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Diagnosis and Management of Invasive Fungal Infections in Critical Care Setting

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Introduction

The incidence of invasive fungal infections is rising worldwide due to an increase in the numbers of susceptible individuals, increase in the usage of broad-spectrum antimicrobials, immunosuppressive therapies and central vascular devices. Improvement in diagnostics as well as therapeutics has led to improved survival of patients with neoplasms, transplant recipients, HIV/AIDS, in post-trauma and age extremes. The challenge is being dealt with the advent of newer antifungals with lesser toxicity and broader activity spectrum. Also, better diagnostic strategies such as improved radiological imaging and rapid serology tests have provided the caregivers with better tools to detect invasive fungal infections earlier, thus improving outcomes. Newer molecular techniques have been devised which can facilitate specific identification of fungal species thus aiding rapid diagnosis. Despite these improvements, results of therapy remain abysmal and resistance levels to existing antifungal agents are on the rise. The mortality rates related to invasive mycoses have been estimated to be 50% to 60% among ICU patients and increases to 75% to 90% among patients with shock. Invasive fungal infections lead to significant stress on healthcare facilities due to prolonged duration of hospitalisation, use of expensive antifungals and increased utilisation of healthcare resources. Recognition of the interaction between the fungal pathogens and host factors is still a major element in the diagnosis and management of fungal infections. Some fungal diseases have typical presentations but many of these occur so infrequently that clinicians may not initially consider them in their differential diagnoses. In the presence of immunocompromised state, invasive fungal infections may manifest unusual signs and symptoms, making their diagnosis a big challenge. Early diagnosis and prompt therapy is the basis to improving disease outcomes in life-threatening invasive mycoses, especially among immunosuppressed patients. Knowledge of the important risk factors and various clinical manifestations of invasive fungal infections may enable both internists and intensivists to develop an inclusive approach towards timely diagnosis of these infections and guiding appropriate therapeutic response.

Epidemiology

Candida species

Candida spp. is the usual flora associated with mucosal surfaces in humans. In the event of mucosal barrier breakdown or immunosuppression, these organisms may become clinically significant pathogens and can cause fulminant infections leading to increased morbidity and mortality. Candidiasis ranges from infections involving mucosal surfaces to more widespread disease (e.g., pyelonephritis, meningitis/encephalitis, ocular, pneumonitis, endocarditis, intra-abdominal infections/ abscesses, sepsis). Invasive candidiasis is a deep-seated mycosis among critically ill patients and is associated with a mortality rate exceeding 50 - 60% in some ICU settings[1]. An estimation of the exact prevalence of these infections is difficult because of variations in study methodologies, number of healthcare institutes involved and type of patients included. However, some studies cite a prevalence of approximately 7 cases per 1,000 ICU patients. The definitive diagnosis of invasive candidiasis is established when candida is isolated in tissue specimens from normally sterile body sites or if cultured from a normally sterile body fluid. Among critically ill individuals, invasive diagnostic methods are often not feasible, and delays in awaiting culture results can deny patients timely institution of specific antifungal therapy. Delay in specific therapy is commonly associated with adverse clinical outcomes in invasive candidiasis[2].

Candida albicans is the most commonly isolated species but accounts for only about 50% of the *Candida spp.* isolated among both ward and ICU patients. Incidence of non-*albicans* species has recently increased with *C. glabrata* being the second most common species isolated. *C. parapsilosis* is commonly seen in patients with chronic indwelling vascular catheters for delivering total parenteral nutrition. The infection rates of *C. tropicalis*, *C. krusei*, and *C. lusitaniae* have somewhat stabilised but are still considered

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as important pathogens. This epidemiological shift has major therapeutic implications as non-*albicans* species often carry either reduced susceptibility or absolute resistance to fluconazole[3]. *C. auris* has been identified in various regions worldwide as a major cause of drug resistant candidaemia in the ICUs. The haploid genome of *C. auris* is approximately 12.5 Mb with a guanine-cytosine content of almost 45%[4,5]. Genomic studies suggest that there are almost 6,500 to 8,500 nucleotide sequences, with a number of these nucleotides coding for proteins considered as virulence factors in other candida species, such as biofilm formation. Many transporter genes as well as protein kinases, contributing to acquisition of drug resistance, have also been identified[5].

Mould Pathogens

Invasive mould infections caused by *Aspergillus spp*. are not uncommon among critically ill patients. Invasive aspergillosis (IA) was considered to be a disease seen mainly in neutropenic patients and hematopoietic stem cell transplant recipients. It's now understood that IA is also an important pathogen in critically sick patients without neutropenia, such as those receiving immunosuppressant therapy and those suffering from any chronic organ system failure[6].

Aspergillus spp. are commonly implicated in sinonasal diseases or chronic pulmonary diseases and also in the dermatologic or CNS infections. Outbreaks of *Aspergillus* have been usually associated with faulty air filtration, construction work material and even infected medical equipment as the infection usually starts from inhalation of the conidia. Diagnosis of IA is particularly challenging in critical patients because typical radiological features (halo- or air crescent-sign) are not usually seen in non-neutropenic individuals. Such patients do not progress rapidly to angioinvasive disease frequently. IA is associated with significantly higher mortality to the extent of 65% to 85%. Such high mortality figures are not usually driven by severity of underlying disease as study findings depict mortality rates to be similar between individuals with intact immune responses and hematopoietic stem cell transplant recipients with IA[7].

The moulds causing disease among immunocompromised patients are *Cryptococcus spp.*, *Fusarium spp.*, *Scedosporium spp.*, and *Mucormycoses spp*. These infections are relatively less common in ICU settings but are seen more frequently among individuals on long-term immunotherapy for various rheumatological and other chronic diseases[8].

Invasive aspergillosis and mucormycosis have been more frequently seen recently among patients who were infected with SARS-CoV-2 virus and treated with high dose steroids or were diabetics.

High-risk individuals

It is well known that invasive mycoses are not restricted to individuals with immunocompromised health status. Critically sick ICU patients have monocytes and macrophages with impaired activity along with dysfunctional neutrophils that put them at higher risk of such opportunistic microorganisms.

Various risk factors for invasive fungal infections in the ICU setting

Broad-Spectrum Antimicrobial Therapy
Candida spp. colonisation
Indwelling Central Vascular or Urinary Tract Catheters
Steroid use
Uncontrolled Diabetes Mellitus
Hematopoietic Stem Cell Transplant
Solid Organ Transplant
Graft-versus-Host Disease
Immunosuppressive chemotherapy
Necrotising pancreatitis
Structural pulmonary disease
Hepatic failure
Renal failure
Haemodialysis
Major surgery (esp. abdominal)
Malignancy
Major burns injury
Mucosal damage
Neutropenia
Higher illness severity (APACHE II > 20)
Prolonged mechanical ventilation
Prolonged duration of ICU stay
Total parenteral nutrition

The presence of the aforementioned risk factors among ICU patients makes the clinical decision of when to use antifungal therapy pre-emptively more difficult. Various clinical decision tools and risk prediction models which were developed, have not been adequately tested in prospective multi-center trials. These algorithms have inadequate diagnostic applicability due to their poor positive predictive values and tendency towards over-prescription of antifungals.

There is a strong correlation between colonisation of candida and it's disease manifestation. Rate of colonisation increases linearly with the presence of various risk factors as enumerated already. Most patients who develop invasive mycoses are already colonised to some extent, but only

about 10% to 35% of the colonised individuals develop clinical manifestations. The Candida Colonisation Index has been formulated in surgical ICU patients to evaluate the risk of developing IC in colonised subjects. Ratio of the number of colonised anatomical sites to the number of cultured sites, if greater than 0.5 is associated with an increased risk of invasive candidiasis. Utilising this threshold to initiate empiric antifungal therapy substantially decreases the incidence of infection as compared with historical controls. The shortcomings with the usage of this index are its poor positive predictive value (8 - 10%) and the greater use of antifungal agents[9]. Some of the other clinical prediction tools incorporating several of the risk factors into a scoring system have been assessed for their capacity to predict invasive candidiasis. Their positive and negative predictive values have been depicted in Table I.

Advances in diagnosis of invasive fungal infections

Limitations of fungal culture and radiographic methods

Clinical symptomatology/signs, radiological studies, tissue/blood cultures and histopathology are the usual methods for diagnosing invasive fungal infections. These approaches have many shortcomings and cause much delays in the initiation of appropriate therapy. Invasive mycoses often have a protracted clinical course with nonspecific clinical features. Typical radiological signs (halo sign or macronodules) are not always visualised, particularly in immunocompromised individuals, and thus may not be detectable earlier on. These radiological features are too non-specific, resulting in

Table I: Clinical prediction scores for invasive candidiasis.

Score (years)	Patient population	Model risk factor	Cut-off value	Sensitivity/specificity (%)	PPV (%)	NPV (%)
Dupont score (1994)	Surgical ICU peritonitis	Female upper GI tract origin of peritonitis, perioperative cardiovascular failure, antimicrobial therapy at least 48 hours before penritonitis onset	Grade C= at least three risk factors	84/50	67	72
Candida score (2006)	Medical/surgical ICUs for ≥ 7 days	Severe sepsis (2 points), major surgery (1 point), total parenteral nutrition (1 point), multi-focal *candida* colonisation (1 point)	Score ≥ 3	81/74	16	98
Ostrosky rule (2007, 2011)	Medical/surgical ICUs for ≥ 4 days	Major criteria: systemic antibiotic use days 1 - 3, central venous catheter	Major factors	89/38	4	99
			Two major + at factor	66/69	6	98
		Minor criteria surgery, immunosuppressants, corticosteroids, pancreatitis, dailysis, total parenteral mulrition	One major + at least two minor factors	34/90	10	97
		Modified to add mechanical ventilation for at least 48 hours as an additional major criteria	Three major factors + at least one minor factor	50/83	10	97
Nebraska medical Center rule (2011)	Medical/Surgical ICUs for ≥ 4 days	Broad spectrum antibiotics (1.5 points), central venous catheter (0.9 points), and total parenteral nutrition days 1 - 3 (0.9 points), steroid use in the 7 days before ICU admission up to day 3 (0.4 points), adbominal surgery (0.9 points), and pre-ICU length of stay x 0.0039	Score ≥ 2.45	84.1/60.2	4.7	99.4
Candidaemia rule (2015)	All hospitalised patients with culture positive severe sepsis or septic shock	Antibiotics with 30 days, central venous catheter, admitted from nursing home, or total parenteral nutrition (2 points each), transferred from outside hospital or receving mechanical ventilation (1 point each), lung as presumed source of sepsis (subtract 6 points)	Score ≥ 3	87.6/55.9	18.5	97.5

NPV = Negative predicative value, PPV = Positive predictive value.

Information from: Dupont H. Can yeast isolation in peritoneal fluid be predicted in intensive care unit patients with peritonitis ? *Crit Care Med* 2003, 31: 752-6. Leon C. A bedside scoring system ("*Candida score*") for every antifungal treatment in non-neutropenic critically ill patients with *Candida* colonisation. *Crit Care Med* 2006; 34: 730-7. Ostrosky-Zeichner L. Multicenter retrospective development and validation of a clinical prediction rule for nosocomial invasive candidiasis in the intensive care setting. *Eur J Clin Microbiol Infect Dis* 2007; 26: 271-6. Ostrosky-Zeichner L. Improvement of a clinical prediction rule for clinical trials on prophylaxis fof invasive candidiasis in the intensive care unit. *Mycoses* 2011; 54: 46-51. Hermsen ED, Zapapas MK, Maiefski M *et al*. Validation and comparison of clinical prediction rules for invasive candidiasis in intensive care unit patients: a matched case-control study. *Crit Care* 2011, 15: R198; and Vasques Gullamet C, Vazquez R, Micek ST *et al*. Development and validation of a clinical prediction rule for candidiaemia in hospitalised patients with severe sepsis and septic shock. *J Crit Care* 2015; 30: 715-20.

inappropriate clinical decisions. Fungal blood culture is the gold standard for the diagnosis of invasive candidiasis but has only 50% sensitivity for candida detection and rarely grows any moulds. Blood cultures do not detect deep seated infections and have significant time lag till results are obtained.

Rapid diagnostic tests

Rapid diagnostic tests may aid in the diagnosis of invasive fungal infections before the signs of infection develop. These modalities have reasonable sensitivity and specificity over usual methods and can be utilised in combination with the various risk assessment models to help guide empirical antifungal therapy to target a particular organism. These tests are explained in detail in Table II.

The β-D-glucan diagnostic test is an assay detecting activation of the coagulation cascade by β-D-glucan. It has a good negative predictive value of about 80%, thus making it a valuable tool to avoid inappropriate antifungal use[10].

The positive predictive value of this test is reported to be 30%, when a cut-off of two consecutive tests greater than 80 pg/ml was used[11]. The recommended cut-off value in a single test result is greater than 80 pg/ml and in two consecutive test results, greater than 60 pg/ml, if serial monitoring is done. Values greater than 150 pg/ml for a single test and greater than 80 pg/ml for two consecutive testing have been suggested for critically ill patients. Two consecutive results (twice within a week) above this value are recommended to enhance the diagnostic accuracy of the test.

Mannan is specific to *Candida spp.* and is a polysaccharide component of the fungal cell wall. Latex agglutination and enzyme immunoassay methods exist for both mannan antigen (Mn) and anti-mannan antibodies (Anti-Mn). These tests are more specific than the β-D-glucan test, but not as sensitive and do not become positive until later in the course of the disease. It is seen that the sensitivity of these tests vary based on the *Candida spp.*, with the highest sensitivity reported for *C. albicans* and the lowest for *C. parapsilosis* and *C. krusei*[12].

Galactomannan is a specific assay for *Aspergillus*. The positive predictive value of this assay is relatively weak in non-neutropenic ICU patients and solid organ transplant recipients. The optimal cut-off value is 0.5 depending upon test optical density. Non-neutropenic patients may show false negative test result because of the slow progression to angioinvasive disease[6,7,13]. False-positive results usually occur when administering β-lactams (piperacillin/tazobactam) or Plasma-Lyte[14]. The test can be performed from bronchoalveolar lavage specimens, which tends to increase both the sensitivity and specificity over serum values.

Detection of fungal nucleic acids by polymerase chain reaction is another method to diagnose invasive mycoses. The test allows for the rapid diagnosis of candidaemia and is better than fungal culture in isolating nonviable organisms. It is reported to have a high sensitivity (96%) and specificity (97%) among ICU patients[15].

A major shortcoming of fungal cultures is the long time lag

Table II: Rapid diagnostic tests for invasive fungal infections.

Test	Application	Sensitivity %	Specificity %	Limitations
β-D-glucan	*Candida* spp. and Aspergillus	57 - 97	56 - 93	False-positive: glucan-contaminated tubes/gauze, cellulose-containing dialysis membranes/filters, contaminated albumin/IVIG with fungal elements, gram-positive infections, gut inflammation, some antibiotics (amoxicillin-clavulanic acid) Controversy surrounding optimal cut-off value
Mannan antigen/ Anti-Mannan antibody	*Candida* spp. only	Mannan: 58 Anti-Mannan: 59 Combination: 83	Mannan: 93 Anti-Mannan: 83 Combination: 86	Positive results occur later in disease course Sensitivity varies depending on species Best results when used together Cut-off value unclear
Nucleic-acid PCR	All Species, but only available currently for *Candida* spp.	96	97	Using test too early may decrease sensitivity Unavailable for many organisms
Galactomannan	Aspergillus and some other molds	Serum: 71 BAL: 76 - 88	Serum: 89 BAL: 87 - 100	False-positive: b-lactams, Plasma-Lyte Not as sensitive in non-neutropenic patients

IVIG = Intravenous immunoglobulin, PCR = Polymerase chain reaction.

Information from: Leon C. What's new in the clinical and diagnostic management of invasive candidiasis in critically ill patients. Int Care Med 2014; 40: 808-19; and Perfect JR. Fungal diagnosis. how do we do it and can we do better ? *Curr Med Res Opin* 2013; 29: 3-11.

to positivity. Post-isolation, it takes many days for speciation and susceptibility testing. Molecular-based identification methods like peptic nucleic acid fluorescence in situ hybridisation (PNA-FISH) differentiate between common candida species within a few hours only[16]. Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) detects candida directly from whole blood specimens, aiding in rapid diagnostics[17].

Antifungal susceptibility testing

Antifungal susceptibility testing (AST) is of utmost importance in defining resistance patterns and in aiding appropriate drug selection and timely de-escalation of antifungal therapy. Clinical breakpoints for *Candida spp.* and selected azoles are described as susceptible, susceptible-dose dependent, and resistant (Table III). Suggested breakpoints are based on pharmacokinetic-pharmacodynamic (PK-PD) relationships and show close correlation with disease outcomes.

Table III: Antifungal susceptibility breakpoints for *Candida* spp.

Antifungal agent	Secies	Susceptible (mcg/ml)	Susceptible-dose dependent (mcg/dl)	Resistant (mcg/ml)
Fluconazole	*C. albicans* *C.parapsilosis* *C. tropicalis*	≤ 2	4	≥ 8
	C. glabrata	n/a	≤ 32	≥ 64
	C. krusei	n/a	n/a	n/a
Posaconazole	All *candida* spp.	n/a	n/a	n/a
Voriconazole	*C. albicans* *C. parapsilosis* *C. tropicalis*	≤ 0.12	0.25 - 0.5	≥ 1
	C. glabrata	n/a	n/a	n/a
	C. krusei	≤ 0.5	1	≥ 2
Antifungal Agent	**Species**	**Susceptible (mcg/ml)**	**Intermediate (mcg/ml)**	**Resistant (mcg/ml)**
Anidulafungin	*C. albicans* *C. tropicalis* *C. krusei*	≤ 0.25	0.5	≥ 1
	C. parapsilosis *C. guilliermondii*	≤ 2	4	≥ 8
	C. glabrata	≤ 0.12	0.25	≥ 0.5
Caspofungin	*C. albicans* *C. tropicalis* *C. krusei*	≤ 0.25	0.5	≥ 1
	C. parapsilosis *C. guilliermondii*	≤ 2	4	≥ 8
	C. globrata	≤ 0.12	0.25	≥ 0.5
Micafungin	*C. albicans* *C. tropicalis* *C. krusei*	≤ 0.25	0.5	≥ 1
	C. parapsilosis *C. guilliermondil*	≤ 2	4	≥ 8
	C. glabrata	≤ 0.06	0.12	≥ 0.25

n/a = not applicable. Information from: Clinical and laboratory standards institute M27-S4.

A definitive dose: MIC relationship for azole therapy has not been established from research data till date. Absence of correlation studies makes it impossible to evaluate appropriate therapeutic options for drugs with susceptible-dose dependent activity, which require higher than usual doses. Susceptibility testing and clinical outcome has not been established for voriconazole to *C. glabrata* and posaconazole to any *Candida spp*. Clinical breakpoints do not exist for *C. krusei* to fluconazole because of intrinsic resistance. The newer breakpoints are now described as susceptible, intermediate and resistant (Table III). These drug breakpoints have been derived primarily from trials in non-neutropenic patients[18].

Antifungal resistance

Detection of resistance between identifiable species is now possible with the availability and increased use of antifungal susceptibility testing. The resistance rates for most fungal species are increasing gradually. Drug resistance in treatment-naive patients is even more disconcerting. This change is a result of selective pressure from increased antifungals usage in the prophylaxis of immunocompromised individuals; increased pre-emptive and empiric use, particularly in ICU patients because of poor diagnostics; inappropriate use of antifungals in the community for treating even minor fungal infections and rampant use of agricultural fungicides.

C. albicans is only rarely resistant to fluconazole therapy (less than 5% of isolates). Antifungal resistance to other *Candida spp.* is rising, with prevalence rates around 10% for several species. Intrinsic drug resistance of some *Candida spp.* (e.g., *C. krusei*, *C. auris*) to fluconazole is well recognised. Approximately 25% to 30% of candidaemia cases involve intrinsically resistant species, and prior use of antifungals is the most common risk factor for selecting these pathogens.

Resistance acquired during treatment is more difficult to predict and remains to be well defined. Acquired resistance has been studied during treatment of *C. glabrata*, particularly with fluconazole. These species are often cross-resistant to other azoles and may even display multi-drug resistant phenotypes. Acquired resistance to echinocandins is also described in individuals receiving antifungals for longer durations[19].

Mechanisms of drug resistance seen in various fungal microbes include genetic mutations, induction of efflux pumps and increased expression of genes encoding for these mechanisms. Biofilms are a major cause of

resistance in *Candida spp.* because of poor penetration of azoles into these complex cellular matrices. *Aspergillus* forms biofilms in pulmonary parenchyma as well as cavities that contribute to the difficulty in eradicating these infections. The usual resistance mechanisms of each of the drug classes are described in the following Table IV[20].

increases the incidence of drug resistance and leads to fluconazole-resistant species selection, resulting in breakthrough drug resistant colonisation and clinical infections. Prophylactic therapy should not be substituted for proper infection control practices, particularly with indwelling vascular as well as urinary catheters.

A retrospective study was conducted in a surgical ICU in

Table IV: Common antifungal resistance mechanisms.

Drug class	Site of action	Resistance mechanism	Implications
Azoles	Inhibit lanosterol-14a-demethylase ERG11 Candida CYP51 Aspergillus	Up-regulation of efflux pump ABC transporters/CDR1, CDR2 genes TAC1 transcription factors Up-regulation of efflux pump MFS transporters/MDR1 gene MRR1 transcription factors ERG11 and CYP51 mutations ERG11 and CYP51 overexpression ERG3 inactivation Biofilm formation Increase in cell wall chitin content	Decrease drug entry into cell (all azoles) Decrease drug entry into cell (fluconazole) Decrease binding affinity, increase MIC Counteract drug effects Ergosterol replaced by another sterol (cross-resistance all azoles) Inhibit drug pentration Increase tolerance to drug
Echinocandins	Inhibit Fksp catalytic subunit of (1,3)-β-D-glucan synthase	FKS1 and FKS2 mutation Increase in cell wall chitin content	Alter catalytic capacity, increase MIC (cross-resistance to entire class) Increase tolerance to drug, paradoxical growth May correlate better with response to therapy than actual MIC
Polyenes	Bind ergosterol Induce oxidative stress	ERG2, ERG3, ERG5, ERG6, ERG1 mutations Increase in anti-oxidative enzymes Alteration in production of free radicals	Decrease ergosterol biosynthesis Decrease oxidative stress

ABC = ATP-binding cassetle; MFS = Major facilitator superfamily..

Information from Spampinato *C. Candida* infections, causes, targets, and resistance mechanisms. Traditional and alternative antifungal agents. *Biomed Res Int* 2013; 204237; Cuenca-Estrella M. Antifungal drug resistance mechanisms in pathogenic fungi: from bench to bedside. *Clin Microbiol Infect* 2014; 20 (Suppl 6): 54-9; and Maubon D. Resistance of *Candida* spp. to antifungal drugs in the ICU: where are we now ? *Int Care Med* 2014; 40: 1241-55.

Approach to invasive candidiasis treatment

Prophylactic therapy

Guidelines from Infectious Disease Society of America (IDSA) for the management of invasive candidiasis support a prophylactic approach to prevent disease in high-risk individuals. Many single-centre studies have suggested that use of prophylactic fluconazole therapy in ICU patients reduces the occurence of invasive candidal infections by about 40 - 50%, however, the approach had doubtful mortality benefit due to inconsistent results and the wide variations in the study cohort. Initiating prophylactic therapy with fluconazole for a large number of ICU patients

France to assess colonisation trends over an 8-year duration and found a substantial increase in the acquired *C. glabrata* colonisation and a decline in *C. parapsilosis* colony clearance in a cohort where 13% of the subjects received prophylactic fluconazole therapy for significant candidal colonisation[21]. The IDSA guidelines (2016) recommend instituting prophylactic therapy with fluconazole only in those individuals who have a 10% or higher risk of infection on risk prediction score assessment. Prophylactic antifungal therapy has been shown to decrease the incidence of intra-abdominal candidiasis in a particular high-risk group including those who are undergoing intra-abdominal surgery with recurrent anastomotic leaks.

Empirical antifungal therapy

Empirical antifungal therapy must be considered in critical ICU patients with risk factors for invasive mycoses and unidentified cause of pyrexia. The decision should be based on evaluation of risk factors, surrogate clinical markers for fungaemia and culture isolates from nonsterile anatomic sites *(strong recommendation; moderate-quality evidence)*. Empirical therapy must be initiated at the earliest in patients with risk factors and having features of septic shock *(strong recommendation; moderate-quality evidence)*. Preferable empirical therapy for suspected candidiasis in non-neutropenic critical patients in ICU is an echinocandin (caspofungin: loading dose of 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose of 200 mg, then 100 mg daily) *(strong recommendation; moderate-quality evidence)*. An acceptable alternative for patients with no prior exposure to azoles and not colonised with azole-resistant Candida species is fluconazole in 12 mg/kg loading dose, followed by 6 mg/kg maintenance dose *(strong recommendation; moderate-quality evidence)*. Lipid formulation Amphotericin B is an option if there is intolerance to the preferred antifungal agents *(strong recommendation; low-quality evidence)*. Recommended duration of empirical therapy for suspected invasive candidiasis in patients who show clinical improvement is 2 weeks, similar to documented candidaemia *(weak recommendation; low-quality evidence)*. For patients who show no improvement to empirical therapy after 4 - 5 days and with no documented evidence of invasive candidiasis after starting empirical therapy, should be considered for discontinuation of antifungal therapy *(strong recommendation; low-quality evidence)*[22].

Pre-emptive antifungal therapy

Screening of high-risk patients before or as soon as the symptoms appear by using diagnostic markers is the basis of this strategy. Screening limits the unwarranted exposure to antifungals but detects patients earlier in the disease course. Similar to prophylactic therapy, the difficulty lies in selecting target patients. In the INTENSE study[23] micafungin was compared to placebo for pre-emptive therapy in high-risk surgical patients with intra-abdominal source of infections. It failed to depict any variation in the incidence of invasive mycoses. Also, there was no difference in the mortality rates or any improvement in organ dysfunction. A study assessing the incidence of resistant *Candida spp*. in subjects with intra-abdominal candidiasis with recent exposure to echinocandins, found the abdomen to be a reservoir of resistant *Candida spp*.[24]. This study found FKS mutant *Candida spp.* in around 25% of individuals with an overall echinocandin failure rate of almost 50%, which explains the lack of utility with micafungin in the INTENSE study.

An approach for empiric/pre-emptive antifungal therapy in suspected invasive candidiasis is outlined below in Fig 1.

Therapeutic strategies for patients with invasive fungal infections

Candida infections

Treatment for candidaemia in non-neutropenic patients[22] recommendations:

1. An echinocandin (caspofungin: 70 mg loading dose followed by 50 mg daily maintenance; micafungin: 100 mg per day; anidulafungin: 200 mg loading dose followed by 100 mg daily maintenance) should be initiated as starting therapy *(strong recommendation; high-quality evidence)*.
2. Fluconazole, 12 mg/kg loading dose followed by 6 mg/kg daily maintenance, is an alternative to echinocandins as starting therapy in patients who are not critically ill and who are not likely to have fluconazole-resistant Candida isolates *(strong recommendation; high-quality evidence)*.
3. Azole sensitivity testing is recommended for all bloodborne and clinically important Candida isolates. Echinocandin sensitivity testing should be done in all patients who have received prior therapy with echinocandins and among those who have been infected with *C. glabrata* or *C. parapsilosis (strong recommendation; low-quality evidence)*.
4. Switchover from echinocandins to fluconazole (mostly within 5 to 7 days) is recommended for patients who have been stable and have specimen isolates sensitive to fluconazole (e.g., *C. albicans*), or are sterile on repeat cultures following administration of antifungal therapy *(strong recommendation; moderate-quality evidence)*.
5. In infections due to *C. glabrata*, switchover to high-dose fluconazole, 12 mg/kg daily; or voriconazole, 3 - 4 mg/kg twice daily; should be considered in patients with fluconazole-sensitive or voriconazole-sensitive isolates *(strong recommendation; low-quality evidence)*.
6. Lipid formulation amphotericin B (3 - 5 mg/kg daily) is an alternative if there is intolerance or resistance to other antifungal agents *(strong recommendation; high-quality evidence)*.
7. Switchover from Amphotericin B to fluconazole is suggested after 5 - 7 days in clinically stable patients who have isolates susceptible to fluconazole and in whom repeat cultures on antifungal therapy have been sterile *(strong recommendation; high-quality evidence)*.

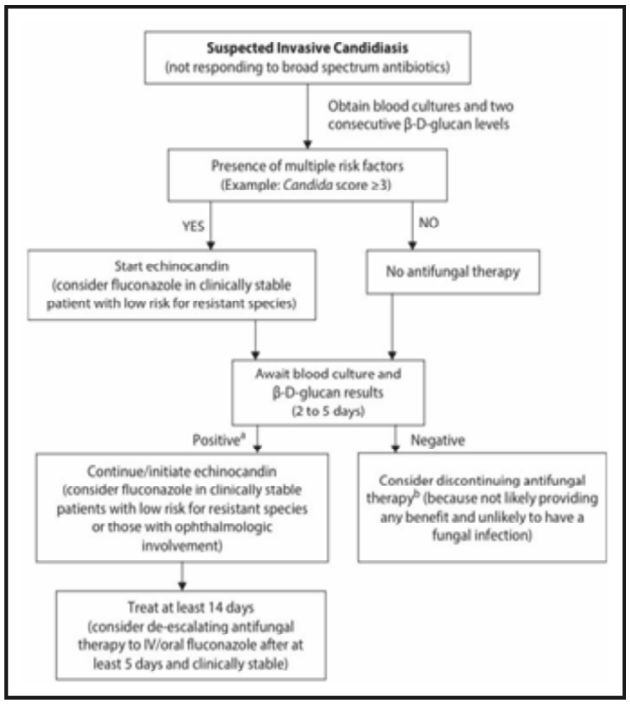

Fig. 1: *General approach to preemptive/empiric antifungal therapy.*

[a]Positive b-D-glucan diagnostic test result is two consecutive tests > 80 pg/ml.

[b]If clinically improving on antifungal therapy, then consider a short course of therapy for no more than 7 days.

Information from: Blot S, Charles PE. Fungal sepsis in the ICU: are we doing better? Trends in incidence, diagnosis, and outcome. *Minerva Anestesiol* 2013; 79: 1396-405.

8. In patients with suspected azole- and echinocandin-resistant Candida infections, lipid formulation amphotericin B is recommended *(strong recommendation; low-quality evidence).*
9. Voriconazole is recommended as step-down oral therapy for selected cases of candidemia due to *C. krusei (strong recommendation; low-quality evidence).*
10. All non-neutropenic patients with invasive candidiasis should have a dilated ophthalmological examination by an expert ophthalmologist, within first week of diagnosis *(strong recommendation; low-quality evidence).*
11. Follow-up blood cultures should be done daily or on alternate days to define the reference point at which candidaemia has cleared *(strong recommendation; low quality evidence).*
12. Recommended duration of therapy for invasive candidiasis without metastatic complications is 2 weeks after documented clearance of organisms from the bloodstream and resolution of clinical features related to infection *(strong recommendation; moderate-quality evidence).*
13. Central vascular catheters (CVCs) must be removed at the earliest in the course of infection when the source is presumed to be the CVC; the decision must be customised for each individual *(strong recommendation; moderate-quality evidence).*

Treatment for candidaemia in neutropenic patients[22] recommendations:

1. An echinocandin (caspofungin: 70 mg loading dose followed by 50 mg daily maintenance; micafungin: 100 mg per day; anidulafungin: 200 mg loading dose followed by 100 mg daily maintenance) should be initiated as starting therapy (strong recommendation; moderate-quality evidence).
2. Lipid formulation amphotericin B (3 - 5 mg/kg daily) is an alternative if there is intolerance or resistance to other antifungal agents (strong recommendation; moderate-quality evidence).
3. Fluconazole, 12 mg/kg loading dose followed by 6 mg/kg daily maintenance, is an alternative to echinocandins as starting therapy in patients who are not critically ill and who are not likely to have fluconazole-resistant Candida isolates *(weak recommendation; low-quality evidence).*
4. Fluconazole, 6 mg/kg daily, is a useful option for stepdown oral therapy in clinically stable patients with persistent neutropenia, who have sensitive isolates and definitive bloodstream clearance *(weak recommendation; low-quality evidence).*
5. Voriconazole, 6 mg/kg twice daily for 2 loading doses followed by 3 - 4 mg/kg twice daily maintenance therapy, can be utilised where additional mould coverage is required *(weak recommendation; low-quality evidence).* Voriconazole can also be used as an oral step-down option in clinically stable patients with neutropenia, who have definitive bloodstream clearance and established voriconazole sensitivity *(weak recommendation; low-quality evidence).*
6. In *C. krusei infection*, echinocandins, lipid formulation Amphotericin B or voriconazole is recommended *(strong recommendation; low-quality evidence).*
7. The minimum duration of therapy recommended for invasive candidiasis without metastatic complications is 2 weeks after established Candida clearance from the bloodstream, alongwith resolution of neutropenia and clinical features related to invasive candidiasis *(strong recommendation; low-quality evidence).*
8. In neutropenic patients, sources of infection other than

vascular access devices (e.g., gastrointestinal tract) predominate. Removal of such infected invasive devices must be done promptly on an individual basis *(strong recommendation; low-quality evidence)*.

9. Granulocyte colony-stimulating factor (G-CSF) transfusions can be considered in cases of persistent candidaemia with predictable prolonged neutropenia *(weak recommendation; low-quality evidence)*.

Treatment for chronic disseminated candidiasis[22] recommendations:

1. Initiation of therapy with lipid formulation Amphotericin B or an echinocandin for several weeks, is recommended. It is followed by oral fluconazole, 6 mg/kg daily, for patients not likely to grow fluconazole resistant isolate on culture *(strong recommendation; low-quality evidence)*.
2. Treatment must be continued till lesions resolve on repeated imaging, can take several months. Premature termination of antifungal therapy commonly leads to relapse *(strong recommendation; low-quality evidence)*.
3. If hematopoietic cell transplantation or anticancer chemotherapy is warranted, it should not be delayed due to the presence of disseminated candidiasis, and antifungal therapy should be continued throughout the period of high-risk to prevent recurrence *(strong recommendation; low-quality evidence)*.
4. In patients with incapacitating persistent fever, brief duration (1 - 2 weeks) therapy with nonsteroidal anti-inflammatory drugs and/or steroids must be considered *(weak recommendation; low-quality evidence)*.

Treatment for intra-abdominal candidiasis[22] recommendations

1. Source control must be achieved in all cases with intra-abdominal candidiasis, with appropriate drainage and/or debridement *(strong recommendation; moderate-quality evidence)*.
2. Choice of antifungal agent is the same as for the treatment of invasive candidiasis or empiric therapy for non-neutropenic patients in the ICU *(strong recommendation; moderate-quality evidence)*.
3. Duration of therapy has to be defined by adequacy of source control and clinical resolution of clinical features *(strong recommendation; low-quality evidence)*.

Treatment of Candida isolates from the tespiratory tract[22] recommendations

Candida growth from respiratory secretions mostly denotes colonisation and seldom requires therapy with antifungal agents *(strong recommendation; moderate-quality evidence)*. Isolation of candida from the respiratory tract of critically ill patients is usual but the occurrence of pneumonia from these organisms is uncommon due to innate mechanisms of defence within the lungs. Clinical decision to treat must be based on evidence of invasive disease or host factors indicating an increased risk of infection with a different source. Definite host factors include neutropenia, hematopoietic stem cell transplant, immunosuppressive therapy, steroids and severe immunodeficiency. Most of the available antifungals penetrate the lung well and are viable options.

Treatment for Candida intravascular infections, including endocarditis and infections of implantable Cardiac devices[22] recommendations

1. Lipid formulation Amphotericin B with or without flucytosine, 25 mg/kg 4 times daily, OR high-dose echinocandins (caspofungin 150 mg daily, micafungin 150 mg daily, or anidulafungin 200 mg daily) are recommended as initial therapy for native valve endocarditis *(strong recommendation; low-quality evidence)*.
2. Fluconazole, 6 - 12 mg/kg daily, is recommended as step down therapy for patients who have sensitive candida isolates with clinically stable condition and have candida clearance from the bloodstream *(strong recommendation; low-quality evidence)*.
3. Oral voriconazole, 3 - 4 mg/kg twice daily, or posaconazole, 300 mg daily, are considered as step-down therapy for isolates that are susceptible to these agents but resistant to fluconazole *(weak recommendation; very low-quality evidence)*.
4. Replacement of affected valve is recommended and drug therapy must be continued for at least 6 weeks after surgery and for an even longer duration in subjects with perivalvular abscesses and other related complications *(strong recommendation; low-quality evidence)*.
5. In patients who are not suitable candidates for valve replacement, long-term suppressive therapy with fluconazole is recommended in drug sensitive cases *(strong recommendation; low-quality evidence)*.
6. In prosthetic valve endocarditis, similar regimens to native valve endocarditis are recommended (strong recommendation; low-quality evidence). Chronic suppressive antifungal therapy with fluconazole is recommended to prevent relapse *(strong recommendation; low-quality evidence)*.

7. In case of pacemaker and implantable cardiac defibrillator infections, whole of the device must be removed at the earliest *(strong recommendation; moderate-quality evidence)*. Antifungal therapy is similar as that recommended for native valve endocarditis *(strong recommendation; low quality evidence)*.
8. Four weeks of antifungal therapy is recommended for infections limited to generator pockets after device removal *(strong recommendation; low-quality evidence)*.
9. Six weeks of antifungal therapy is recommended for infections involving the wires after wire removal *(strong recommendation; low-quality evidence)*.
10. Antifungal regimen is the same as that recommended for native valve endocarditis for ventricular assist devices that cannot be removed *(strong recommendation; low-quality evidence)*. Chronic suppressive therapy with fluconazole is recommended if the isolate is susceptible, for as long as the device remains in place *(strong recommendation; low-quality evidence)*.

Treatment for central nervous system Candidiasis and other yeasts[22] recommendations

1. Liposomal Amphotericin B, 5 mg/kg daily, with or without oral flucytosine, 25 mg/kg 4 times daily is recommended for initial treatment *(strong recommendation; low-quality evidence)*.
2. Fluconazole 6 - 12 mg/kg daily is recommended for step-down therapy after the patient has responded to initial therapy *(strong recommendation; low-quality evidence)*.
3. Treatment must be continued until all the clinical features alongwith CSF and radiological abnormalities have resolved completely *(strong recommendation; low-quality evidence)*.
4. Infected CNS devices, including ventriculostomy drains, shunts, stimulators, prosthetic reconstructive devices, and biopolymer wafers that deliver chemotherapy must be removed whenever possible *(strong recommendation; low-quality evidence)*.
5. In patients where ventricular device cannot be removed, Amphotericin B deoxycholate must be given through the device into the ventricle at doses ranging from 0.1 mg to 0.5 mg in 2 mL of 5% dextrose *(weak recommendation; low-quality evidence)*.

Treatment for urinary tract infections due to Candida species[22] recommendations

1. Treatment of asymptomatic candiduria with antifungals is NOT warranted unless the subject belongs to a category at high risk for dissemination; patients at high risk include neutropenic patients and patients undergoing urological instrumentation *(strong recommendation; low-quality evidence)*.
2. Patients undergoing urological instrumentation must be treated with oral fluconazole, 6 mg/kg daily, OR Amhotericin B deoxycholate, 0.3 - 0.6 mg/kg daily, both before and after the instrumentation *(strong recommendation; low-quality evidence)*.
3. In symptomatic ascending candida pyelonephritis, oral fluconazole 3 - 6 mg/kg daily for 2 weeks is recommended for fluconazole susceptible isolates *(strong recommendation; low-quality evidence)*.
4. In fluconazole-resistant *C. glabrata*, Amphotericin B deoxycholate, 0.3 - 0.6 mg/kg daily for 7 days with or without oral flucytosine, 25 mg/kg 4 times daily, is recommended *(strong recommendation; low-quality evidence)*.
5. For *C. krusei*, Amphotericin B deoxycholate, 0.3 - 0.6 mg/kg daily, for 1 - 7 days is recommended *(strong recommendation; low quality evidence)*.
6. Removal of urinary tract obstruction is strongly recommended *(strong recommendation; low-quality evidence)*. Patients with nephrostomy tubes or DJ stents in situ should be considered for device removal or replacement, if possible *(weak recommendation; low-quality evidence)*.

Invasive mould infections

Prophylactic and empirical therapy

The guidelines for management of infections due to invasive moulds in critical patients are largely derived from clinical studies involving treatment of haematological malignancies. Lipid formulations of Amphotericin B remain the most widely considered for empiric therapy in the setting of an unidentified invasive mould and in subjects with history of recent azole therapy. Voriconazole is suggested as first-line therapy in infections with *Aspergillus*. The echinocandins are also active against *Aspergillus*. But only caspofungin has been approved for this indication. Prophylactic treatment in immunocompetent or non-neutropenic patients cannot be strongly recommended based on current clinical studies. Empirical therapy must be instituted even in those without traditional risk factors, at the earliest clinical suspicion for invasive aspergillosis (IA). There is no well-defined duration of therapy, although a prolonged course is usually required.

Combination antifungal therapy

Combination therapy for invasive aspergillosis is an alternative for rescue therapy in individuals unresponsive to a single agent or with breakthrough symptoms while on therapy. Approximately 25 - 35% of critical patients in ICUs are suspected to have resistant mycotic infections, and meta-analysis of various studies has shown that around 45 - 55% of patients are usually administered combination antifungal therapy. Usual regimens include two agents with dissimilar action mechanisms, like an echinocandin (acting on fungal cell wall) with either amphotericin B (acting on fungal cell membrane) or an azole. Because of the high probability for antagonism, both amphotericin B and azole together are not used in combination therapy. A randomized clinical study that compared the combination of voriconazole with anidulafungin versus voriconazole alone established a tendency towards decreased mortality in haematopoietic stem cell transplant recipients with combination antifungal therapy[25].

COVID-19 associated pulmonary aspergillosis (CAPA)

COVID-19 associated pulmonary aspergillosis (CAPA) has been reported to worsen the disease course of COVID-19, resulting in increased mortality. Usual risk factors are corticosteroid therapy or anti-interleukin 6 therapy usually given for cytokine release syndrome. Also, the initially reported few cases have been found to be azole-resistant. The first line recommended therapy is either voriconazole or isavuconazole. But in azole-resistant CAPA, liposomal amphotericin B is the drug of choice. CAPA is defined as possible, probable or proven, based on sample validity and thus diagnostic certainty. Radiological findings are not sufficient to define patients with CAPA, but multiple pulmonary nodules or lung cavitation should prompt thorough investigation for CAPA in COVID-19 patients. Frequently observed radiological features of invasive pulmonary aspergillosis, such as the halo sign, are not sufficient to define CAPA without mycological evidence. This feature is insufficient because the halo sign suggests local infarction, and can be seen in severe COVID-19 due to endothelialitis and thrombosis. Fungal biomarkers have poor predictive value in diagnosing CAPA as compared to invasive pulmonary aspergillosis. Histopathological or direct microscopic detection of fungal hyphae, showing invasive growth with associated tissue damage; or aspergillus recovered by culture or microscopy or histology or PCR obtained by a sterile aspiration or biopsy from a pulmonary site, showing an infectious disease process are the only ways to conclusively prove CAPA.

Voriconazole treatment (loading dose 6 mg/kg twice a day for two doses, followed by 4 mg/kg twice a day) has a better outcome than does treatment with amphotericin B deoxycholate. However, liposomal amphotericin B can be considered for initial therapy if, epidemiologically, drug-resistant patterns support this treatment, before the results of susceptibility testing for voriconazoles are available.

Isavuconazole (loading dose 200 mg three times a day for six doses, followed by 200 mg once a day, 12 - 24 h after the last loading dose) has similar clinical efficacy to voriconazole but less hepatotoxicity and neurotoxicity and decreased risk of corrected QT-interval prolongation. Posaconazole has excellent *in-vitro aspergillus* activity and has been successfully used as salvage treatment in patients without COVID-19. Duration of therapy recommended is upto 6 to 12 weeks depending upon the clinical response[26].

COVID-19 associated mucormycosis (CAM)

Mucormycosis is a medical emergency even when clinically suspected. During COVID-19 pandemic, there have been various reports of mucormycosis among patients with COVID-19, especially in those who are diabetic or those who have received steroids. Also, patients who received empirical voriconazole therapy were found to be at an increased risk for getting mucormycosis. COVID-19 associated mucormycosis (CAM) has resulted in high morbidity and mortality, exorbitant treatment costs and shortage of antifungal drugs. There are two usual clinical presentations: rhino-orbito-cerebral mucormycosis (ROCM) and pulmonary mucormycosis.

ROCM may manifest as nasal blockade or congestion, nasal discharge (bloody or brown/black), local pain, facial pain or numbness or swelling, headache, orbital pain, toothache, loosening of maxillary teeth, jaw involvement, blurred or double vision with pain; paresthesia, fever, skin lesions, thrombosis or necrosis (eschar).

Pulmonary mucormycosis may manifest as fever, cough, chest pain, pleural effusion, haemoptysis, worsening of respiratory symptoms. Lung CT may be confused with COVID-related shadows; suspect mucormycosis in patients with thick-walled lung cavity (need to differentiate from covid-associated pulmonary aspergillosis), reverse halo sign, multiple nodules, pleural effusion (not usually seen in aspergillosis). Also, there are repeated negative galactomannan and beta-glucan tests in CAM.

Suspected patients should undergo appropriate radio-imaging study: MRI paranasal sinuses with brain contrast study for ROCM, CT thorax for pulmonary mucormycosis. Histopathological examination of biopsy (endoscopic or CT-guided) specimen from affected tissues/sites, and staining with hematoxylin-eosin, periodic acid-Schiff stain or Grocott-Gomori's methanamine-silver stain reveals aseptate or pauci-septate hyphae (ribbon like) which are irregularly branching at 90^0 angle, indicates mucorales.

Surgical debridement and antifungal therapy are the mainstay of treatment and usually require a multi-disciplinary approach. Liposomal amphotericin B (5 mg/kg/day), diluted in 5% dextrose may be given over 2 - 3 hours infusion. Slow escalation should be avoided and higher doses upto 10 mg/kg/day may be given with brain involvement. In patients who are intolerant to amphotericin B, alternative agents like posaconazole or isavuconazole may be used. Posaconazole: 300 mg twice a day on first day, followed by 300 mg once a day. Check posaconazole trough level after 7 days of therapy and avoid interacting drugs. Isavuconazole: 200 mg three time a day for two days, followed by 200 mg once a day. Isavuconazole fared better than posaconazole in mucormycosis in various clinical studies. After 3 to 6 weeks of amphotericin B therapy, consolidation therapy with either posaconazole or isavuconazole should be given for atleast 3 to 6 months or as per clinical response[27].

Pharmacological aspects of antifungal agents

Amphotericin B

Amphotericin B is the most important therapeutic option for treatment of invasive fungal infections among critically ill patients. It has wide spectrum anti-fungal activity against a variety of fungal microbes causing infections in ICU patients. Amphotericin B is recommended for disseminated infections in immunocompromised patients, in CNS involvement, or when resistance to other antifungal agents is seen. Amphotericin B deoxycholate used to be the main formulation available before the advent of three newer adjuvant products (i.e., amphotericin B lipid complex, liposomal amphotericin B and amphotericin B colloidal dispersion). All three formulations have similar efficacy but significantly favourable toxicity profile than the parent compound. The liposomal product has lesser nephrotoxicity compared to the other two lipid-based products. Nephrotoxicity can be mitigated with all the amphotericin B formulations by appropriate hydration of the patient with a normal saline loading (250 - 500 ml) prior to each administered dose and by avoiding concomitant use of other nephrotoxic drugs, particularly diuretics. Nephrotoxicity can also be prevented by using continuous drug infusions but should be avoided because the concentration-dependent pharmacodynamics of amphotericin B must not be compromised. Lipid formulations of amphotericin B, except amphotericin B colloidal dispersion, have more than 50% reduced rate of infusion-related reactions than deoxycholate formulation. Infusion-related adverse events can be reduced further with the use of diphenhydramine and acetaminophen 30 minutes before starting the infusion. Other noteworthy reactions defined with the liposomal products are flushing, retrosternal chest heaviness, hypoxia, flank pain and urticarial rashes. Amphotericin B usage is commonly associated with hypokalaemia and hypomagnesaemia. Amphotericin B binds to the fungal cell wall ergosterol, thereby altering its permeability; tissue binding of the drug may also occur in mammalian renal cells and cause potassium loss. Regular electrolyte monitoring and correction are recommended.

Echinocandins

The first available echinocandin was approved by the FDA in 2001 and changed the entire approach to management of disseminated fungal infections. Echinocandins have a unique mechanism of action specific to the fungal cell wall. They have fungicidal activity against *Candida* species and fungistatic activity against moulds, and are also active against the biofilms. They are not recommended for treatment of fungal urinary tract infections as they are not well excreted in urine, thus having poor drug concentration there. Adverse effects associated with the echinocandins are usually benign, with few reports of hepatotoxicity and infusion related reactions. The infusion reactions are mostly histamine-mediated and are similar to the red-man syndrome usually seen with vancomycin. The echinocandins do not have any significant interactions with the hepatic CYP enzymes; thus, drug interactions are negligible. Caspofungin and micafungin are reported to increase serum levels of tacrolimus and cyclosporine but dose reductions are not usually required; monitoring of serum drug concentrations is useful.

Extended-spectrum triazoles

These agents offer increased activity against many *candida* and other yeasts, alongwith a variety of moulds. Availability of these agents has enhanced the therapeutic options for management of invasive mycoses by providing an effective oral alternative. Oral absorption of triazoles is modified depending upon how they are administered. Voriconazole should be given before meals as presence of food reduces absorption by almost 25%. On the contrary, posaconazole must be given with high-fat meals. Gastric acid also increases the absorption of posaconazole. Therefore, concomitant gastric acid suppression therapy must not be used alongwith posaconazole therapy. Proton pump inhibitors should also be avoided. There is lack of recommendations for dose adjustment with these agents; thus, therapeutic drug monitoring (TDM) may be required for ensuring clinical efficacy.

These agents may cause liver toxicity, adrenal suppression, and QT_c prolongation. Also, significant visual disturbances occur with use of voriconazole. Both oral as well as intravenous therapy cause visual impairments but are usually transient, with patients adjusting to them within 1 - 2 weeks

of therapy. These vision issues are typically-related to the initiation and temporal administration of the drug and have been described as bright flashing lights or hallucinations.

The intravenous formulation of voriconazole contains a second-generation cyclodextrin-solubilising agent. First generation cyclodextrins were reported to cause nephrotoxicity and accumulate in renal failure. It is recommended to use oral voriconazole in patients with creatinine clearance below 50 ml/min.

Clinical interaction with CYP hepatic enzymes is seen with all azoles, with voriconazole and posaconazole being strong inhibitors of CYP3A4. It can lead to significant increase in the serum levels of tacrolimus, sirolimus and cyclosporine. The interaction with sirolimus is totally unpredictable and warrants frequent concentration monitoring, if the combination cannot be avoided. Drug interactions may occur in critical patients receiving triazoles with excessive exposure to midazolam, fentanyl, phenytoin, steroids, warfarin and quetiapine.

Voriconazole is a moderate inhibitor of CYP2C19. It must be used cautiously in combination with strong inhibitors or inducers of CYP2C19 enzyme, like rifampin, and with drugs metabolised by CYP2C19, like clopidogrel. Non-linear pharmacokinetics make voriconazole dose adjustments difficult in the setting of complex critical care regimen. Concomitant administration of azoles with other QT_c - prolonging drugs must be monitored strictly or altogether avoided.

The FDA approved isavuconazonium sulfate for the treatment of patients with invasive aspergillosis and mucormycosis in 2015. Isavuconazonium is a prodrug which is hydrolysed to isavuconazole, the active form which can be dosed once a day. Isavuconazole is reported to be noninferior to voriconazole in the treatment of invasive aspergillosis and mucormycosis resistant to other antifungal agents. The spectrum of activity includes *C. glabrata* (including fluconazole-resistant strains) and *C. krusei*, alongwith Cryptococcus, Coccidioides, Blastomycoses and Histoplasmosis. Isavuconazole is available in an intravenous formulation which does not contain the cyclodextrin excipient, but it does require an in-line filter. Oral formulation is also available, but these capsules cannot be opened and administered via nasogastric tube. Isavuconazole is a moderate inhibitor of CYP3A4 enzyme. The drug interactions are clinically not relevant with isavuconazole as compared to other azoles. Adverse effects are mostly gastrointestinal in the form of nausea, vomiting, diarrhoea, constipation. Hypokalaemia, hepatotoxicity, shortened QT_c interval and infusion reactions are reported in clinical trials. Longer half-life makes management of drug interactions and adverse effects difficult.

An inhibitor of β-(1,3)-glucan synthase is biafungin which is a novel long-acting echinocandin under development. It's long half-life should allow for a once a week dosing and can be used against drug resistant fungal isolates.

Dosing considerations

Inappropriate dosing of antimicrobials is not uncommon in the ICU due to pharmacokinetic-pharmacodynamic variations in critical patients (e.g., increment in distribution volume and increased drug filtration). It commonly leads to increased resistance, treatment failure and poor clinical outcomes. Fluconazole is commonly under-dosed by omission of loading doses and by prescribing fixed (400 mg) versus weight based (6 mg/kg) dosing.

Another important factor in the alteration of kinetics of antifungal agents is obesity. Obese patients have larger variations in the distribution volume of drugs. Both fat as well as lean body mass are more in obese patients, and the blood flow is decreased to the adipose tissue. Such individuals have reduced hepatic/splanchnic blood flow and metabolism due to the hepatic fat infiltration. Also, metabolism of CYP3A4 is reduced in obese patients leading to disturbances in therapeutic drug concentration. Renal clearance increases with increased lean body mass, leading to greater renal clearance of these drugs. As amphotericin B formulations do not get distributed into the adipose tissue, they should be dosed as per lean body weight. On the other hand, fluconazole should be dosed at the higher end of the dose range, based on total body weight. Dosing of voriconazole and posaconazole should be based on lean body weight, whereas dosing should be increased by upto 25% to 50% for echinocandins.

Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) helps in guiding antifungal therapy in a more effective manner. Although routine monitoring is not recommended in all cases, there may be numerous clinical scenarios where TDM is very important, especially in the critical patients.

When to consider therapeutic drug level monitoring[28]

Extremes of age
Questionable compliance
Multiple drug interactions
Concomitant use of acid suppressing therapy
Malabsorption
Morbid obesity
Extensive or disseminated disease (e.g., CNS, Mediastinal)
Multiple organ dysfunction
Renal replacement therapy
Prophylaxis in high-risk patients
Use of ECMO or cardiopulmonary bypass

Monitoring of therapeutic concentration levels of antifungal drugs is mainly restricted to the triazole class which covers moulds (i.e., itraconazole, voriconazole, and posaconazole) and flucytosine. The target trough levels, serum sampling timings and recommendations for dose modifications for these drugs are listed in Table V. Monitoring of concentrations of amphotericin B or the echinocandins is not warranted. Monitoring is also not routinely suggested for fluconazole; although, it is considered when the MIC of the pathogen is increased, in CNS disease, or in patients requiring renal replacement therapy.

Itraconazole demonstrates non-linear pharmacokinetics with wide variations in the oral absorption because of changes in the formulations (30% higher AUC with the oral solution vs. capsules), food, and gastric pH. Therefore, monitoring is warranted in most patients receiving this agent to ensure adequate absorption.

Non-linear pharmacokinetic variability with voriconazole is mainly due to genetic polymorphisms of the CYP2C19 hepatic enzyme and saturable hepatic metabolism. There is also evidence suggesting that the FDA-approved non–weight-based fixed dose oral formulations of voriconazole could be insufficient to achieve effective serum concentrations. It is of prime concern in obese patients and those with active disease, in whom the maximal recommended dose may need to be exceeded, thus warranting monitoring.

Posaconazole TDM is recommended in most patients receiving the suspension because of poor bioavailability from saturable absorption and reduced absorption in the setting of mucositis, graft-versus-host disease, the concomitant administration with acid-suppressing therapies, or administration without a high-fat meal. Drug levels are reported to be suboptimal in 50% of subjects receiving posaconazole suspension for fungal prophylaxis in few studies.

Flucytosine is a pyrimidine analogue used in combination with amphotericin B to treat cryptococcal meningitis. TDM is needed due to its toxicity profile, significant intra- and inter-patient pharmacokinetic variability, dependence on renal elimination where nephrotoxicity is common, and high-risk of developing resistance. Evidence from pharmacodynamics studies indicates a significant exposure-toxicity correlation with a higher incidence of bone marrow suppression and hepatotoxicity seen at peak concentrations exceeding 100 mg/l. Maintaining concentrations above the MIC for at least 50% of the dosage interval is associated with improved clinical outcomes and may prevent the emergence of resistance. It is recommended that peak concentrations be performed to prevent toxicity and minimise the risk of resistance.

Antifungal stewardship

Antifungal stewardship consists of coordinated efforts for surveillance and guidance of appropriate usage of antifungal agents (in terms of both the choice of the selected agent as well as the correct dosage) in order to achieve the best possible clinical results and reduce the incidence of

Table V: Recommendations for therapeutic drug monitoring.

Drug	Minimum target Concentrations[a]	Timing of Concentrations[b]	Concentrations Associated with toxicity	Strategies to increase low concentrations
Itraconazole	P: 0.5 mg/l T: 0.6 - 1 mg/l	7 - 14 days	≥ 17 mg/l[c]	Change to solution Avoid acid suppressants with capsules Take solution in fasting state Increase dose from 200 mg twice daily to 300 mg twice daily
Voriconazole	P: > 1 mg/l T: > 1 mg/l Trough: MIC of 2 - 5	Within 7 days[d]	> 5.5 mg/l	Increase dose: IV: up to 6 mg/kg twice daily PO: up to 300 mg twice daily
Posaconazole[e]	P: 0.35 mg/l P: > 0.7 mg/l	At 48 hours Within 7 days	Unknown	Increase total daily dose to 800 mg Administer total daily dose divided four times daily Switch to the delayed-release tablets Avoid acid suppressants Take with food or high-fat supplement
Flucytosine	T: Peak 20-40 mg/l	Within 72 hours	Peak >100 mg/l	Increase dose by 50%, use caution due to toxicity

[a]Trough concentrations measured using high performance liquid chromatography (HPLC)/mass spectrometry unless otherwise specified. [b]Time listed is the number of days after the initiation of the therapy. [c]Concentration measured with bioassay, would expect 5-fold lower concentration with HPLC/mass spectrometry. [d]Repeat level may be necessary because of fluctuations in concentrations due to Michaelis-Menten kinetics. [e]Recommendations are for oral solution only.

IV = Intravenous; P = Prophylaxis; PO = Oral; T = Treatment.

Information from Ashbee HR. Therapeutic drug monitoring of antifungal agents: guidelines from the British Society of Medical Mycology. *J Antimicrob Chemother* 2014; 69: 1162-76.

selective errors and adverse events. Antifungal utilisation has gradually increased over time in conjunction with an increase in the number of immunosuppressed individuals at high-risk for invasive mycoses. Difficulty in diagnosis of invasive fungal infections leads to delays in institution of specific therapy, and subsequently worse clinical outcomes. There is also emerging data correlating prior antifungal exposure and suboptimal dosing to emergence of antifungal resistance. Antimicrobial stewardship programs must constitute a multi-disciplinary bundle based approach to ensure appropriate utilisation of antifungals via post-prescription review, feedback and prior authorisation from the infectious disease specialist. Institutional guidelines should also be formulated to guide diagnostic testing in high-risk individuals; appropriate selection, dosing, and duration of the antifungal agent; therapeutic drug monitoring, if warranted; and opportunities for de-escalation and stepping down of therapy.

Conclusion

Critical components in the management of invasive fungal infections that are clinician mitigated include (1) prompt antifungal therapy, (2) risk factor analysis to identify patients at greater risk than the usual ICU population for IFI and therefore in need of prophylactic or pre-emptive therapy given the current lack of prompt accurate diagnostics, (3) choice of the appropriate antifungal agent and dosing regimen, and (4) source control. Currently, until a species diagnosis or susceptibility is known, an echinocandin is the recommended first-line therapy for most of the patients with IFI. PK/PD studies suggest that the currently recommended regimens would be useful for most infections. Once the fungal speciation is done for *C. albicans*, *C. parapsilosis* or *C. tropicalis* and if the patient is responding to initial therapy, the appropriate therapy would be to step down to fluconazole. For other species, the therapy should be directed based on susceptibility profile.

Key words: *Fungal Sepsis, Invasive Mycoses, Candidaemia, Aspergillosis, Mucormycosis, Antifungal Stewardship.*

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