Chronic aspergillus infections of the respiratory tract: diagnosis, management and antifungal resistance

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ABSTRACT

Purpose of review:

Chronic pulmonary aspergillosis (CPA) is a relatively rare, slowly progressive pulmonary syndrome due to *Aspergillus* spp. which requires specific knowledge in terms of disease entity, diagnosis, management and azole-resistance. This review focuses on the recent understanding of CPA entity and the emergence of azole-resistance in CPA.

Recent findings:

Due to complexities related to patients' background and limited pathological evidence, the disease entity of CPA was incomprehensive and numerous names were previously used. The disease entities and nomenclature of subtypes of CPA have recently been proposed, though previous literature had grouped several different forms of CPA together. Recent advances in the methodology of susceptibility testing have indicated increasing azole-resistance in *Aspergillus* spp. CPA is potentially involved in producing azole-resistance and associated with poor response to azoles.

Summary:

Since there are few publications regarding CPA, there are still many unanswered questions. However, updating of disease entity will promote the clinical and basic research in this field. Moreover, the emergence of antifungal drug resistance of *Aspergillus* is becoming major concern. Thus, more evidence and research regarding drug-resistance are required to improve the outcome of CPA.

INTRODUCTION

Chronic pulmonary aspergillosis (CPA) is defined as slowly progressive pulmonary syndrome. Compared to invasive pulmonary aspergillosis (IPA), the pathophysiology of CPA ranges widely from aspergilloma to chronic necrotizing pulmonary aspergillosis (CNPA), also known as, sub-acute IPA, semi-invasive aspergilloma, symptomatic pulmonary aspergilloma or *Aspergillus* pseudotuberculosis in previous literature [1-4]. Allergic bronchopulmonary aspergillosis is a hypersensitivity disease of the lungs associated with inflammatory destruction of respiratory tract to *Aspergillus* spp. [5] and distinct from CPA though its chronicity. Due to the complex of CPA patients' clinical background, minimal pathological evidence and a low number of cases, these forms of CPA sometimes mixed up and the disease entity is currently confused. For example, Du made comment that CNPA is suitable diagnosis instead of IPA in certain published case report article [6, 7]. Thus, the disease entity of *Aspergillus* infection especially chronic forms of aspergillosis is sometimes vague, particularly in the absence of typical clinical features and course. As described before, subcategorizing CPA is difficult, such that CPA can represent "complex pulmonary aspergillosis" rather than "chronic pulmonary aspergillosis".

This lack of standardization with CPA leads to difficulties in diagnosis, establishing treatment regimens and conducting other clinical studies. Additionally, antifungal drug resistant *Aspergillus* spp. is becoming a major clinical concern that will certainly influence the morbidity and mortality of CPA patients who usually require long term antifungal treatment.

This review focuses on the current understanding of CPA, including its diagnosis, management and drug resistance.

DISEASE ENTITY

The chronic forms of pulmonary aspergillosis were originally established in early 1980s by Binder as chronic necrotizing pulmonary aspergillosis (CNPA) [1] and as semi-invasive aspergillosis by Gefter [2]. It is characterized as slowly progressive inflammatory pulmonary syndrome due to *Aspergillus* spp. Patients usually possess underlying pulmonary diseases (e.g., tuberculosis sequelae, bronchiectasis, chronic obstructive pulmonary disease, cystic lesions and pulmonary fibrosis) with destruction of lung tissues, usually with low-grade immunosuppression (e.g., low-dose steroid administration, diabetes, collagen diseases, renal disorders or alcohol).

In the last decade, new nomenclature and definition of chronic forms of aspergillosis have been proposed [4, 8, 9] and recent guidelines from Infectious Diseases Society of America (IDSA) have indicated three major subtypes of chronic forms of pulmonary aspergillosis, namely CNPA (categorized in invasive form of aspergillosis), chronic cavitary pulmonary aspergillosis (CCPA) and aspergilloma [10]. Aspergilloma was traditionally classified as simple or complex in the surgical literature and complex aspergilloma is recently treated as CCPA in current IDSA guidelines [10, 11]. CCPA is defined as the occurrence of multiple cavities with or without fungus ball, in association with detectable serum *Aspergillus* antibodies, pulmonary and systemic symptoms, and elevated inflammatory markers. Aspergilloma is defined as a conglomeration of *Aspergillus* hyphae, fibrin, and cellular debris within a preexisting pulmonary cavity or an ectatic bronchus, with positive serum *Aspergillus* antibodies. As described before, the distinction between aspergilloma and other forms of CPA may be semantic but reflect the singularity of the aspergilloma against to the multiple cavities which is typical for CPA [9]. An apparent pathological difference between aspergilloma (simple) and other forms of CPA is that there is no absolute tissue invasion of hyphal elements of *Aspergillus* in simple aspergilloma, while hyphal invasion to lung parenchyma but not to blood vessels, may occasionally be seen in CNPA and CCPA [3, 9]. As Hope et al. indicated that apparent distinct entities do not exist for this syndrome and these forms usually overlap [9] and previous articles regarding CNPA included these chronic forms of aspergillosis. Recent IDSA guidelines and textbook have indicated the differences between CNPA and CCPA are the prolonged time frame (CNPA: 1-3 months vs. CCPA: more than 3 months) [12], although this difference is arbitrary, and the genetic predisposition of defects in innate immunity such as mannose-binding lectin and surfactant protein A in CCPA patients [13-15]. Toll-like receptors (TLRs) play critical roles in innate immunity and Carvalho et al. recently reported that polymorphism (Asp299Gly) of the TLR-4 gene is significantly associated to CCPA (odds ratio, 3.46; P=0.003) [15, 16]. Establishing the precise disease entity of CPA, while challenging, is important in conducting clinical trials, developing tools for diagnosis and treatment.

Complexities regarding the background of patients with CPA, co-infection with other microorganisms and status of immunosuppression may interfere with establishment of the simple entity of CPA. Due to these reasons, previous and even current articles regarding CPA may include all forms of CNPA, CCPA and even aspergilloma. The authors describe CCPA and CNPA as CPA in this review, as this approach has been used by others [11, 12].

EPIDEMIOLOGY

There are at least a hundred species in *Aspergillus* spp., and most common pathogenic species to humans are *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. *A. fumigatus* is the most common cause of the vast majority of CPA cases. Though recent trend in etiological agents of CPA is not known, *A. fumigatus* occurred in 82.6-91.3% of CPA cases in previous studies [4, 17-19]. *A. flavus* is the second most common cause of all forms of aspergillosis [20]. Although the clinical presentation of CPA caused by *A. flavus* does not differ from CPA by other *Aspergillus* spp., it rarely causes CPA including aspergilloma for unknown reasons [21, 22]. *A. lentulus* and *A. udagawae* are classified together with *Aspergillus* section *Fumigati* and relatively low-susceptible to antifungals including amphotericin B [23-25]. More importantly, these species used to be misidentified as *A. fumigatus* by morphological observation, however, recent molecular techniques enables identification of these species [26]. Although no data is currently available for the frequency of infections with these species in CPA patients, previous epidemiological data might include and label these uncommon species as *A. fumigatus*.

CLINICAL FEATURES AND DIAGOSIS

CPA and aspergilloma usually occur in middle-aged to elderly patients with chronic pulmonary underlying diseases. Pre-existing or residual cavities after mycobacterial infection are common dwelling site of *Aspergillus* and these cavitary lesions located mostly in upper lobes [27-29]. Other pulmonary diseases that resulted in forming cavitation such as emphysematous bullae, chronic obstructive pulmonary diseases, bronchiectasis, pulmonary cysts sarcoidosis, histoplasmosis and rheumatoid nodules can be a cause of CPA and aspergilloma [9, 29]. CPA slowly progress in months and even years, causing lung destruction such as progressive cavitation, fibrosis and pleural thickening [2, 30, 31]. CPA patients often present with chronic pulmonary or systemic symptoms (usually for longer than three months), such as weight loss, productive cough, chronic sputum, hemosputum or hemoptysis [4, 32]. Single or multiple fungus balls might be seen in these cavitary lesions. While patients with aspergilloma usually demonstrate no disease-specific symptoms, however, hemoptysis occurs in 50-90% of patients, occasionally becoming life threatening massive hemoptysis in 30% [9, 28]. Though no large-scale epidemiological data, Nam et al. reported a five-year survival rate in CPA patients of around 50% [32].

Serum *Aspergillus* precipitins test detecting antibodies (IgG) to *Aspergillus* are usually positive [8, 33] in CPA patients, including those with aspergilloma. Recent study indicated that the test was positive in more than 95% of cases [32], while another study indicated 89.3% positivity compared to only 50% with *Aspergillus* galactomannan antigen ELISA tests [34]. Although *Aspergillus* galactomannan antigen ELISA tests have been approved for the diagnosis of IPA, with a sensitivity of 79% and a specificity of 86% in meta-analysis [35], little data is available for its utility in CPA patients and, hence, it is currently not considered useful in CPA diagnosis. The utility of testing bronchoalveolar lavage specimens by *Aspergillus* galactomannan antigen ELISA tests varies in the reports and is still controversial; there being no available data for CPA cases. Other serological tests, such as β-D-glucan tests, have not been evaluated in CPA patients. There is an urgent need to develop newer serological tool for diagnosis and for reflecting clinical progression of CPA cases.

Sputum cultures, bronchoalveolar lavage cultures, and surgical biopsy specimens usually reveal the causative organism and may support diagnosis. However, as Aspergilli are ubiquitous in the environment, careful evaluation is required to confirm colonization or infection in the culture positive results. Pathological examination by transbronchial biopsies, surgical biopsy and computed-tomography guided biopsies are confirmatory methods for CPA diagnosis, however, they may not be applied to all cases. Thoracoscopic or open-lung biopsies are rarely performed mainly due to the risk of complication from underlying pulmonary diseases [27, 33].

Although various histopathological alternations in CPA have been reported, apparent histopathological definition of CPA has not been established to date [3, 36, 37]. Generally, CPA is characterized by chronic inflammation of the cavity wall, the presence of hyphae consistence with *Aspergillus* spp. and necrotic lung tissue with or without fungus ball [36]. Hyphae invading to adjacent lung parenchyma, but without angioinvasion (common findings with IPA) by *Aspergillus*, can be seen in CPA except simple aspergilloma [4, 9]. The presence of organizing lesions without fungal components around the cavity is also characteristic of CPA but not IPA [37].

Diagnosis of CPA is made synthetically based on these findings of clinical, radiological, mycological and serological factors. Denning et al. proposed enrollment criteria for prospective clinical studies of CPA and much of recent literature basically follow this criteria [4].

MANAGEMENT

Due to the recent development of new antifungal drugs, such as voriconazole (VRCZ) and echinocandins, the management of CPA has changed. Before 2000, only itraconazole (ITCZ) capsules and intravenous amphotericin B formulations were available for treatment of CPA [27].

The latest IDSA guidelines for the treatment of aspergillosis recommend oral VRCZ or ITCZ for CNPA and CCPA as primary treatment [10]. Surgical resection is recommended for simple aspergilloma cases [10, 28, 38]. Attempts to resect CPA lesions, however, are difficult due to pre-existing underlying diseases, such as the sequelae of tuberculosis infections and other factors (e.g. poor general status due to complications), which contribute to the morbidity and mortality. Recent studies indicated lower mortality and better survival rates in selected CPA (complex aspergilloma) cases compared to previous literature [39, 40]. However, evidence is insufficient and, hence, surgery should be reserved for those who develop severe hemoptysis. Bronchial artery embolization (BAE) to occlude the causative vessels in CPA patients with hemosputum or hemoptysis is another option for treatment of CPA in case surgical treatment is contraindicated. However, BAE is only temporarily effective due to the presence of collateral vascular channels at the site of bleeding.

Although no large scale randomized studies for the management of CPA have been conducted, medical therapy is the standard therapy for CPA. Additionally, the following clinical factors have not been standardized or even described in IDSA guidelines: timing of the initiation of treatment, duration of treatment and timing of discontinuation of treatment for CPA. Only few case series reports are currently available. From the early 1990s to date, the reported efficacy of oral ITCZ is somewhat variable, with a range of 30-82.1%, a duration of administration of approximately 4-12 months and adverse effects being seen in 16-33% [4, 32, 41-43]. In the last decade, the reported efficacy of oral VRCZ in terms of response rate ranged from 53-65% with several months' administration, and the frequency of adverse effects which resulting in discontinuation of therapy ranged from 9-27% [44-46]. The efficacy of ITCZ and VRCZ in these studies was mostly assessed by clinical, radiological and mycological improvement at the end of treatment or regular interval, regardless of whether there was a partial or complete response. Posaconazole is a new triazole drug with a wide spectrum of activity including zygomycoses. Although its efficacy has been proved in salvage therapy for IPA patients and prophylaxis of invasive fungal diseases in patients with neutropenia and transplantation, no clinical data is as yet available for CPA cases [47, 48]. Its activity against to *Aspergillus* spp. and oral formulation are both suitable for CPA treatment.

Since relatively prolonged administration of triazoles is the mainstay of CPA

treatment, appropriate use of drugs based on pharmacokinetics/pharmacodynamics (PK/PD) properties and therapeutic drug monitoring (TDM) is logically important. The PK/PD parameter of triazoles is the ratio of the area under the concentration-time curve (equal to total exposure per dosing interval) to the minimum inhibitory concentration (AUC/MIC) [49, 50]. The purpose of TDM is to maximize the efficacy and minimize the toxicity of therapeutic agents. Pascual reported the relationship between trough concentrations and successful treatment as well as toxicity of VRCZ [51]. A trough level of 1mg/L was associated with a 70% probability of successful outcome in invasive mycoses, while that of 6 mg/L resulted in approximately 20% probability of central nervous system toxicity [51]. In recent reports regarding TDM studies of ITCZ and VRCZ, trough level of between 1 to 2 mg/L and 0.5 to 1.5 mg/L, respectively are recommended for successful treatment of fungal diseases [52]. A trough level of 5 mg/L of ITCZ is associated with a 26% probability of an adverse effect [53]. These proposed target concentrations are however, based upon limited data and not from prospective randomized studies. Thus, recent data suggests that TDM of triazoles may be useful in management.

There is limited published data on the use of other classes of antifungal agents in the treatment of CPA. The efficacy of intravenous micafungin (MCFG) was reported in two small series of CPA cases by Kohno and Izumikawa, in which the success rate of treatment was 12/22 (duration of treatment; 11-57 days) and 7/9 (duration of treatment; 29-96 days) cases, respectively [54, 55]. Only one randomized control study of CPA therapy, comparing intravenous VRCZ and intravenous MCFG was presented, which indicated favorable response rate with both MCFG (60%) and VRCZ (53%) [56], though the utility of intravenous antifungal drugs for CPA treatment is not known. No studies on the use of combination use of antifungals to treat CPA, besides a small number of case reports, have been reported.

Overall crude response rate of CPA to antifungal drugs such as ITCZ, VRCZ and MCFG is approximately 50-70%, however, we should realize that the definition of CPA, evaluation of response to drugs, endpoints of each study and the duration of treatment were varied in these published reports.

Evaluating response to antifungal drugs in CPA is challenging, since it usually take several weeks to months for noticeable improvement of respiratory signs, symptoms and radiological findings. Additionally, no recommendations for commencement and discontinuation of treatment, dose and route of drugs are established. More data from case series or randomized clinical trails are urgently required.

There is small number of case studies using interferon-γ as adjunctive therapy for CPA with or without co-use of antifungals. Although all four cases responded well [4, 57], its utility has not been established.

ANTIFUNGAL RESISTANCE

Azole-resistant *A. fumigatus* is increasing and becoming one of major clinical concerns in the treatment of *Aspergillus* infection [58-61]. The data regarding azole-resistance is mainly originated from United Kingdom and Netherland and no worldwide epidemiological data is available. Azole resistance reported by Howard et al. from the United Kingdom group is largely in the groups of patients with CPA [61]. Azole resistance in *A. fumigatus* has not been focused in the last decade, there being only few studies after the first report of ITCZ resistant strains in 1997 by Denning et al [62]. Recent advances in standardizing susceptibility testing by Clinical and Laboratory Standards Institute (M38-A2) and European Committee on Antimicrobial Susceptibility Testing [63, 64], and establishment of interpretative cutoffs [65] as well as clinical breakpoints [59], have greatly enhanced clinical and basic research. The resistant mechanisms against antifungal drugs including azoles are described in elsewhere [66]. The primary resistance mechanism to azoles is the mutation in the target protein, 14 alpha-demethylase coded by *cyp51A,* and several hotspots of mutation are already confirmed [61]. It is notable that many isolates resistant to ITCZ are usually cross-resistant to posaconazole (74%) and VRCZ (65%) [61]. Although the molecular approach for revealing azole-resistance is well studied, it is still unknown how resistance is evolved in nature. Verweij et al. reported the possibility that environmental fungicide (azoles) use may induce mutations in *cyp51A* and these environmental strains become a cause of aspergillus infection of human [67, 68]. On the other hand, some molecular epidemiological analyses indicated that drug-resistance is acquired in infecting strains within the human lung rather than inhalation of environmental azole-resistant strains [61, 69]. Importantly, many azole-resistant strains were isolated from CPA patients with aspergilloma and many of whom had been exposed to azoles for an extended duration (1-30 months) [61, 70]. Although oral administration of azoles is the mainstay of treatment of CPA, long term administration potentially induces azole-resistance. Due to higher cross-resistance rate to azoles, intravenous amphotericin B or echinocandins are only alternative options for the treatment of CPA with azole-resistant strains. Studies on optimizing triazole regimens with PK/PD and TDM data to prevent mutations of *cyp51A* and acquire maximum efficacy are desired. Developing new oral or intravenous antifungal drugs which do not show cross-resistance to current azoles are also required.

A. fumigatus is sensitive to amphotericin B and echinocandins in vitro and no emergence of resistant to these drugs has been reported [71]. Other species of *Aspergillus,* such as *A. terreus* and *A. nidulans,* are resistant to amphotericin B [71], although, the incidence of CPA due to these species is not high.

CONCLUSION

Studies regarding CPA are quite limited. Newer disease entities of CPA will somewhat increase and promote clinical and basic studies. The emergence of azole-resistant *Aspergillus* is becoming a major issue in the treatment of aspergillosis, such that new antifungal drugs should be developed and new regimens of antifungal drugs will be required.

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