patients, the recommended duration of treatment is 21 days while it is usually 14 days in non-HIV patients. 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 (Fisk et al., 2009). 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 (Benfield et al., 2008; Helweg-Larsen et al., 2009). The development of new PcP treatment options and prospective, randomized controlled trials
comparing second-line PcP treatments are both important needs.

Trimethoprim-sulfamethoxazole is also the recommended first-line prevention for primary and secondary prophylaxis against PcP. However, the widespread use of this medication for PcP prophylaxis has been associated with increases in trimethoprim-sulfamethoxazole-resistant bacteria and has raised concerns over potential trimethoprim-sulfamethoxazole drug resistance in *P. jirovecii*. Trimethoprim-sulfamethoxazole drug resistance might also result in resistance to trimethoprim plus dapsone (a sulfone), thereby further limiting the therapeutic options available to treat PcP. The inability to culture *P. jirovecii* has hindered efforts to document drug-resistance in *Pneumocystis* but researchers have explored this question by examining genetic mutations within the dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) genes, the enzymatic targets of trimethoprim and sulfamethoxazole (sulfamethoxazole and dapsone) medications, respectively (Huang et al., 2004). In other micro-organisms, DHFR and DHPS mutations have been shown to cause drug resistance. Although DHFR mutations are infrequently found, non-synonymous DHPS mutations are found in up to 81% of HIV-infected patients with PcP and the use of sulfamethoxazole for PcP prophylaxis is strongly associated with the presence of these mutations (Crothers et al., 2005; Huang et al., 2000). DHPS mutations continue to be reported worldwide and there are geographic differences in the observed frequency of mutations (Alvarez-Martinez et al., 2008; Beard et al., 2000; Crothers et al., 2005; Esteves et al., 2008; Huang et al., 2000; Magne et al., 1989; Rabodonirina et al., 2006; Wissmann et al., 2006). Furthermore, the presence of DHPS mutations has been associated with increased mortality in one study and increased risk of trimethoprim-sulfamethoxazole PcP treatment failure in a second study, although other studies have failed to find these associations and instead have reported that risk factors such as low serum albumin and early ICU admission were stronger predictors of PcP mortality (Crothers et al., 2005; Helweg-Larsen et al., 1999; Kazanjian et al., 2000).

At present, there exists a seeming paradox regarding the clinical significance of DHPS mutations. Studies consistently report that the majority of patients with PcP and DHPS mutations who are treated with trimethoprim-sulfamethoxazole respond to this treatment (Crothers et al., 2005; Helweg-Larsen et al., 1999; Kazanjian et al., 2000; Navin et al., 2001). However, patients with DHPS mutations who are treated with trimethoprim-sulfamethoxazole tend to have worse outcomes compared to those with wild-type DHPS who are treated with trimethoprim-sulfamethoxazole and those with DHPS mutations who are treated with a non-sulfa-based regimen (Crothers et al., 2005). The precise explanation for these observations remains a focus of current investigation.

**PcP MORTALITY AND INTENSIVE CARE**

Despite differences in geography and demographic characteristics, in-hospital mortality among HIV-infected patients with PcP is similar (ranging from 10.3 to 13.5%) in different cohort studies from Los Angeles, London, and San Francisco (Fei et al., 2009b; Radhi et al., 2008; Walzer et al., 2008). Each of these studies also reported on predictors of mortality. In the Los Angeles study of 262 HIV-infected patients diagnosed with PcP from January 2000 through December 2003, need for mechanical ventilation, development of a pneumothorax, and low serum albumin were independent predictors of increased mortality (Radhi et al., 2008). In the London study of 494 consecutive HIV-infected patients with 547 episodes of laboratory-confirmed PcP from June 1985 through June 2006, increasing patient age, subsequent episode of PcP, low hemoglobin level, low partial pressure of oxygen breathing room air, presence of medical comorbidity, and pulmonary Kaposi sarcoma were independent predictors associated with increased mortality (Walzer et al., 2008). Mortality was comparable during the periods from June 1985 through December 1989, January 1990 through June 1996, and July 1996 through June 2006 (p = 0.14). Finally, in the San Francisco study of 451 consecutive HIV-infected patients diagnosed with 524 episodes of microscopically-confirmed PcP from January 1997 through December 2006, increasing patient age, recent injection drug use, increased total bilirubin, decreased serum albumin, and increased alveolar-arterial oxygen gradient were independent predictors of increased mortality (Fei et al., 2009b). Using these five predictors, a six-point PcP mortality prediction rule was derived that stratified patients according to increasing risk of mortality: score 0-1, 4% mortality; score 2-3, 12% mortality; and score 4-5, 48% mortality. Studies are being conducted to validate these single-institution findings in other cohorts.

A few institutions have tracked PcP in HIV-infected patients requiring critical care in the Intensive Care Unit (ICU). Among these institutions, a study from San Francisco General Hospital in the current combination antiretroviral therapy era reported that critically ill HIV-infected patients with PcP who received combination antiretroviral therapy had a significantly lower mortality compared to patients who did not receive antiretroviral therapy (Morris et al., 2003). The use of combination antiretroviral therapy was an independent predictor
that was associated with lower mortality (odds ratio, OR = 0.14; 95 % confidence interval, CI = 0.02-0.84; p = 0.03). A study from London, however, failed to find a mortality difference associated with combination antiretroviral therapy (Miller et al., 2006). In this study, mortality improved from 71 % before mid-1996 to 34 % after mid-1996 (p = 0.008). This improvement in mortality was ascribed to general improvements in care as no patients were started on combination antiretroviral therapy. In the absence of data from randomized clinical trials, the pros and cons of initiating antiretroviral therapy in critically ill HIV-infected ICU patients have been debated. Recently, the National Institutes of Health (NIH)-funded AIDS Clinical Trials Group (ACTG) published its results of a prospective, multicenter randomized clinical trial of HIV-infected non-ICU inpatients hospitalized with an acute opportunistic infection (Zolopa et al., 2009). Overall, 282 subjects were enrolled and 63 % had PcP. The study found no difference in their primary endpoint. However, subjects randomized to the early antiretroviral therapy arm had fewer AIDS progressions/deaths (OR = 0.51, 95 % CI = 0.27-0.94, p = 0.035) and a longer time to AIDS progression/death (stratified Hazard Ratio, HR = 0.53, 95 % CI = 0.30-0.92). Importantly, there was no increase in adverse events in subjects randomized to early antiretroviral therapy in this study but cases of severe immune reconstitution inflammatory syndrome (IRIS, also called immune reconstitution syndrome, IRS, and immune reconstitution disease, IRD) resulting in respiratory failure and requiring invasive mechanical ventilation have been reported and serve as a cautionary note (Jagannathan et al., 2009). Whether the ACTG results from hospitalized but non-critically ill patients can be extrapolated into the ICU, where patients are often receiving mechanical ventilation and have renal and/or hepatic insufficiency, is an important but largely unanswered question (Huang et al., 2008).

PNEUMOCYSTIS COLONIZATION
AND POTENTIAL PERSON-TO-PERSON TRANSMISSION

The presence of Pneumocystis organisms or P. jiroveci DNA detected in the absence of PcP has been termed Pneumocystis colonization (also called carriage and sub-clinical infection). Pneumocystis colonization has been increasingly reported and documented to occur in infants and children, pregnant women, immunocompetent adults with underlying pulmonary disease, non-HIV immunocompromised individuals, and HIV-infected persons (Morris et al., 2004). For example, in one study of 58 infants < 1 year of age who died, Pneumocystis colonization was detected in 100 % (Beard et al., 2005). HIV-infected inpatients hospitalized with non-PcP pneumonia also appear to have a high prevalence of Pneumocystis colonization, with one study reporting a prevalence of 68 % (Davis et al., 2008).

Colonization with Pneumocystis has also been associated with airways obstruction and chronic obstructive pulmonary disease (COPD) and possibly with other pulmonary diseases as well (Vidal et al., 2006). In one study, Pneumocystis colonization was detected in 36.7 % of HIV-negative patients with very severe COPD (Global Health Initiative on Obstructive Lung Disease [GOLD] Stage IV) compared with 5.3 % of smokers with normal lung function or less severe COPD (GOLD Stages 0, I, II, and III) (p = 0.004) and with 9.1 % of control subjects (p = 0.007) (Morris et al., 2004). Pneumocystis colonized subjects exhibited more severe airway obstruction (median FEV1 = 21 % predicted vs 62 % predicted in non-colonized subjects, p = 0.006). In a second study, patients colonized with Pneumocystis had higher proinflammatory cytokine levels than did those patients without evidence of Pneumocystis colonization (Calderon et al., 2007). Finally, HIV-infected outpatients who were colonized with Pneumocystis had worse airway obstruction and higher sputum matrix metalloproteinase-12 levels, suggesting that Pneumocystis colonization may be important in HIV-associated COPD (Morris et al., 2009). Studies to further evaluate the role of Pneumocystis colonization in the development and the progression of HIV-associated COPD are ongoing.

Although the clinical significance of Pneumocystis colonization remains to be elucidated completely, important insights can be gained from studies in laboratory animals. An animal model has been developed to study Pneumocystis colonization in immunocompetent and simian immunodeficiency virus (SIV)-infected cynomolgus macaques and the potential role of Pneumocystis colonization on COPD (Kling et al., 2009). Animal studies have also been used to study disease transmission. These studies demonstrate that animals are a reservoir for the specific Pneumocystis species that affects them, that animals carrying Pneumocystis develop PcP after being immunosuppressed (reactivation), and that immunocompromised Pneumocystis-free animals develop PcP after exposure to not only immunocompromised animals infected with Pneumocystis but also immunocompetent animals that are colonized with Pneumocystis. Animal studies also demonstrate that animal-to-animal transmission occurs and occurs via an airborne route.

Person-to-person transmission of Pneumocystis via an airborne (aerosol) route has been suggested. However,
a precise understanding of the factors involved in disease transmission remains unclear and there are no universal recommendations for the respiratory isolation of persons with active PcP. Even if such recommendations become standard practice, disease transmission could hypothetically occur via persons who are colonized with Pneumocystis as is the case in animal studies. Molecular epidemiology and serology studies to examine potential person-to-person transmission are ongoing.

CONCLUSIONS

Over the past 100 years, significant advances have been made in our understanding of both Pneumocystis and Pneumocystis pneumonia (PcP). This mini-review described recent advances in our understanding of Pneumocystis and PcP, ongoing areas of clinical and translational research, and offered recommendations for future clinical research from researchers participating in the First centenary commemorative Conference on the discovery of Pneumocystis. The attendees of the conference sincerely hope that the next 100 years bring continued advances in our understanding of the organism and the disease.

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