

Invasive Fungal and Bacterial Infections: new challenges and developments
The 24th European Congress of Clinical Microbiology and Infectious Diseases 2014
Barcelona, Spain (10-13 May 2014)

Emerging resistance in fungi and anti mould prophylaxis

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The European Congress of Clinical Microbiology and Infectious Diseases, the annual meeting of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID,) is the most attended infectious disease conference worldwide. More than 10,000 clinicians and scientists attended the 2014 congress to present and share the latest research breakthrough in infectious diseases.

Part 1. Aspergillosis guidelines and invasive fungal infections (IFI)

Aspergillosis guidelines

The new ESCMID guidelines for the diagnosis and treatment of Aspergillus disease [1] developed in co-operation with the ESCMID Fungal Infection Study Group (EFISG), ESCMID Study Group for Infectious in Compromised Hosts (ESGICH), European Confederation of Medical Microbiology (ECMM) and the European Respiratory Society (ERS) and European Academy of Allergy and Clinical Immunology (EAACI) were presented at the ECCMID 2014. The levels of evidence and strength of recommendations in 2012 were the same as previous ESCMID guidelines, for example, Candida guidelines [2] and those on managing invasive fungal infections caused by rare and emerging fungi in 2013 [3].

Andrew J Ullmann (University Clinic Würzburg, Germany) presented an overview of the guidelines, which include eight modules: diagnostic procedures, resistance, pharmacokinetics/therapeutics, invasive aspergillosis in children, invasive aspergillosis in haematology, oncology and HCT, invasive aspergillosis in patients without malignancies,

and chronic pulmonary aspergillosis and aspergilloma. Cornelia Lass-Flörl (Innsbruck Medical University, Austria) discussed the recommendations on diagnostic procedures for invasive aspergillosis.

Andreas Groll (University Clinic Münster, Germany) emphasised the similarities and the differences between fungal infections in children and in adults, especially the increased risk of IA in children with acquired immunodeficiencies including haemato-oncology populations (alloHSCT), HIV and chronic granulomatous.

David Denning (University of Manchester, UK) explained that while the optimal duration of therapy in CPA is unknown, indefinite suppressive therapy may be appropriate in selected patients. Alternative antifungals such as micafungin, amphotericin B, liposomal AmB or caspofungin, should be considered in CPA patients with progressive disease, those who fail, are intolerant of antifungal triazoles or who have triazole resistance. These guidelines were open to comments and suggestions after the ECCMID meeting.

IA in kidney transplant patients

Kidney transplant patients who develop invasive aspergillosis (IA) post-transplant have a poor prognosis and increased mortality rate and loss of graft, especially if they received high corticosteroids dose >0.2 mg/kg/day before the onset of IA. This was the conclusion of a 10-year study presented by Anne Claire Desbois (Paris, France) which analysed 17 kidney transplant patients. Desbois proposed the use of galactomannan antigen (GA) and *Aspergillus fumigatus* polymerase chain reaction (aPCR) in combination for an early diagnosis [4].

Anti-mould- prophylaxis in IPA

In the case of anti-mould prophylaxis, not all azoles are alike, as explained by Donald Sheppard (McGill University, Montréal, Canada) [5]. There are differences between the pharmacokinetics and intracellular concentration of voriconazole and posaconazole with plasma C_{max} of voriconazole higher than plasma C_{max} of posaconazole but posaconazole increases its concentration highly inside the alveolar cells. Sheppard described how in animal models of invasive candidiasis and invasive aspergillosis, posaconazole serum concentrations never exceeded the minimum inhibitory concentration (MIC). Cellular posaconazole inhibits *Aspergillus* growth for 30 hours and has a long time post-antifungal effect for several days. Posaconazole accumulates in the intra-cellular membrane at a high concentration so the resistant strains are treated. It is active against TR34/L98H strains and it transfers from host to fungal membrane with a relapsed time of 5 minutes [6]. *Aspergillus*

spores are very hydrophobic and posaconazole is also very hydrophobic with a possible transfer from cells facilitated by hydrophobin. Posaconazole concentration is higher in tissues than in serum.

The results of phase IB study of the pharmacokinetics and safety of posaconazole tablet in patients at risk for invasive fungal infection (IFI) were presented at ECCMID 2014 by Rafael Duarte (ICO-Hospital Duran i Reynals, Barcelona, Spain) [7]. This open-label, uncontrolled, global phase IB study was the first study to use the new oral (tablet) formulation, posaconazole 300mg daily, and included two sequential dosing cohorts: posaconazole tablet 200mg in 20 patients with AML/MDS and 300mg tablet in 30 patients with AML/MDS. The primary endpoint of the study was to determine the posaconazole plasma PK parameters. Patients with a history of type I hypersensitivity or idiosyncratic reactions to azoles, those with moderate or severe liver dysfunction, prolonged QTc interval (>500msec), those who received posaconazole less than 10 days before the start of the study drug administration or have received other antifungals (whether oral, intravenous, inhaled) in the past 30 days for other reasons than antifungal prophylaxis, those who had possible, probable or proven IFI, and those with urine clearance under 30 mL/min were excluded from the study.

Posaconazole 300mg oral tablet achieved target exposures in the agreed range for safety and efficacy in 97 percent of the patients with a steady state C_{avg} between 500 ng/mL and 2500 ng/mL irrespective of food intake while mean posaconazole concentrations exceeded the target of 500 ng/mL after two doses of 300 mg tablets within 24 hours from the start of therapy. The safety profile of posaconazole 300 mg tablet formulation was similar to that of posaconazole oral suspension with no new safety concerns reported.

Oliver Cornely (University of Cologne, Germany) presented the results of the phase III study of the pharmacokinetics (PK) and safety of posaconazole tablet in patients at risk of invasive fungal infection (IFI) at the ECCMID 2014 [8]. This was a single-armed study in 210 high-risk patients with prolonged neutropenia from chemotherapy who had either haematological malignancies such as AML (n=113) and MDS (n=6), or had had an allogeneic haematopoietic stem cell transplant (HSCT; n=91), of which 79 were female and 93 percent Caucasian. Only 186 subjects were considered PK evaluable, of which 107 had AML/MDS and 79 had a HSCT. The loading dose on day 1 was two 300mg posaconazole tablets and then, from day 2 to day 28, one 300 mg tablet/day. The mean duration (SD) of the study was 22.7 days and the median duration was 28 days with 21 patients exposed to the treatment for more than 28 days. This global study was conducted in centres across Europe, North America and Mexico. In the POS 300mg cohort, there was a strong correlation between POS trough concentration (C_{min}) and observed C_{avg} ($R^2 = 0.92$). The distribution of trough

levels on day 8 and beyond showed a steady-state C_{\min} following multiple dosing with a target corridor.

The researchers identified treatment-emerging adverse events (TEAEs) of posaconazole in 207 patients (99 per cent), with severe or life-threatening events in 111 patients (53 per cent); 20 deaths and treatment-related adverse events (TRAEs) were reported in 84 patients (nausea, diarrhoea, abdominal pain). There was no signal or no pattern in the TEAEs or TRAEs or life-threatening adverse events.

Currently available as an oral suspension, posaconazole has been approved by the USA FDA as a tablet formulation, and in February 2014 received positive opinion recommendation from the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA). The tablet is a solid dispersion of posaconazole in a pH-sensitive polymer (HPMCAS) at a one to three weight ratio using a hot melt extrusion process. It has the advantage of the drug being released in the small intestine, and achieves maximum systemic absorption; thus it can be administered at any time of day independent of food intake; the tablet can be stored at room temperature.

Azole resistance in Aspergillus

Azole resistance develops by two mechanisms: during treatment (mutations) and through the environment due to presence of resistant spores in the air. The frequency of azole resistance varies up to 5 per cent among countries. Speaking at the integrated symposium, Paul Verweij (Nijmegen Medical Centre, The Netherlands) explained that 'all azoles are affected by resistance' [9]. Itraconazole resistance has increased while voriconazole is the most active azole.

While resistance to azoles in *Aspergillus fumigatus* has been reported in patients previously treated with azoles, it has been encountered more recently in azole-naïve patients and due to the environment (azoles used in agriculture). It is now thought to involve point mutations in the 14a-sterol demethylase gene (*cyp51A*) and/or increased *cyp51A* expression due to a tandem repeat (TR) promoter alteration. Reports of TR34/L98H mutation were previously encountered in the Netherlands, other Western European countries as well as Iran and China. [10]. Speaking at the ECCMID 2014, Emilie Frealle (University Lille Nord de France, Lille, France) presented the first report of TR34/L98H *A. F=fumigatus* isolates in the North of France and described the resistance to *Aspergillus fumigatus* in 33 azole-naïve patients with COPD [11]. In this study, 31 sputa and 29 environmental isolates were analysed for *Aspergillus fumigatus* by culture and by *Aspergillus* qPCR and using an electrostatic dust fall collector (EDCs). Ten patients had *Aspergillus* colonization by culture and/or qPCR. Two out

of 34 environmental isolates (5.9 per cent) from two different patients had TR34/L98H mutation. The prevalence of TR34/L98H *A. fumigatus* isolates in patient's homes was 6.9 percent and the prevalence in *A. fumigatus* culture positive EDCs was 16.7 percent. However, *cyp51A* mutated isolates were found in environmental samples, justifying the link between the emergence of azole resistance and the use of azoles in agriculture.

Candine resistance of candida

FKS mutations are associated with *Candida* echinocandin (EC) resistance. Researchers from the University of Pittsburgh, USA, have identified intra-abdominal invasive candidiasis (IAC) as an important reservoir for emergence of EC resistance in a study of 256 isolates collected from patients with bloodstream or IAC [12]. An ongoing systematic multicentre study from Denmark found a significant difference between blood and post treatment mucosal isolates in the azole treated patients, and linked to both an emergence of intrinsically fluconazole resistant species but also an increase in fluconazole MICs for *Candida glabrata*. Rasmus Hare Jensen (Statens Serum Institut, Copenhagen, Denmark) commented on the high level of intrinsic or acquired resistance to azoles post-treatment among colonizing *Candida* isolates and patients with candidaemia [13].

Host-immune response in fungal infections

In a keynote lecture organised with the ESCMID Fungal Infection Study Group (EFISG), Emmanuel Roilides, University of Thessaloniki, Greece, discussed the progress made in fighting against fungal infections from the perspective of cells on the front line [14]. He explained that until recently not much was known about fungal immunopathogenesis. *In vitro* and *in vivo* studies have demonstrated that 'all fungi are not the same' as the host-immune response varies for different fungi and moulds due to the influence of immunomodulatory factors. While most studies have concentrated on fungi such as *Candida* and *Aspergillus*, there are other rare fungi that require treatment. Roilides presented the results of a study of posaconazole in combination with GM-CSF on *Scedosporium prolificans*, which is most difficult to treat. Currently, no antifungal has activity against *S. prolificans*. In this study, cells of neutropenic mice that were incubated with the organism were treated *in vivo* with posaconazole in combination with GM-CSF. The combination of posaconazole and GM-CSF caused the highest hyphae damage *in vivo*. However, the results were not confirmed *in vitro* as the combination did not increase survival. Future studies are needed to follow what has been already acted on, for example, using pattern recognition receptors.

Part 2. Antibacterial resistance and antibiotic stewardship

Antibacterial resistance was one of the main topics of ECCMID 2014. In a keynote lecture, Fernando Baquero (Ramón Cajal University Hospital, IRYCIS, Madrid, Spain) addressed this issue from a past and current knowledge and social norms point of view including aetiological thinking - 'the classic simple causal chain in antibiotic resistance', resistance genes and frequent misinterpretation of antibiotic resistance by patients [15]. He thought that prescribers' knowledge on resistance varies from some putting the society first while others had the individual patient at heart. Baquero made the case for antimicrobial resistance being looked at as a global problem that may be solved through resolution of local problems through local solution. He called for 'the beginning of a time of great expectations' including novel strategies to shorten the timelines required to bring a new antibiotic to market, diversification and specialisation of antimicrobial therapy, developing complex antimicrobial therapy for very severe cases and severe epidemiological threats as well as advances in design and reports of trials, analysis of evidence, statistical methods and knowledge engineering.

Antimicrobial stewardship

Multidrug resistance has increased worldwide. To meet this challenge, surveillance programmes such as the Alexander Project [16], the WHO Global Strategy for containment of antimicrobial resistance (AMR) [17] and the WHO response on the benefits of AMR [18], published on April 30, 2014, have been set up. Speaking at an integrated symposium at the ECCMID 2014, Rafael Canton (Ramon y Cajal University Hospital, IRYCIS, Madrid, Spain) emphasised that the programmes focus on the ability of countries with appropriate surveillance systems to detect emerging problems, follow the effects of intervention, collect information and participate in surveillance [19].

E. coli and *Klebsiella pneumoniae* have the highest resistance rates reported worldwide [17]. There are international surveillance studies on AMR, some funded by the pharmaceutical industry - SENTRY, FOCUS, GLOBAL, and other publicly funded and initiated such as EARS-Net, WHONET, NARMS.

SMART (Study for Monitoring Antimicrobial Resistance Trends) is a worldwide surveillance study sponsored by MSD that monitors in vitro susceptibility of Gram negative organisms to identify early changes in the spectrum of microbial pathogens and trends in the antimicrobial resistance patterns in nosocomial and community-acquired intra-abdominal infections [20]. Researchers monitoring AMR for the SMART studies, found a high prevalence of ESBLs in Asia, Latin America and the Middle East and a low prevalence in Africa. However, the

prevalence of ESBLs varies with different species, as shown in the case of intra-abdominal infections [21], and among countries within different geographic areas [22].

Turkey has the highest rate of ESBL positive *E. coli* in Europe. Unpublished data from within the SMART study and presented at the ECCMID 2014 showed the emergence of carbapenem resistance using molecular testing of the isolates. The European Antimicrobial Resistance Surveillance Network (EARS-net) determined the increment of *K pneumonia* carbapenem resistance and the increase in third generation cephalosporins resistant *K pneumonia* [23]. There is a direct correlation between antibiotic use and resistance. Patients who have infections due to multiresistant organisms are treated with more antimicrobials while areas of high antibiotic consumption have increased resistance. In 2013, the ECDC report analysed the pattern of antimicrobial use in acute care hospitals across Europe and found that more antibiotics were used in Southern Europe than in Northern Europe [24]. Canton explained that future surveillance resistance programmes and studies on antimicrobials use will include sequencing technologies to obtain more information.

Role of international collaboration

George Karam (Louisiana State University School of Medicine, New Orleans, Louisiana, USA) emphasised the urgency of international collaborations on antibiotic stewardship with examples of programmes from France where antibiotics are not prescribed automatically, Canada (*Do bugs need drugs?*), the EU (*Antibiotic Stewardship Awareness Day*) and the USA (*Get Smart for Healthcare – Know When Antibiotics Work*) [25].

Major factors influencing the clinical approach to managing resistance include selective antibiotic pressure (polyclonal epidemiology) and clonal or oligoclonal epidemiology. Selective pressure involves minimising the use of unnecessary broad-spectrum activity antibiotics (*Pseudomonas aeruginosa* -sparing antibiotics), prevention of over-the-counter (OTC) purchases of antibiotics and examining the role of generic antibiotics.

The Chennai Declaration created a roadmap to tackle the challenge of antimicrobial resistance including rationing OTC sale of antibiotics and selective use of broad-spectrum antibiotics. However, strategies for infection control such as addressing overcrowding, poor sanitation and hand washing are still essential as well as the selective use of cephalosporins and fluoroquinolones. Karam suggested targets for a global strategy to include risk stratification, combination therapy and optimised dosing.

Risk stratification should include: patient characteristics such as demographics, advanced age, comorbidities, and immunodysregulation; stratification based on types of infection with type 1 being young with no comorbidities and type 3 being prolonged hospitalization; major

interventions and repeat multiple antibiotics. Karam believes that 'anybody could be at risk of infection resistance' due to strong contributions of selective pressure of antibiotics via OTC and non-predictive bioavailability of generic antibiotics in addition to the colonisation of healthy people with antimicrobial resistant organisms.

'It is difficult to contemplate a post-antibiotic era', commented Matteo Bassetti (University Hospital, Piazzale Misericordia, Udine, Italy) [26]. While antimicrobial stewardship is about the right drug at the right time, infection control is about the right dose for the right duration of time. 'Start with the adequate treatment', explained Bassetti. The stewardship approach to carbapenem use includes the use of ertapenem (group 1) as first line antibiotic in complicated community-acquired infections, low risk *Pseudomonas/Acinebacter* infection, intra-abdominal infection, while group 2 drugs include imipenem, meropenem and doripenem for serious hospital-acquired infections.

Virginia Villegas (International Centre for Medical Research and Training (CIDEIM), Cali, Colombia) explained the practical considerations of an antimicrobial surveillance (AMS) programme that included checking the local epidemiological data (for example, local hospitals) and understanding the mechanism of resistance [27]. The epidemiological profiling of one of the 28 hospitals which are part of a network in Columbia identified the five most common micro-organisms involved in antibacterial resistance as being *E coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida* and *Klebsiella pneumonia*. Villegas explained that the hospitals report on epidemiological data with recommendations every six months, which enables the clinicians to distinguish a clonal outbreak from selective pressure of third generation cephalosporins. She emphasised that 'the concept of selective pressure is essential when selecting an antibiotic'. The resistance monitoring programme in the selected hospital resulted in the restriction of ceftriaxone use and a reduction in the *Klebsiella pneumonia* incidence rates as well as in savings of almost 2 million USD.

Key words: antifungal, invasive fungal infection, aspergillosis, antimicrobial stewardship

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