“Invasive Pulmonary Aspergillosis: An Old Kid On The Block”: Report Of Three Cases And Review Of Literature

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Citation

Abstract
Since the start of the acquired immunodeficiency syndrome (AIDS) epidemic in the 1980's, respiratory diseases have been an important cause of morbidity and mortality among these patients. Majority of the human immunodeficiency virus (HIV) infected patients encounters a pulmonary complication during the course of their illness. Invasive pulmonary aspergillosis is the most common invasive fungal infection worldwide, and a major cause of mortality among immunosuppressed patients despite adequate therapy. The diagnosis, although difficult to achieved, should be consider in immunosuppressed patients with fever no responding to antibiotic therapy, and those with typical findings on thoracic imaging. Whenever possible, diagnosis should be confirmed by histopathologic examination. Incidence has risen due to more intensive anticancer chemotherapy, organ transplantation, aggressive surgical interventions and prolonged survival among HIV-infected patients due to highly active antiretroviral therapy. We report 3 cases of invasive pulmonary aspergillosis in severely immunocompromised hosts, in whom the diagnosis was made by autopsy.

CASE 1
A 38-year-old hispanic male had a medical history significant for HIV/AIDS, Kaposi's sarcoma of the skin and substance abuse. He was admitted to the hospital with 3 weeks history of cough, tachypnea, pleuritic pain, fever and weight loss.

Vital signs on admission were: blood pressure of 100/50 mmHg, heart rate of 92 beats/min, respiratory rate of 22 breaths/min, and temperature of 101°F. Physical examination showed a cachectic male, in mild respiratory distress with bilateral crackles on auscultation. He had several dark pigmented skin lesions in the trunk and upper extremities.

The complete blood count showed a white blood cells of 1,320/mm³, a hemoglobin level of 7.5 g/dl, and a platelet count of 164,000/mm³. Relevant serum chemistry included a sodium of 150 mEq/L, a creatinine level of 0.5 mg/dl, and a lactate dehydrogenase enzyme level of 301 units/L. His CD₄ count was 14/cumm. Serum cryptococcal antigen was negative, and urine antigen for Histoplasma was negative. Chest radiography demonstrated diffuse increased interstitial markings with bibasilar opacities. Chest computed tomography revealed ground-glass appearance with diffuse interstitial air space disease and bilateral pleural effusions.

Intravenous vancomycin (1 g Q12h), cefepime (2 g Q8h) and pentamidine (4 mg/kg Q24h) were initiated empirically. After 48 hours developed respiratory failure and was placed on mechanical ventilation. Intravenous voriconazole (4 mg/kg Q12h) was added. His hospital course was complicated with acute renal failure, septic shock and, ultimately, pulseless electrical activity and cardiac arrest.

All cultures done during hospitalization were negative. Autopsy showed angio-invasive aspergillosis of the lungs as the main cause of death. (FIGURE 1) Also, Kaposi's sarcoma of the lungs and skin was found.
CASE 2

An African-American female was admitted for generalized papular-vesicular rash, lethargy, cough and weight loss for 3 weeks. She was 48-year-old with unknown past medical history at the time of admission, but her husband died from acquired immunodeficiency syndrome (AIDS).

Vital signs were: blood pressure of 90/67 mmHg, heart rate of 126 beats/min, and respiratory rate of 22 breaths/min. She was afebrile. Remarkable findings on physical examination included vesicular lesions with excoriation in the face, anterior thoracic and abdominal walls, oropharyngeal thrush, and bilateral rales on auscultation. She was alert but confused.

Complete blood count showed a white blood cells of 15,500/mm$^3$, a hemoglobin level of 12 mg/dl, and a platelet count of 232,000/mm$^3$. Relevant chemistry results included a sodium level of 125 mEq/L, bicarbonate level of 15 mEq/L, and a creatinine level of 2.5 mg/dl. Alkaline phosphatase level was 346 units/L. Arterial blood gas results were compatible with hypoxemic respiratory failure. Lactic acid level was 4.7 mg/dl. Chest radiograph showed multiple diffuse densities. (FIGURE 2)

Intravenous vancomycin (500 mg Q24h), cefepime (1 g Q24h), acyclovir (350 mg Q24h), fluconazole (200 mg Q24h), azithromycin (500 mg Q24h) and trimethoprim/sulfamethoxazole (15 mg/kg Q24h) were initiated empirically along with vasopressors and steroids.

Histoplasma and Legionella urine antigens were negative. Tzanck smear of the skin lesions showed cytopathic changes compatible with herpesvirus infection. CD4 count was 35/cumm. Blood and urine cultures were negative.

Hospital course was complicated with respiratory failure requiring mechanical ventilation and multi-organ dysfunction syndrome. On day 6th of admission developed bradycardia and cardiac arrest.

Post-mortem histopathology examination showed angioinvasive pulmonary aspergillosis with hemorrhagic infarction of the lungs as the main cause of death. Cytomegalovirus pneumonia was also reported. (FIGURES 3-4-5)

Figure 2
Figure 2: Multiple diffuse densities showed in the chest radiograph
Invasive Pulmonary Aspergillosis: An Old Kid On The Block: Report Of Three Cases And Review Of Literature

CASE 3

A 41-year-old African-American female was admitted for shortness of breath. On arrival to emergency room was intubated and placed on mechanical ventilation due to severe respiratory distress. Her past medical history was significant for HIV/AIDS, diabetes mellitus and gastro-esophageal reflux disease.

Vital signs on admission were: blood pressure of 100/60 mmHg, heart rate of 136 beats/min, respiratory rate of 26 breaths/min, and temperature of 99°F. She had bilateral rales on lung auscultation. Complete blood count showed white blood cells of 6,900/mm³, a hemoglobin level of 10.5 mg/dl, and a platelet count of 405,000/mm³. Serum sodium level was 130 mEq/L, and a creatinine level was 1.9 mg/dl. Aspartate aminotransferase and alanine aminotransferase levels of 90 and 96 units/L respectively, lactate dehydrogenase enzyme level of 236 units/L, and alkaline phosphatase level of 555 units/L. CD4 count was 55/cumm. Legionella and Histoplasma urine antigens were negative as well as serum cryptococcal antigen.

Intravenous vancomycin (1 g Q24h), piperacillin/tazobactam (3.375 g Q8h), pentamidine (4 mg/kg Q24h) and prednisone were empirically initiated. During hospitalization she developed cardiac arrest and anoxic encephalopathy. Blood cultures were positive for Mycobacterium-avium complex. Tracheal aspirate culture showed Aspergillus fumigatus and bronchoscopy with bronchoalveolar lavage demonstrated Mycobacterium-avium complex and Pneumocystis jiroveci.
Plain chest radiograph showed bilateral increased interstitial markings with a left upper lobe infiltrate. Chest computed tomography showed extensive bilateral infiltrates with multiple non-uniform cystic spaces and minimal bronchial dilatation. (FIGURES 6-7) Amphotericin B (50 mg Q24h), isoniazide, rifabutin, ethambutol, pyrazinamide and clarithromycin were added to the therapy. Patient expired after 1 week. Autopsy demonstrated bilateral pulmonary aspergillosis with tracheal involvement and Cytomegalovirus pneumonia as the major causes of death. (FIGURES 8-9)

**Figure 6**
Figure 6: CXR showing bilateral increased interstitial markings

**Figure 7**
Figure 7: Bilateral infiltrates on a chest computed tomography

**Figure 8**
Figure 8: Multiple cytomegalic inclusions in the lung tissue
Fig. 9: Several branching hyphae in a cross-section of the lung tissue

**DISCUSSION**

Aspergillus species are ubiquitous molds commonly found in humid areas, foods and rotting vegetation, especially during autumn and winter. It is closely related to Penicillium spp in the fungal kingdom. More than 150 species exist but only a few are facultative pathogenic: fumigatus (causing more than 90% of infections), flavus, niger, terreus, nidulans and amstelodami among others. Enters the host primarily through the respiratory tract by inhalation of airborne spores, and is associated with building hygiene and construction works. Following hematogenous dissemination from the lungs may cause central nervous system infection (seizures, ring-enhancing lesions, epidural abscesses, cerebral infarction and meningitis), sinusitis (acute or chronic rhinosinusitis, granuloma), endophthalmitis and keratitis, endocarditis (second most common cause of fungal endocarditis following candida spp), and fungal abscesses in myocardium, liver, spleen and bone. Also, cutaneous lesions resembling pyoderma gangrenosum, usually around intravenous catheter insertion sites, burns or surgical wounds, can be seen. Onychomycosis and vertebral osteomyelitis or diskitis has also been reported, the latter in patients with chronic granulomatous disease and intravenous drug users. It is primarily an opportunistic pathogen in immunosuppressed patients, especially those with neutropenia, where lungs are commonly affected and the risk of infection increased when granulocyte count is less than 500 cells/mm³. Also, solid organ transplantation, drugs such as myeloablative chemotherapy and steroids, chronic granulomatous disease and allogeneic bone marrow transplantation for hematologic malignancies, especially acute myelocytic leukemia, are known risk factors. Use of marijuana increased the risk for aspergillosis. Aspergillus spp can cause disease in HIV-infected patients and the incidence is increasing due to the use of certain drugs such as ganciclovir, zidovudine and steroids. These patients usually have a low CD₄ count, in most of the cases less than 50/cumm.

Antifungal defenses in humans are based on normal mucosa barriers, macrophages and neutrophil function as well as tumor necrosis factor alpha and macrophage inflammatory protein (MIP-1 alpha), that are reduced in neutropenic hosts. Aspergillus spores release factors that suppress the synthesis of pro-inflammatory cytokines such as interleukin-1 and tumor necrosis factor-alpha in macrophages. T-cell function is another important mechanism of defense, because Aspergillus antigens are able to induce T-helper (th) 1 and 2 types reactivities.

There are different types of infection caused by Aspergillus species. In Allergic bronchopulmonary aspergillosis (ABPA) fungal spores trigger an asthma-type allergic reaction with cough, wheezing and shortness of breath in patients with pre-existing asthma or cystic-fibrosis. Several syndromes are related to allergic bronchopulmonary aspergillosis: mucoid impaction, bronchocentric granulomatosis, eosinophilic pneumonia and hypersensitivity pneumonitis. Aspergilloma is the most common form of infection, usually non invasive. Is generally found incidentally on a routine chest radiograph or during an evaluation of hemoptysis. Presents with cough, shortness of breath and hemoptysis in patients with a pre-existing lung cavity, generally secondary to tuberculosis, sarcoidosis, cystic fibrosis or emphysematous bullae. Fever is uncommon unless there is an associated infection with bacterial pathogens. Infections caused by other fungi such as Zygomycetes and Fusarium may cause the formation of fungus ball. Chronic necrotizing pulmonary aspergillosis (CNPA), also known as semi-invasive pulmonary aspergillosis, is seen in mildly immunocompromised patients receiving prolonged steroids therapy. Also, diabetes mellitus, alcoholism and chronic lung disease are risk factors. Chronic cough, fatigue and weight loss are common symptoms, and chest imaging shows cavitory consolidations in the upper lobes and pleural...
thickening with destruction of lung parenchyma (22, 23). Invasive pulmonary aspergillosis is a life-threatening condition though to be an emerging complication in HIV-infected patients, with an incidence of about 12% among AIDS patients (23). Most commonly affects males, although some autopsy findings showed higher prevalence among women (23). Presents as pneumonia-like illness in people with altered immune system (24, 28, 37, 38). Lungs are infected, with vascular invasion and subsequent infarction with tissue necrosis. Risk factors for invasive pulmonary aspergillosis are ischemic airway injury, altered alveolar phagocytic function and impairment of mucociliary clearance (38). Approximately 25%-30% of patients have no symptoms. The more immunosuppressed, the lesser the symptoms (1). Fever despite appropriate antibiotic therapy for more than 96 hours in a neutropenic patient should alert the physicians about the possibility of invasive aspergillosis (39).

Pneumothorax can be the initial presentation in neutropenic patients (1). Also, pleuritic pain, cough (1, 31) and hemoptysis, that is more frequent when granulocytopenia resolves and is a poor prognostic sign suggesting vascular invasion, can be observed (31, 33, 34). Hypoxemia and hypocapnea are frequently found in patients with diffuse disease (1).

Different classification criteria have been proposed for the diagnosis of aspergillosis: Definite, when there is a histopathologic evidence of invasive aspergillosis in a tissue specimen and a documented Aspergillus spp in culture; Probable, if there is any histopathologic evidence of invasive aspergillosis but not documented Aspergillus spp in culture or when, in absence of histopathology, a positive culture is obtained from a clinical or radiographically suspicious lesion, and Disseminated when more than 1 non-contiguous sites are involved, reflecting blood dissemination (33, 36, 37).

Several radiographic patterns could be found in invasive pulmonary aspergillosis. The commonest radiologic finding is a thick-walled cavity with or without an intracavitary mass, probably due to hemorrhagic infarction resulting from angioinvasion (33, 38). HIV-infected patients with lung cavities and prolonged hospitalizations are at increased risk for invasive pulmonary aspergillosis (40). The appearance of a hemorrhagic pulmonary nodule or “halo sign” is typical but not pathognomonic of invasive pulmonary aspergillosis, since it can be present in other pulmonary disorders such as alveolar hemorrhage, bronchiolitis obliterans organizing pneumonia, viral infections, Kaposi's sarcoma, Wegener's granulomatosis and angiosarcoma. “Halo sign” is also found with other fungi infections such as Mucorales, Trichosporon, Blastoschizomyces and Fusarium (1). “Halo sign” can be undetectable by simple chest radiograph in early stage of the disease, with a specificity of about 80% (41, 42, 43). Early recognition of central hypodensity or “hypodense sign” in immunocompromised patients is valuable in the diagnosis of invasive pulmonary aspergillosis (44). Other radiographic findings have been described, especially bilateral lower lobes atelectasis and consolidations (45), and mycetoma formation in patients with pre-existing cavities due to tuberculosis or pneumocystis (44). Pleural effusions are uncommon (1, 40, 41). Necrotizing tracheobronchitis with nodular thickening of the tracheal and bronchial walls, and allergic bronchopulmonary pattern with bronchial obstruction and plugs of endoluminal fungus are not uncommon, especially in patients with AIDS and those undergoing lung transplantation (1, 46). Thoracic computed tomography remains as the most sensitive radiological method to detect early changes of invasive pulmonary aspergillosis. The routine use of high-resolution chest computed tomography scans in patients with suspected invasive pulmonary aspergillosis has been associated with better outcomes, probably due to earlier diagnosis (46). Magnetic resonance imaging is not useful in the early diagnosis of invasive pulmonary aspergillosis (43). Imaging findings should be interpreted in conjunction with clinical presentation and severity of the disease, since individual disease processes may manifest in a variety of different radiological appearances, and there is considerable overlap between the radiographic findings of the numerous infectious and neoplastic entities that are known to occur in AIDS patients (46).

Definite diagnosis of invasive aspergillosis is established by tissue examination. The histopathology will show a tissue reaction with an acute suppurative inflammation, areas of ischemic necrosis, and the presence of septate hyphae that branches at 45\(^\circ\) angles. The septated hyphae of Aspergillus spp are best detected by Gomori methenamine silver and periodic acid-Schiff stains. The histologic response in patients with aspergillosis ranges from a florid granulomatous to a minimal tissue response, depending upon the degree of immunosuppression (1). When angioinvasion occurs, bland thrombi and acute necrotizing arteritis can be found (47). Similarities in tissue section can be seen in infections caused by Pseudallescheria boydii, Fusarium spp and Scopulariopsis spp (1, 34, 32, 33). Tissue specimens could be obtained from transbronchial or open-lung biopsy, which
is, in general, the gold standard in the diagnosis. Due to the risk of bleeding, open-lung biopsy is reserved for patients in whom the diagnosis of invasive pulmonary aspergillosis remains uncertain, and when the procedure is expected to have an impact on the patient’s management and outcome. A computed tomography-guided percutaneous lung biopsy could be performed, with a diagnostic yield of 80%-100%.

Another diagnostic tool is bronchoscopy with bronchoalveolar lavage, especially in patients with diffuse lung involvement, with a diagnostic yield of 50%-75% but with lower specificity than transbronchial biopsy (34, 52).

Aspergillus spp can be found in the bronchoalveolar lavage by culture (Saboraud medium), by microscopic detection of mold hyphae (hematoxylin-eosin, Grocott’s staining), by detection of the Aspergillus antigen, and by polymerase chain reaction (PCR). A PCR performed in the bronchoalveolar lavage fluid is associated with a specificity of about 90% for the diagnosis of invasive pulmonary aspergillosis (51). In HIV-infected patients Aspergillus can be found in the bronchoalveolar lavage as a colonizer (53).

Aspergillus of the lungs may coexist with tuberculosis, cryptococcosis, cytomegalovirus and pneumocystis infection (26, 54).

The most commonly used serologic test to diagnose invasive pulmonary aspergillosis is based on the detection of antigens, especially galactomannan (GM) antigen, a polysaccharide component of the fungal wall released into surrounding tissue during hyphal growth. It has a sensitivity of more than 90% and specificity of 95% using a sandwich enzyme-linked immunosorbent assay (55). There is an improved sensitivity of the sandwich ELISA assay compared with the latex-agglutination assay, but up to 14% of false-positive results has been reported (56). Sensitivity of antigen testing is dependent of the extension and severity of the disease, and increased when a low index cutoff value of <1.0 is used to define positivity (57). In patients receiving mold-active antifungal therapy there is a decrease sensitivity of the serum assay, impairing the ability of the test to provide an early indicator of breakthrough infection (58). Cross-reactivity has been reported against Penicillium spp, Alternaria, Paecilomyces, beta-lactam antibiotics, patients on dialysis and chronic graft-versus-host disease. Galactomannan levels should be monitored before the administration of beta-lactam antibiotics. Positive results in patients treated with such antibiotics, in the abscense of signs of invasive aspergillosis, must be confirmed by a bi-weekly follow up, and at least 5 days after the cessation of treatment (60, 61, 62, 63). The use of echinocandins may paradoxically increased galactomannan levels by inducing hyphal fragmentation (64). Serial samples are necessary to maximize the antigen detection, and it can be useful for clinical decision making because positivity correlates with mortality (34, 52). Test results correlate with fungal burden, suggesting that it may be clinically important to follow up the response to antifungal therapies (65).

Another serologic test useful in the diagnosis is the detection of Aspergillus spp by polymerase chain reaction (PCR). It is more sensitive than the antigen detection methods but it can not be used to monitor antigen titers during treatment. It has sensitivity and a negative predictive value of about 100%. False-positive results may be caused by accidental contamination of DNA and bronchoalveolar fluid specimens by Aspergillus conidia (66). According to some studies, PCR assay is more useful than serum galactomannan antigen detection in the diagnosis of invasive aspergillosis (67, 68). Blood cultures rarely yield Aspergillus species (69).

Early diagnosis and appropriate therapy are imperative in patients with invasive pulmonary aspergillosis. An aggressive diagnostic approach in patients at risk and prompt institution of antifungal therapy is essential for survival (70). Initial therapy is very important and intravenous treatment may be preferred in acutely ill patients (2). Amphotericin B or lipid-based formulations in patients with altered renal function has been the standard of treatment for patients with invasive aspergillosis (4). Better outcomes have been seen when voriconazole is selected as initial treatment (71).

Voriconazole is recommended as initial therapy because of better clinical responses and improved survival when compared with other azoles, amphotericin B formulations and echinocandins. Also, voriconazole have better tolerance and less side effects than the conventional/standard therapy with amphotericin B (72). Great response is obtained when voriconazole plasma levels are more than 250 ng/µl. Most of the adverse effects such as visual disturbances, confusion, skin rash and abnormal liver function test are associated with plasma levels of more than 6000 ng/µl (73).

For salvage therapy, amphotericin B formulations should not be recommended when amphotericin B formulations were used as initial therapy (74). Caspofungin has been used effectively as an alternative for salvage treatment of invasive pulmonary aspergillosis (75). The underlying medical condition and previous treatment exposure substantially
contributes to the clinical outcome for antifungal therapy. Patients with persistent neutropenia and underlying malignancy have the worst prognosis (86).

Although not clear clinical data is available, some physicians are using combination antifungal therapy because of the persistently high mortality rate associated with aspergillosis, even among patients treated with voriconazole for salvage therapy. In some studies, the combination of voriconazole and caspofungin was associated with improved 3-month survival rate compared with voriconazole as a single therapy in patients receiving salvage therapy to treat aspergillosis (52). Other possible combinations include azoles or amphotericin B with echinocandins such as caspofungin, micafungin and nikkomycin Z, the latter only active against fumigatus and flavus species. Also, amphotericin B with flucytosine or rifampin has been used. Flucytosine may exacerbate myelosuppression in patients with neutropenia, and rifampin may have significant drug interactions (76, 87). Successful treatment of invasive aspergillosis with the combination of caspofungin and itraconazole have been reported (88). Combination of antifungal agents may confer the benefit of increasing efficacy and sparing toxicity, especially with echinocandins-triazoles combination (91). The improved survival in groups using combination therapy may support the use of combination regimens in patients who experienced treatment failure with other agents (89). Triple combination is another alternative to treat invasive aspergillosis (92). The optimal duration of therapy is unknown and depends on the extension of the disease, the response to therapy, and the patient's underlying disease or immune status. Duration of therapy should be guided by clinical response and should be prolonged beyond resolution of the disease and reversible underlying predispositions. A reasonable course would be to continue therapy to treat microfoci, after clinical and radiographic abnormalities are resolving, and when obtained cultures are negative (93).

Additive therapy to antifungal treatment include cytokines, such as granulocyte-macrophage colony stimulating factor, interferon-gamma and granulocyte transfusions, but these are not recommended for routine therapy (56, 82, 83). Surgical treatment is an option in cases of localized disease that is unresponsive to antifungal therapy (94). Surgery is usually done to prevent severe hemoptysis, especially after the neutropenia had resolved, although there is not contraindications for thoracic surgery in these patients (11, 84). Aggressive surgical management improves the prognosis of invasive pulmonary aspergillosis in some patients (95). Cases of recurrent pulmonary and pleural aspergillosis have been reported after surgery (96).

Mortality remains high in patients with invasive aspergillosis despite appropriate treatment. The overall case-fatality rate is about 60%, and is higher among bone marrow transplant recipients and for patients with central nervous system involvement or disseminated aspergillosis (97). Patients with HIV-infection have a case-fatality rate of about 85%, probably because the underlying immunosuppressive condition is progressive, irreversible, and because these patients are more likely to have diffuse invasive pulmonary aspergillosis (97, 98). Autopsy findings in patients with AIDS revealed that mortality from fungal infections has been increasing or, at least, remains unchanged (98, 99). Kaposi's sarcoma and Cytomegalovirus have been a frequent findings at autopsy in patients with AIDS (100, 101). Overall prognosis will depends upon the type and severity of the disease as well as the immune status of the host (100).

CONCLUSIONS

Invasive pulmonary aspergillosis is an important and commonly fatal complication of advanced HIV infection. Although invasive pulmonary aspergillosis remains a life-threatening complication among immunocompromissed patients, with a persistently high case-fatality rate, aggressive diagnostic evaluation and initiation of treatment are essential to improve its prognosis.

Clinically, the onset of invasive pulmonary aspergillosis is highly variable and plain chest radiographs are nonspecific in early stages of the disease. Therefore, clinicians should suspect the diagnosis in the setting of immunosuppressed patients with persistent fever, prolonged neutropenia, and those with persistent pulmonary infiltrates. There has been a substantial increased in the number of cases documented at autopsy. Causes of death established postmortem have been frequently shown to differ from those suspected by clinicians. However, invasive aspergillosis is a devastating infection, and the fact that all our cases were diagnosed post-mortem confirms the perception of the emergence of invasive aspergillosis in AIDS patients.
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11 of 12

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