

Report from the 15th International Symposium on Infections in the Immunocompromised Host: Thessaloniki, Greece, June 22–25, 2008

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Recent changes in the epidemiology of life-threatening opportunistic invasive fungal infections (IFIs) are challenging diagnosis and treatment strategies. However, although more research is needed, 400 delegates from 44 countries at the 15th International Symposium on Infections in the Immunocompromised Host (ICHS) heard that new approaches are starting to make an impact.

Changing patterns of infection

IFIs not only have increased in incidence in the last 10 years but their aetiology has changed. Professor George Petrikkos, University of Athens, told delegates that the increase is predominantly due to a rise in the frequency of *Aspergillus* and *Candida* infections, the leading causes of IFIs in patients with haematological malignancies.

However, candidaemias caused by *C. albicans* actually have decreased ($p < 0.001$) while those due to *C. glabrata* have increased ($p = 0.05$). Data from the US Transplant-Associated Infection Surveillance Network (TRANSNET) indicate that *C. glabrata* now accounts for 32% and *C. albicans* for only 22% of all IFIs. This switch in the species balance has been attributed to the increased prophylactic use of voriconazole, to the widespread use of antifungal agents for febrile neutropenia, and to prolonged echinocandin therapy, Prof. Petrikkos said.

Invasive aspergillosis now occurs in 20% of allogeneic haematopoietic stem cell transplantation (HSCT) recipients, in 10% of patients with acute myeloid leukaemia (AML), in 5% with acute lymphoblastic leukaemia (ALL) and in 2% receiving autologous HSCT. *A. fumigatus* accounts for about half of all isolates and *A. flavus*, *A. niger* and *A. terreus* for 9%, 7% and 5%, respectively, he reported.

Emerging rare species

The previously rare zygomycoses are now more common. Prof. Petrikkos explained that their incidence per 1000 HSCTs increased from 1.7 in 2001 to 6.2 in 2004. By early 2006, Zygomycetes were responsible for around 24% of all invasive mould infections among transplant recipients. *Fusarium* and *Scedosporum* species are also becoming important aetiological agents.

Whereas the mortality of IFI due to candidosis is falling, that due to aspergillosis and other mycoses is increasing, he reported. A 2006 survey of nearly 12,000 patients from 18 centres showed an overall mortality of 42% for *Aspergillus* species, 64% for Zygomycetes and 52% for *Fusarium* species [1].

A new global registry, Fungiscope (www.fungiscope.net) has already documented 18 cases of these and other rare, but emerging, IFI. A further 10 cases are under investigation. The registry's coordinators, Professor Oliver Cornely and Dr. Maria Rüping, from Cologne, Germany, have invited other researchers to contribute to their project.

Paediatric Invasive Fungal Infections

Children and adolescents are as vulnerable as adults to IFI, but there are differences in the epidemiology and the populations at risk, said Dr Andreas Groll, University Children's Hospital, Münster, Germany. IFI caused by *Candida*, *Aspergillus* and other opportunistic yeasts and moulds are mostly due to deficiencies in phagocytosis or to barrier breakdown, whereas deficiencies of acquired immunity are a prerequisite for IFIs caused by *Cryptococcus neoformans* and dimorphic fungi.

Patients at risk include premature neonates and surgical patients as well as those with congenital immunodeficiency, malignancy-associated neutropenia or recipients of HSCT and solid-organ transplants. In neonates, IFI frequency is related to gestational age, prolonged rupture of membranes, intubation, the use of H2 blockers and third-generation cephalosporins, and colonisation of more than one body site. *Candida* species now account for 9–13% of all bloodstream isolates in neonatal intensive care units. The associated crude mortality is 15–

30%, and the attributable mortality 6–22%, despite appropriate therapy, Dr Groll reported.

The frequency of invasive aspergillosis in paediatric patients is 4.5–10% with an associated crude mortality of 40–94%. In some centres, he added, the dominating species now is *A. terreus* which is less susceptible to amphotericin B.

In children, as in adults, the prevalence of zygomycoses appears to be increasing, at least in cancer patients. Both the incidence of disseminated infection and the mortality appear to be higher in paediatric than adult patients: a mortality rate of 79% in neonates was reported recently.

Evolving treatment strategies

The changing epidemiology of fungal infections has led clinicians to rethink their treatment approaches. Prof. Petrikos reported that amphotericin B has activity covering all the major fungi and which includes *Mucor*, *Rhizopus* and *Fusarium* species. Of the other available agents, however, only posaconazole has such an extended spectrum.

Furthermore, emerging resistance has rendered some previously effective treatments less useful. Resistance to both fluconazole and itraconazole has reached 10–15% among *C. glabrata* and half of all strains show dose-dependent susceptibility, he noted. Amphotericin B MICs are higher for *C. glabrata* than *C. albicans*. Echinocandin MICs for *C. parapsilosis* have also increased recently.

Prof. Petrikos also warned of a possible link between the increasing incidence of zygomycosis and the prolonged use of voriconazole. Amphotericin B, preferably in a lipid formulation, remains the treatment of choice for zygomycoses, although posaconazole appears to be a well-tolerated and effective salvage treatment, he added.

Paediatric treatment

Less is known about the treatment of IFI in children than adults, explained Dr Theoklis Zaoutis, The Children's Hospital of Philadelphia, USA. Although there has been an increase in the overall use of antifungal drugs in the USA, from 2000 to 2006, the use of amphotericin B deoxycholate has declined whereas that of voriconazole and lipid formulations of amphotericin B has increased. These latter agents are now the most common treatments for aspergillosis in children, despite the dearth of data in this patient population, he said.

Dr Zaoutis emphasised the different azole pharmacokinetics in children; the optimal dose of fluconazole may be 12mg/kg/day and 6mg/kg/day may be too low in neonates. Similarly, children may need higher voriconazole doses than adults because of their faster metabolic clearance.

"The vast majority of US physicians use voriconazole as prophylaxis or empiric therapy in their high-risk leukaemic [paediatric] patients in the complete absence of data, so the dose is completely wrong," asserted Dr Joseph Wiley, the Herman and Walter Samuelson Children's Hospital at Sinai, Baltimore, USA. The European Medicines Agency recommends a maintenance dose of 7mg/kg twice daily.

Studies of posaconazole, one of the few antifungal agents with activity against Zygomycetes, have included only small numbers of children. "We do not at this moment know the right dose," Dr Zaoutis said. Experience with voriconazole suggests that the adult dose of posaconazole, too, may be suboptimal for paediatric patients. A study of posaconazole is now under way in children, Professor Cornely told the meeting.

Current problems in diagnosis

Early accurate diagnosis and species identification could allow directed antifungal therapy despite this changing epidemiology, but unfortunately all the current diagnostic tools have some limitations, said Professor Per Ljungman, Karolinska University Hospital, Stockholm, Sweden.

CT scanning in suspected invasive aspergillosis has speeded diagnosis and cut mortality – in one series from 50% to 17% – and patients presenting with the pathognomonic halo sign on radiology have better treatment outcomes than those who do not. However, many of those (39% in one study) with pulmonary aspergillosis show no halo sign. In addition, the halo sign evolves over time into the ‘air-crescent’ sign which is non-species specific, he said.

Histopathology can give positive results even when cultures are negative and can rule out non-infectious aetiology. However, sampling is invasive and the technique cannot definitively establish the identity of the pathogen(s) involved. Culture-based methods are more sensitive but slow and show mixed accuracy, Prof. Ljungman reported.

The enzyme-linked immunosorbant assay (ELISA) avoids the need to culture the organism. But the galactomannan ELISA for aspergillosis shows high rates of false-negative results for patients receiving mould-active fungal agents, false-positive results in those on piperacillin-tazobactam and variable sensitivity. The (1,3)- β -D-glucan ELISA cannot detect cryptococcal infections or zygomycoses and has high false-positive and false-negative rates. PCR-based DNA assays have not yet been clinically validated and lack standardisation.

Diagnosis more difficult in children

The diagnosis of IFIs is even more problematic in children than in adults. The halo or air-crescent signs are found in only 8% of paediatric patients with aspergillosis, Dr Groll explained. The galactomannan assay has lower sensitivity in children than in adults and there are few paediatric data, noted Dr Emmanuel Roilides, Aristotle University, Thessaloniki. Available information suggests a higher false-positive rate than in adults: 10.1–44% compared with 0.9–2.5%. False-negative results are also common in some subgroups of paediatric patients. PCR data are scarce in infants and children, said Dr Roilides.

All three speakers recommended combining the various diagnostic modalities to reach a definitive diagnosis. In one series of 88 patients, this approach reduced the rate of empirical antifungal therapy from 35% to 7.7%, and missed no cases

of invasive aspergillosis or (except for one case of zygomycosis) other IFI. Twelve-week survival rate for patients with invasive aspergillosis was 63.6%. However, not all centres can adopt this scheme due to lack of the appropriate resources, said Prof. Ljungman. There is therefore a need for other strategies to protect high-risk patients, such as antifungal prophylaxis.

Antifungal prophylaxis

In two recent, multicentre, randomised trials, posaconazole proved as effective as, or superior to, fluconazole for antifungal prophylaxis in patients with severe GVHD [2] and superior to fluconazole or itraconazole in AML/MDS patients with neutropenia [3].

In the GVHD trial, posaconazole and fluconazole were similarly effective in preventing all IFI (5.3% and 9.0%, respectively, $p=0.07$) and better than fluconazole in preventing proven or probable invasive aspergillosis (2.3% vs. 7.0%; $p=0.006$). There were also fewer breakthrough IFI in the posaconazole group (2.4% vs. 7.6%; $p=0.004$).

In the AML/MDS trial, the incidence of proven or probable fungal IFI was significantly lower in the posaconazole group (2%) than in the patients receiving the other azoles (8%) ($p<0.001$). Invasive aspergillosis was significantly less frequent in the posaconazole group ($p<0.001$) and these patients had a significantly longer survival ($p=0.04$). Tolerability profiles for the various agents were similar.

The findings of the AML/MDS study directly led to the European Conference on Infections in Leukaemia (ECIL) A1 recommendation for posaconazole prophylaxis 200 mg/kg tid orally for patients with leukaemia [4]

Prof. Cornely, co-investigator for the neutropenia study, presented some new data on numbers needed to treat (NNT). NNT to prevent an IFI, invasive aspergillosis, death due to fungal infection or due to any cause, were 16, 17, 27 and 14, respectively [5]. These, as he pointed out, are “pretty small numbers”

and considerably lower than the NNT of around 50 that cardiologists consider acceptable for their therapies.

“The effect on survival was even stronger than the effect on preventing invasive fungal infections,” he emphasised. This suggests that a higher number of definitive diagnoses would not have reduced the observed benefits of posaconazole. Moreover, although posaconazole was discontinued at ≤ 12 weeks, the probabilities of IFI and/or death were reduced until at least day 100 post-randomisation, indicating a persisting protection. This may be related to the much higher posaconazole levels achieved in the alveolar cells than in the plasma [6].

Prof. Cornely emphasised “I hate prophylaxis because it’s over-treatment.” But he added: “With posaconazole in this population we have a survival advantage and that is why we use it. I’m convinced it would work in other patient populations as well.”

He also presented data comparing results in his own unit before and after the introduction of posaconazole prophylaxis. Before prophylaxis, 9 (15%) of patients had proven or probable IFI compared with only 1 (3%) subsequently. One-year survival has also improved: from 58.6% to 70.1%.

New approaches

The increased incidence of IFIs, their continuing high mortality and the emergence of rare fungi, despite advances in antifungal therapy, have prompted development of adjunctive strategies. Several approaches are under investigation including the use of white blood cell transfusions, growth factors, Th1 and proliferative cytokines, calcineurin inhibitors, antibody therapy and vaccination.

Dr Roilides reported that although most of these techniques are still experimental, efungumab, a monoclonal antibody against heat shock protein 90 (Hsp90) has been studied in a randomised, double-blind comparative trial in combination with a lipid formulation of amphotericin B in patients with culture-

confirmed invasive candidosis. Complete overall, clinical, and mycological response rates were all higher with the combination than with lipid amphotericin B alone: 84% vs. 48%; 86% vs. 52%; and 86% vs. 54% respectively [7].

Research using the nematode worm *Caenorhabditis elegans* is helping to identify new antifungal targets and agents, reported Dr Eleftherios Mylonakis, Harvard Medical School, Boston, USA. Mechanisms of fungal infection are similar in humans and invertebrates, he explained.

Studies in *C. elegans* are particularly attractive because they may allow concurrent evaluation of toxicity and antifungal activity, and of fungal biofilms. This work has demonstrated that photosensitisation can induce caspofungin sensitivity in the usually resistant *Cryptococcus neoformans*. The model also has the potential to screen new compounds for antifungal activity.

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