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Editors' welcome

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In this issue, we are happy to feature presentations from the Medical Mycology Training Network (MMTN) Malaysia Conference 2019 held in Penang, Malaysia, in July 2019. The conference was a remarkable 2-day affair, with an excellent gathering of mycologists, clinicians, laboratory technicians and other medical mycology professionals from Malaysia.

Our esteemed AFWG Board Members generously shared their expertise through presentations, and clinical and diagnostic mycology workshops on challenging cases. In the following pages we feature some of the interesting sessions, including discussions on controversies and common mistakes in basic and clinical mycology, and interpreting *Candida* in different specimens.

We are also looking forward to the first International Society for Human and Animal Mycology (ISHAM) Asia Congress, jointly organized by ISHAM and AFWG. The event will assemble the world's leading experts from 18 countries to provide global updates in medical mycology, and will be another fantastic opportunity to network with like-minded professionals in the region and showcase your latest research. Register and submit your abstract now at www.ISHAMAsia.com and see you in Bangkok, Thailand, on 7–9 May 2020!

As always, please visit www.AFWGonline.com to keep up with our latest activities, or drop us a message on Facebook, Instagram or LinkedIn. Thank you!

Five controversies in mycology

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1. Who should receive antifungal prophylaxis?

Studies have demonstrated the benefit of antifungal prophylaxis in high-risk patients with hematologic diseases. For instance, posaconazole was shown to prevent invasive fungal diseases (IFD) significantly more effectively than fluconazole or itraconazole, and improved overall survival in neutropenic patients undergoing chemotherapy for acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).[1] Posaconazole, voriconazole, fluconazole and itraconazole were similarly found to be beneficial as antifungal prophylaxis in high-risk patients after allogeneic hematopoietic stem cell transplantation (HSCT).[2-4] In HSCT recipients, although these agents showed efficacy to prevent IFD, they did not seem to have survival benefit according to several clinical studies. Data regarding cost-effectiveness of antifungal prophylaxis in patients with hematologic diseases or after HSCT in resource-limited settings or developing countries are lacking and should be considered before universal implementation.

Recent data have also shown that new treatment strategies in chronic lymphoproliferative disorders (CLD) have increased the risk of IFD.[5] Given these findings, patients with CLD could possibly be considered eligible for antifungal prophylaxis. However, drug-drug interactions between azoles and vincristine are a major obstacle in this issue. More data are needed before generalized recommendations can be made.

The underlying disease is not the only factor that places a patient at high risk of IFD; risk modulators that should also be considered include transplant-related factors, environmental factors, treatment and even the organism itself (Figure 1).[6]

2. Should we use antifungal prophylaxis in all high-risk individuals?

Dr Chayakulkeeree discussed considerations for using antifungal prophylaxis:

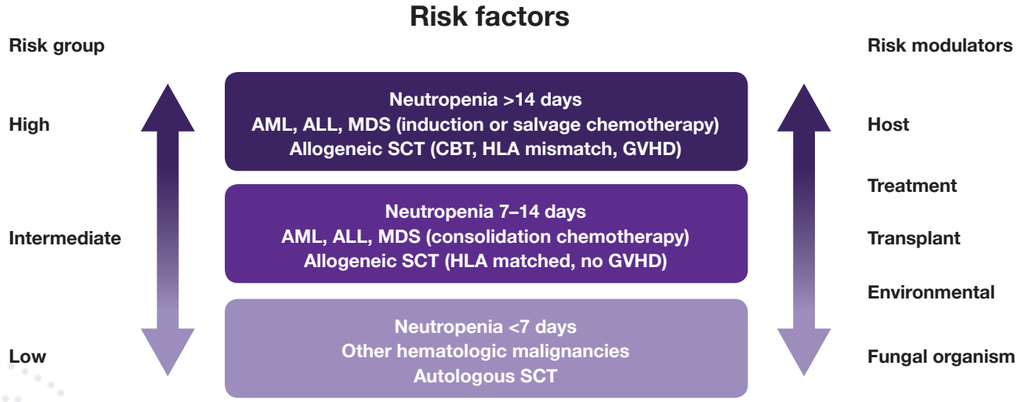
- Only in high-risk patients
- Mold-active agents should be used – ECIL-5 guidelines strongly recommend posaconazole as antifungal prophylaxis in allogeneic HSCT recipients with graft-versus-host disease (GVHD) and in those with AML/MDS undergoing chemotherapy[7]
- Epidemiology of IFD in hematologic patients in each country
- Cost-effectiveness
- Choice of diagnostic tools – Mold-active antifungal drugs have been shown to decrease the sensitivity of the *Aspergillus* galactomannan enzyme immunoassay[8]
- Ability to deal with breakthrough infection – Treating a breakthrough infection in a patient already given antifungal prophylaxis entails using another antifungal class that could be more expensive and have more adverse effects

3. Which is better in neutropenic patients: pre-emptive or empirical antifungal therapy?

Pre-emptive antifungal therapy, based on galactomannan enzyme immunoassay and high-resolution computed tomography (HRCT), has been shown to reduce the rate of antifungal use and yielded a 12-week survival rate of 63.6% for patients with IFD.[9]

In another trial that compared empirical versus pre-emptive antifungal therapy for high-risk, febrile neutropenic patients, pre-emptive treatment decreased treatment costs by 35%, but was associated with more IFD than empirical treatment. Survival rates were similar between the two groups.[10]

Figure 1. Risk groups for invasive fungal infections[6]



ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CBT, cord blood transplant; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; MDS, myelodysplastic syndrome; SCT, stem cell transplantation

Some limitations of the pre-emptive diagnosis-driven antifungal approach must be noted: availability of the galactomannan assay (twice a week); availability of HRCT within 24 hours when requested; false-positive and false-negative galactomannan results; and a biomarker for candidiasis may need to be included.

4. Should we suggest empirical antifungal therapy in high-risk ICU patients?

The León score[11] and the Ostrosky-Zeichner score[12] were designed to identify patients at high risk of invasive candidiasis in the ICU. However, both these scores are not highly sensitive and may not be reliable predictors of invasive candidiasis.

In the EMPIRICUS study of patients with ICU-acquired sepsis, *Candida* colonization and multiple organ failure, empirical treatment with micafungin did not increase fungal infection-free survival compared with placebo.[13] As such, empirical antifungal treatment in high-risk ICU patients cannot be strongly recommended at this time.[14] In some circumstances, for patients' safety, empirical antifungal treatment in high-risk ICU patients with sepsis may be considered but need to be modified or discontinued upon availability of fungal culture and susceptibility results. Therefore, development of rapid and accurate non–culture-based diagnostic tests is crucial to facilitate appropriate empirical antifungal treatment in ICU patients.

5. What is the place for combination antifungal therapy?

The combination of amphotericin B and flucytosine was shown to be effective as induction therapy for HIV-associated cryptococcal meningitis, and was noninferior to 2 weeks of amphotericin B-based therapy.[15]

However, combination antifungal therapy is still controversial in other fungal infections, such as invasive candidiasis, invasive aspergillosis and mucormycosis, and more data are needed to demonstrate the clear role of combination antifungal therapy in these infections.[16]

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How do I interpret *Candida* in the urine?

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Candiduria in patients without an indwelling catheter

With a *Candida*-positive urine culture, clinicians must always differentiate colonization from infection. A clean-catch urine sample should be repeated for culture. If the second culture is sterile, no treatment is necessary. If the same *Candida* spp. is isolated from a symptomatic patient, it is considered significant; symptomatic and persistent candiduria indicates an infection. However, it remains a diagnostic challenge in critically ill or paraplegic patients. Under the circumstances of unexplained fever, candiduria may be considered significant in the critically ill.

Presence of pyuria does not always indicate *Candida* urinary tract infections (UTI). In fact, a quarter of patients with funguria have concomitant bacteriuria.[1] Unlike bacterial UTI, in candiduria, quantitative urine cultures do not reliably predict infection.[2]

Managing candiduria in patients with an indwelling urinary catheter

Candida colonization of urinary tract is expected with prolonged catheterization. Studies have shown that candiduria may resolve if the urinary catheter is removed (35–40%) or replaced (20%).[3] However, if candiduria persists and the patient remains febrile after removal or replacement of catheter, and there is no other obvious source of infection, antifungal treatment may be considered.

Does colonization predict invasive candidiasis?

With prolonged ICU stay, 80% of critically ill patients were found to be colonized with *Candida* spp.[4] Nevertheless, only 1–8% of colonized patients develop candidemia.[3] Colonization does not always culminate in infection, hence antifungal treatment is mostly not required. Empirical treatment may be needed in symptomatic patients with multiple sites of *Candida* colonizations and with significant risk factors for invasive candidiasis.[3]

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How do I interpret *Candida* in respiratory tract cultures?

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Candida in the human microbiota

Candida is part of the human microbiota of the respiratory tract. As such, *Candida* can be cultured from the mouth of people with and without pneumonia, and appears rapidly in the lower respiratory tract (LRT) in patients admitted to the ICU.[1] Unfortunately, there is still no culture-based or molecular test of respiratory specimens that can distinguish between *Candida* contamination, colonization and invasive disease.[2]

Significance of *Candida* in the lower respiratory tract

An observational study found no relationship between *Candida* spp. in the LRT and invasive candidiasis. Moreover, there was also no link between *Candida*-dominated LRT microbiota and intra-hospital or 30-day overall mortality.[1]

A prospective study that assessed cultures of immediate postmortem lung biopsies showed extensive *Candida* colonization throughout the lungs in patients with and without pneumonia.[3] *Candida* pneumonia is extremely rare in ICU patients, as shown by a prospective study of autopsies performed on patients who died in the ICU: there was no histopathologically proven case of *Candida* pneumonia found in patients with pneumonia on autopsy and a *Candida*-positive airway culture prior to death.[4]

Treating patients with *Candida* in the respiratory tract

There is no benefit to treating patients with *Candida* in the airways. Results from a retrospective study showed that in critically ill patients with pulmonary *Candida* spp. colonization, antifungal therapy did not affect the incidence of new pneumonia or mortality.[5] A randomized, placebo-controlled trial similarly demonstrated that antifungal therapy did not have a significant impact on hospital/ICU length of stay and mortality in critically ill patients with *Candida*-positive airway secretions.[6]

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10 common mistakes in laboratory mycology

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Pre-analytical errors

Mistake 1: Improper collection and processing

Proper sample collection is crucial for efficient laboratory diagnosis. For instance, samples from dermatophyte infections must be collected from the margins instead of the center of a lesion. For mycetomas, a simple swab collection will not yield a good reading. Granules must be collected from the serosanguinous sinus discharge or from just under the surface of a scab covering a sinus. Granules can also adhere to the dressing, so this can be sent to the laboratory as well.

Mistake 2: Incorrect transport conditions

The table below shows some recommendations for proper transport of specimens.

Specimen	Transport conditions
Sputum	Sterile screw-capped container
Bronchoscopy fluid	Sterile screw-capped container
Cerebrospinal fluid	If a delay is anticipated, specimen should be left at room temperature
Urine	If a delay beyond 2 hours is anticipated, refrigerate at 4°C
Blood	Biphasic agar broth bottles designed especially for fungal cultures or automated culture bottle
Tissue biopsy	Specimen should not be frozen or allowed to dehydrate prior to culture

Analytical errors

Mistake 3: Lack of awareness or a low index of suspicion

Clinicians may not always suspect fungal infections and may ignore sending samples to mycology laboratory. However, a vigilant laboratory person may pick up fungal infections while processing a sample for the desired test of the clinician. For example, a clinician suspecting a patient of tuberculosis (TB) sends samples for mycobacterial smear and culture. An expert laboratory person may detect *Cryptococcus* or *Histoplasma* spp., which produce similar clinical presentations. Similarly, laboratory personnel should be knowledgeable enough to accept or disregard any laboratory observations. For example, blood culture that grows all mycelial fungi may not be contaminant. They could be *Fusarium* or *Scedosporium* spp. isolated from the blood. Timely communication between laboratory staff and clinicians is important in the optimal management of fungal infections.

Mistake 4: Lack of expertise in the laboratory

Training of mycologists and the technical staff is essential to prevent errors in identifying fungi microscopically (eg, knowing the difference between real fungi and artifacts, contamination, etc).

Mistake 5: Errors involving blood cultures

It is important for laboratory staff to be familiar with handling blood cultures, particularly in centers with no commercial blood culture system. After sub-culture on solid medium, Professor Chakrabarti recommends incubating the medium for at least 48 hours and examining it macroscopically for pin-point yeast growth, which may otherwise be missed.

Mistake 6: Errors in identification

An overdependence on commercial systems may lead to errors in identifying fungi, as in the case of *Candida auris*. It has been shown that *C. auris* cannot be reliably identified by standard biochemical identification systems because the organism is not in their databases.[1] Updating the database of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems may help in proper identification. Histopathologic identification of fungi in tissue sections and cytologic preparations can also be prone to error.[2] Fungal identification is challenging even for the best laboratories, according to Professor Chakrabarti.

Mistake 7: Errors in antifungal susceptibility testing

Most laboratories now use the commercial Vitek 2 system for antifungal susceptibility testing of yeasts. However, its results may not perfectly correlate with results using the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution reference methods. Several factors can affect CLSI/EUCAST results, such as errors in testing media, incubation times, etc. Any resistance detected by commercial systems may be confirmed by standard micro-broth dilution technique.

Mistake 8: Errors involving galactomannan and beta-D-glucan assays

Mistakes can occur during:

- Blood collection (contamination, collecting from an intravenous line, collecting <5 days post-dialysis)
- Serum separation (hemolysis should be avoided, use of calibrated centrifuge at optimum speed, serum transfer in biosafety cabinet)
- The test itself (should be done only in biosafety cabinet, plasticware should be sterile and pyrogen-free)

Mistake 9: Errors in therapeutic drug monitoring (TDM)

Inappropriate medical indication, sample collection, method and interpretation can affect TDM results. For instance, in collecting samples, the clinician or laboratory staff must know the correct timing of collecting a peak sample and a trough sample.

Post-analytical errors

Mistake 10: Errors in interpretation

Not all positive findings require treatment, so mycologists should be familiar with guideline recommendations to know when to treat or report fungal findings. For instance, the Infectious Diseases Society of America (IDSA) guidelines state that if *Candida* is found in respiratory secretions or urine, antifungal treatment is not recommended unless true infection is proven.[3]

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10 common mistakes in clinical mycology

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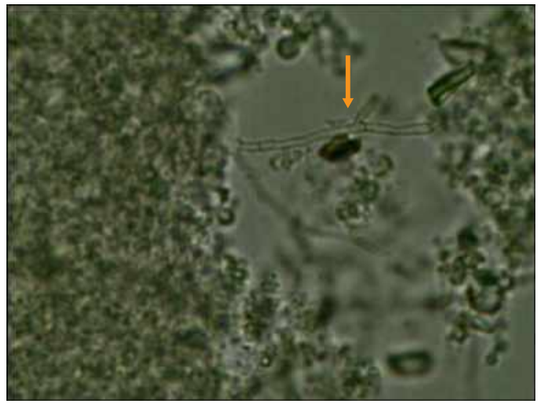
Mistake 1: Improper collection and processing

Clinicians overlook the value of direct microscopic examination from a clinical sample and fail to send a request to the microbiology laboratory for direct microscopic examination with common stains, including wet preparation potassium hydroxide (KOH) or calcofluor white stains. Direct microscopic examination is cheap and gives a quick result, which is useful in initiating early therapy with turnaround time of around 30 minutes. Fungal morphology on direct microscopic exam also provide valuable information to clinicians for initiating appropriate antifungal treatment. Figure 2 shows KOH preparation from brain abscess aspirate as an example.

Figure 2. KOL preparation from brain abscess aspirate



Mistake 2: Not requesting fungal cultures from biological samples

When clinicians do not suspect an invasive fungal infection, they can neglect to request for a fungal culture. For instance, a patient with fever of unknown origin, constitutional symptoms, and weight loss with lymphadenopathy along with adrenal enlargement (Figure 3) could be suspected of having TB in countries where TB is common like India, but this could also be a case of histoplasmosis. The patient's clinical profile and imaging results could raise the index of suspicion. A diagnosis of histoplasmosis would be missed unless the histopathologist identifies yeast in the tissue, and on hematoxylin and eosin (H&E) stain (Figure 4). Histoplasmosis is a great mimic of TB.

Figure 3. CT scan showing adrenal enlargement

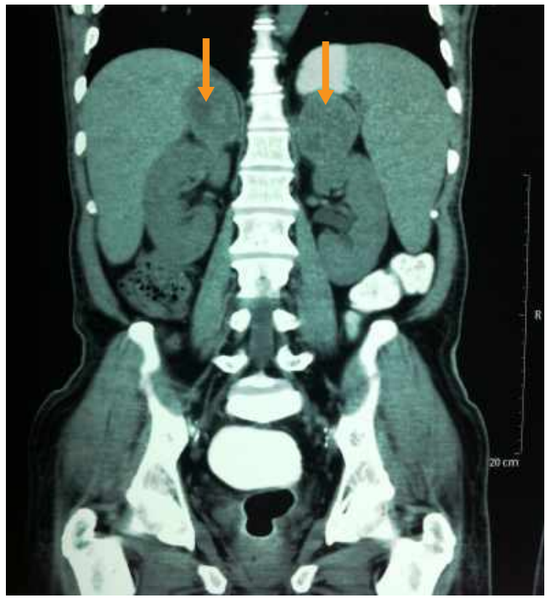
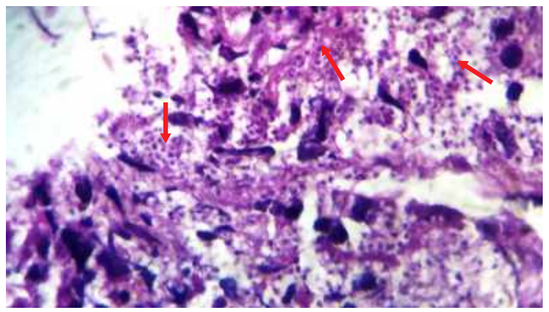


Figure 4. Histopathologic examination of adrenal biopsy showing multiple yeast



Mistake 3: Not being aware of drug-drug interactions

Drug-drug interactions can affect the efficacy of antifungal agents and some drug-drug interactions can be life-threatening. For instance, co-administration of rifampicin with voriconazole, fluconazole and caspofungin can significantly reduce the plasma concentrations of these antifungals, resulting in loss of antifungal activity. Concomitant use of H2 blockers or proton pump inhibitors can significantly decrease the plasma concentration of itraconazole, resulting in treatment failure. Azole antifungal agents could also significantly increase the plasma concentrations of amiodarone, which can lead to serious cardiac arrhythmias and sudden cardiac death. Similarly, azoles increase warfarin exposure and the concomitant use can lead to life-threatening bleeding.

Mistake 4: Using nephrotoxic agents concomitantly

Clinicians must be careful to avoid adding other nephrotoxic drugs in patients, particularly those in the ICU, already receiving amphotericin B. Giving aminoglycosides or other nephrotoxic agents together with amphotericin B could potentially result in severe nephrotoxicity, and renal function should be monitored frequently.

Mistake 5: Failing to adjust antifungal dosage according to renal replacement therapy

In patients with renal failure and unstable hemodynamics, the dosage of fluconazole may have to be adjusted regularly. This is a common situation in patients receiving intermittent hemodialysis. For example, an ICU patient with acute kidney injury and creatinine clearance of 10–50 mL/min requires 50% of the fluconazole dosage. During the ICU course when the patient needs hemodialysis, fluconazole needs to be administered at full calculated dose after hemodialysis. Dose should be halved on the days when the patient is not receiving hemodialysis. Hence, dose adjustment in such situation is a continuous process. Higher fluconazole dosage is required along with drug level monitoring in patients who are receiving continuous venvenous hemofiltration.

Mistake 6: Not monitoring QTc in ICU patients on azole antifungals

Clinicians need to be careful when managing critically ill patients receiving azoles and who also have concomitant hypomagnesemia or are also taking quinolones, clarithromycin and other drugs with potential effect on QTc prolongation. These patients could develop arrhythmias or sudden cardiac death. Electrocardiogram (ECG) monitoring should be the standard of care in ICU patients with multiple risk factors for QTc prolongation.

Mistake 7: Not practicing TDM

TDM is a very important component of antifungal therapy in patients with invasive fungal infections and should be encouraged particularly for patients receiving voriconazole, posaconazole and itraconazole. Some clinicians believe that simply using the recommended weight-based dosage of antifungals is enough, and run the risk of treatment failure or drug toxicities. TDM ensures that patients receive the maximum benefit of antifungal treatment.

Mistake 8: Using posaconazole oral suspension as first-line treatment for mucormycosis

Mucormycosis is a life-threatening infection, and any delay in effective therapy contributes to morbidity and mortality. Amphotericin B is the usual treatment. Giving posaconazole as oral suspension should be avoided because it will take a week before steady-state level is achieved. In amphotericin B-intolerant patients, posaconazole injection or tablet formulation has better pharmacokinetics than its suspension formulation. In cases where clinicians wish to use posaconazole suspension, it should overlap with amphotericin B treatment for at least 1 week before discontinuation of amphotericin B. This ensures patients have uninterrupted antifungal coverage.

Mistake 9: Treating UTI caused by non-*albicans Candida* spp. with voriconazole/echinocandins

In long-term care facilities, isolating *Candida* in urine is common. However, when an antifungal is indicated, using voriconazole or echinocandins is not recommended because of their poor urinary system penetration.

Mistake 10: Treating patients with *Candida* cultured from bronchoalveolar lavage

Candida pneumonia is very uncommon; *Candida* is generally a colonizer in the respiratory system. Antifungal treatment is not required in these cases.

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