Report from the 4th Advances Against Aspergillosis Conference

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Traditionally, the patients believed to be at highest risk of invasive aspergillosis (IA) are those who are neutropenic due to chemotherapy for hematological malignancy or those undergoing allogeneic hematopoietic stem cell transplantation. However, emerging data show that other patients are vulnerable to IA, even though some are not classically immunocompromised. These include: solid organ transplant recipients; patients with TB, chronic obstructive pulmonary disease and patients in the intensive care unit for other reasons. The conference highlighted the diagnostic and therapeutic challenges facing physicians treating this diverse group, not least of which include the unreliable estimates of IA incidence due to poor surveillance and inadequate data collection. Moreover, although there is now considerable experience of IA in neutropenic patients, much less is known about the management of those who are non-neutropenic. Nevertheless, approaches that have proven effective in neutropenic patients may also benefit others in this growing population. The meeting, attended by more than 500 delegates from almost 50 countries, also provided the opportunity to hear how basic scientific research may improve the understanding of the pathogenic mechanisms and therapy of IA.

Incidence

Poor surveillance hinders accurate estimates of invasive aspergillosis (IA) incidence, as Malcolm Richardson (University of Manchester, UK) explained. Only 60 outbreaks of nosocomial aspergillosis were reported in the English language literature between 1967 and 2007. Moreover, the source of the outbreak was unknown in many of these cases. Comparisons of levels of airborne exposure are difficult as there is no standardized protocol for aerobiological surveillance, despite many recommendations. Dionissios Neofytos (Johns Hopkins University, MD, USA) pointed out that many surveillance databases are inadequate. Single centers provide less reliable data than multicenter databases such as the US Transplant-Associated Infection Surveillance Network (TRANSNET) and Prospective Antifungal Therapy (PATH) Alliance registries. TRANSNET reports a yearly cumulative incidence of IA in hematopoietic stem cell transplantation (HSCT) recipients of 4%, compared with 10–20% observed in single-center studies, while the PATH Alliance records a 12-week survival rate of 70% compared with 50% at a single center. However, these multicenter databases are far from perfect, due to heterogeneity in case capture, case definitions and clinical practices, differences in endemicity, limited clinical data, inadequate follow-up and an inability to capture late events related to transplant-associated complications, underlying disease relapse and infections. Diagnostic accuracy may vary between patient groups because different tests are used: in PATH Alliance, diagnosis in most solid organ transplant (SOT) recipients was based on culture, but nonculture tests such as a CT scan or a galactomannan (GM) assay were used in HSCT recipients. The introduction of antifungal (AF) prophylaxis, combination AF therapy and PCR techniques to detect fungal-specific DNA present further challenges to surveillance registry design, Neofytos cautioned.

Non-neutropenic patients

At-risk groups

Non-neutropenic, nonhematological patients now account for up to 40% of all those with IA. Their mortality is higher than that of those with neutropenia: approximately 90% compared with 60% in those with neutropenia, reported Patricia Muñoz (Universitario Gregorio Maranon, Spain). Intensive care unit (ICU) patients are not necessarily neutropenic, but 90% receive steroids and so are immunocompromised, explained Elie Azoulay (Hôpital Saint-Louise, Paris, France). They may develop an immunoparesis characterized by decreased monocyte

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HLA-DR expression and dendritic cell anergy, leaving them susceptible to secondary infection, added Guillaume Monneret (Université Lyon, France). In SOT recipients, graft dysfunction, intensive immunosuppressive therapy, polyclonal and monoclonal antibodies for induction or treatment of rejection, renal failure and the need for hemodialysis, are the main risk factors, explained Faouzi Saliba (Université Paris-Sud, France). IA in SOT patients often presents with a different clinical picture than that in hematological patients. There are few typical clinical or radiological signs because immunosuppression impairs the inflammatory response. By the time IA is diagnosed, the infection is often far advanced. Reliable surrogate markers are few. In lung transplant recipients, positive cultures are obtained from sputum and bronchiolar lavage fluid in only 8–34% and 45–62% of patients, respectively. Pulmonary imaging is usually abnormal, but the halo sign is extremely rare. The sensitivity of the GM assay is a mere 22%, increasing to only 62% with a real-time PCR assay of the first positive GM sample.

Chronic obstructive pulmonary disease (COPD) and pulmonary TB are also risk factors for IA. COPD accounts for up to 10% of cases, and is associated with a 70% mortality, said John Baddley (University of Alabama, AL, USA). In a recent retrospective study of 14,618 COPD patients, 239 had positive respiratory-tract cultures for *Aspergillus* spp., and 53 (22%) of these had probable IA – a rate of 3.6 cases per 1000 COPD admissions. Diagnosis is difficult, although corticosteroid therapy, previous antibiotic use, late-stage disease and viral infections are known risk factors and should raise the index of suspicion. Pulmonary TB may lead to chronic pulmonary aspergillosis (CPA). This is defined as the presence of at least one pulmonary cavity on chest imaging with or without a fungal ball, together with symptoms for at least 3 months and serology or cultures implicating *Aspergillus* species. David Denning (University of Manchester, UK) has estimated that, as a result of TB, there may be 350 new cases of CPA in the UK and 250,000 worldwide every year [1]. All pulmonary TB patients should have a chest x-ray at the end of treatment and those with radiological changes should undergo further follow-up, including an *Aspergillus* antibody test, he advised.

Prophylaxis & treatment

Targeted prophylaxis represents a major advance in the management of high-risk SOT patients in whom toxicity and drug interactions limit the efficacy of therapy. Out of 198 high-risk liver transplantation recipients treated by Saliba's group, 146 (21.9%) received amphotericin B lipid complex (ABLC) for days 1–7 and 50 received fluconazole for 18 ± 7 days. Although this prophylaxis significantly reduced the incidence of *Candida* infection, it showed no benefit in the prevention of IA, possibly because the study lacked sufficient power. However, prophylaxis with caspofungin for 21 days was successful in preventing IA in 89% of patients in another recent study, he said. After liver transplantation, a lipid formulation of amphotericin B or an echinocandin are now recommended for at least 3–4 weeks [2]. For lung transplant recipients, the recommendations are inhaled amphotericin B, inhaled lipid formulations of amphotericin B or, in high-risk patients, voriconazole or itraconazole [2]. Finally, Monneret suggested that augmenting patients' immunity with IFN-γ or granulocyte macrophage-colony stimulating factor can reduce ventilation time and ICU and hospital stay.

The utility of combination antifungal therapy in non-neutropenic patients with IA received considerable attention. Muñoz and her colleagues reported their experience using combination therapy in COPD-associated IA. They studied a total of 53 patients of whom 49 received AF therapy with voriconazole; 23 out of 49 also received an echinocandin. The overall mortality rate was 78%. This was lower than the median mortality of 91% seen in seven other studies in a total of 108 patients of whom none received an echinocandin and only one received voriconazole. Muñoz acknowledged that the slightly better survival in their study is not necessarily attributable to the use of combination therapy; however, few other data are available on the newer AF agents in this population. There is more evidence – but as yet no well-designed comparative study – for combination therapy in SOT recipients. A multicenter observational study of voriconazole/caspofungin versus a lipid formulation of amphotericin B as primary therapy for IA found a lower mortality with the combination regimen at 12 weeks: 51 versus 67.5% (log-rank $p = 0.13$). The study did not have the power to demonstrate a clear benefit at this early time point, Muñoz explained. In another study (conducted in both SOT and bone-marrow transplant [BMT] recipients) combination therapy was also superior to single-drug regimens, with mortality rates of 42 and 83%, respectively, among patients with definite and probable diagnoses of invasive pulmonary aspergillosis (IPA).

The two large comparative trials – of voriconazole versus amphotericin B and of standard versus high doses of liposomal amphotericin B (the Ambiload trial) – largely excluded ICU patients, explained Raoul Herbrecht, (Hôpitaux Universitaires de Strasbourg, France). Some clinical studies suggest improved survival with combination therapy but have a limited sample size or used historical controls; others have produced conflicting results. Consequently, the use of combination therapy in non-neutropenic patients remains controversial and is not supported by current guidelines. Even so, half of all US centers and a quarter of those in Spain use combinations as primary therapy for SOT patients, Muñoz reported.

A different perspective was offered by Herbrecht, who suggested optimizing monotherapy in ICU patients rather than using unproven combinations. He recommended earlier use of azoles in those at risk, and perhaps prophylaxis in patients found to be colonized with *Aspergillus*. Importantly Herbrecht emphasized that drug response varies with mean serum levels of both voriconazole and posaconazole. In a study of posaconazole, in which the patients were divided into four quartiles according to their serum drug concentration, those in the highest quartile showed a 71% response rate compared with only 24% for those in the lowest quartile. This highlights the importance of therapeutic drug monitoring (TDM). TDM is essential to ensure adequate serum levels in SOT patients receiving azoles, Muñoz agreed. Further support for azole TDM comes from Aniket Vadnerkar and associates from the University of Pittsburgh, PA, USA. In their study of 12 heart/lung transplant recipients receiving posaconazole for either prophylaxis or treatment of IFI, serum posaconazole levels were inadequate in three-quarters of the 32 samples tested. Coauthor Cornelius Clancy stressed that most of the data for posaconazole use are based on hematological patients and may not be relevant to SOT recipients. The Pittsburgh group urges clinicians to correct modifiable risk factors that may affect oral absorption and to use TDM to guide posaconazole therapy [3]. Indeed, managed appropriately, posaconazole may be a rational option for lung transplant recipients. Adequate serum levels of posaconazole result in alveolar cell concentrations that remain above the MIC_{90} for *Aspergillus* spp. during the entire 12-h dosing interval and for 4 h after the last dose, reported John Conte and colleagues (American Health Sciences and the University of California, San Francisco, CA, USA) [4]. Thus, it is not clear if measuring serum posaconazole levels is the ideal strategy for determining therapeutic tissue levels.

Cost–effectiveness of posaconazole prophylaxis in high-risk neutropenic patients

Although the use of azole prophylaxis in SOT and other nonhematological patients needs more supporting data, research continues to confirm its cost–effectiveness in the neutropenic setting. Carlo Lazzaro (Studio di Economia Sanitaria, Milan, Italy) based his recent economic analysis on data from the clinical trial in which posaconazole was superior to fluconazole and itraconazole in high-risk patients with acute myeloid leukemia or myelodysplastic syndrome [5]. Lazzaro found that posaconazole prophylaxis in these patients represents good value for money [6].

Aspergillus & asthma

Asthmatic patients may develop allergic bronchopulmonary aspergillosis (ABPA); aspergillus sensitivity is associated with increased severity of nocturnal symptoms and reduced lung function, said Ritesh Agarwal (Postgraduate Institute of Medical Education and Research, India). Marianne Skov (The Children's Hospital at Westmead, Denmark) found azoles useful in children with ABPA. She added that posaconazole has proved effective where itraconazole or voriconazole has failed. In addition to ABPA, Denning reported on research suggesting that AF therapy can induce 'dramatic' responses in some asthma patients who do not have documented fungal colonization. Whether the efficacy of azoles in ABPA and asthma patients is due to direct antifungal effects or an immunomodulatory activity remains unknown according to Agarwal and Denning.

Aspergillus fumigatus: pathogenic insights & implications

Most *Aspergillus* species, like all other filamentous fungi, are generally regarded as obligate aerobes. But research, reviewed by Robert Cramer (Montana State University, MT, USA), shows that *A. fumigatus* grows extremely well in low oxygen environments (~0.1%); an adaptation with important implications for both pathogenicity and therapy. Work by Cramer's own group [7,8] has surprisingly revealed the presence of significant hypoxia at the site of the pulmonary lesions in animal models of IPA. They have also discovered

that *A. fumigatus* has multiple mechanisms, including lactic acid and ammonium fermentation pathways, to generate energy under hypoxic conditions. Exposure to hypoxia induces SrbA, a conserved transcription factor in the sterol regulatory element binding protein family. *A. fumigatus* mutants lacking SrbA are avirulent in murine models of IPA and need at least 5% oxygen to resume normal growth. These mutants also have a very low MIC for fluconazole which is normally ineffective against the wild-type strains. Cramer said these findings offer the potential to elucidate virulence mechanisms and to improve AF efficacy. Moreover, they could also explain why laboratories sometimes fail to isolate *Aspergillus* from patients with IA. This implies that culturing patient samples under anaerobic as well as aerobic conditions might improve IA detection rates, although Cramer did not specifically mention this strategy.

Additional insights into the pathogenicity of IA come from studies with a bioluminescent *A. fumigatus* strain constructed by Oumaima Ibrahim-Granet and colleagues in Paris, Seattle and Jena [9]. They used bioluminescence imaging and histopathological analysis to examine the development and progression of IA in mice with different types of innate immune defect. They found an *in vivo* correlation between the onset, peak and kinetics of hyphal tissue invasion in the lungs of animals with depleted or dysfunctional phagocytes. Depletion of neutrophils in the lung led to rapid conidial germination and an early rise in bioluminescence after infection, an effect not seen following alveolar macrophage depletion. These findings, the researchers point out, are consistent with the idea that neutrophil recruitment rather than alveolar macrophage activity is essential for early host defense. The group is now using the model to monitor AF efficacy *in vivo* and believe it will allow substantial cost savings compared with conventional methods.

Financial & competing interests disclosure

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