Review

Diagnosis of Breakthrough Fungal Infections in the Clinical Mycology Laboratory: An ECMM Consensus Statement

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Abstract:

Breakthrough invasive fungal infections (bIFI) cause significant morbidity and mortality. Their diagnosis can be challenging due to reduced sensitivity of conventional culture techniques, serologic tests, and PCR-based assays in patients on antifungal therapy, and their diagnosis can be delayed contributing to poor patient outcomes. In this review, we provide consensus recommendations on behalf of the European Confederation for Medical Mycology (ECMM) for the diagnosis of bIFI caused by invasive yeasts, molds, and endemic mycoses, to guide diagnostic efforts in patients receiving antifungals and support the design of future clinical trials in the field of clinical

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mycology. The cornerstone of lab-based diagnosis of breakthrough infections for yeast and endemic mycoses remain conventional culture, to accurately identify the causative pathogen and allow for antifungal susceptibility testing. The impact of non-culture-based methods are not well-studied for the definite diagnosis of breakthrough invasive yeast infections. Non-culture-based methods have an important role for the diagnosis of breakthrough invasive mold infections, in particular invasive aspergillosis, and a combination of testing involving conventional culture, antigen-based assays, and PCR-based assays should be considered. Multiple diagnostic modalities, including histopathology, culture, antibody and/or antigen tests and occasionally PCR-based assays may be required to diagnose breakthrough endemic mycoses. A need exists for diagnostic tests that are effective, simple, cheap, and rapid to enable the diagnosis of bIFI in patients taking antifungals.

Keywords: breakthrough invasive fungal infections, invasive candidiasis, invasive mold infections, endemic mycoses, diagnostics

1. Introduction

Invasive fungal infections (IFIs) cause significant morbidity and mortality, particularly in patients with compromised immune systems, such as patients with underlying hematologic malignancies, hematopoietic stem cell transplant recipients (HSCT), solid organ transplant (SOT) recipients, and others who are critically ill in the intensive care unit (ICU). In many of these patients, antifungal prophylaxis and/or early empirical treatment are used during the greatest period of risk for IFI to decrease morbidity and mortality from these infections. Still, some patients will develop a breakthrough IFI (bIFI) (1, 2), defined as any IFI that occurs during exposure to an antifungal agent, including from fungi outside the spectrum of activity of the antifungal agent, as recently defined in detail by the European Confederation for Medical Mycology (ECMM) and Mycoses Study Group Education and Research Consortium (MSGERC) consensus criteria (3).

The diagnosis of bIFI can be challenging. Overall, while they remain the cornerstone of IFI diagnostics, culture-based approaches are limited by low sensitivity in patients exposed to antifungals, and delays in diagnosis are common (4). In addition, conventional biomarkers that have become the mainstay of diagnosis of invasive fungal infections and invasive aspergillosis, such as 1,3-β-d-glucan (BDG) and galactomannan (GM), respectively, are negatively influenced in patients receiving mold-active prophylaxis or treatment (5-9).

Here we review the literature on the diagnosis of bIFI, including conventional diagnostics such as culture, serologic tests, nucleic-acid based assays, and other modalities for the diagnosis of bIFI from both yeasts and molds. Lastly, we provide consensus recommendations on behalf of the ECMM.

2. Materials and Methods

Executives of the ECMM selected a group of authors based on content expertise, including individuals involved with the ECMM either as council members, fellows, or via the worldwide guideline initiative, with expertise in the diagnosis of yeast, mold infections, and endemic mycoses. ECMM is the umbrella organization of 28 national mycological societies, comprised of one delegate from each of the 28 nations forming the ECMM council (www.ecmm.info) (10, 11).

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According to their expertise authors were divided into 3 groups and assigned to breakthrough IFI caused by yeasts (n=3), molds (n=3), and endemic mycoses (n=2). The authors searched PubMed for relevant English language articles on clinical studies of antifungal prophylaxis and treatment through July 2020. Search terms included "diagnosis", "antifungal prophylaxis", "antifungal treatment", "breakthrough fungal infection". The references of articles retrieved were reviewed for additional relevant reports. Study selection and data extraction were performed separately for yeast infections, mold infections, and endemic mycoses. There was no intent to grade the quality of the studies. The executive committee reviewed each study and drafted a consensus statement for diagnosis of breakthrough IFI.

A draft proposal for definitions was developed and sent out by the president of the ECMM to all council-members for comments and suggestions. The authors addressed all comments received and circulated the updated document again for final approval.

3. Consensus Definitions

3.1. Diagnosis of Breakthrough Infections caused by Yeasts

3.1.1. Conventional Diagnostics

Clinical samples analyzed when an invasive yeast infection is suspected depends on the suspected location(s) of fungal infections and typically include blood, urine, cerebrospinal fluid, or tissue biopsies for deep or systemic infections. Skin scrapings, shaved nail or hair, vaginal secretions and swabs allow the detection of superficial infections (12).

Fungal culture is one of the primary lab-tests used to diagnose bIFI as it allows the identification of the fungal pathogen and supports antifungal susceptibility testing. Species identification and antifungal susceptibility profiles can help guide antifungal treatment. The most commonly used culture media are Sabouraud dextrose and malt extract agar plates. Additional specialized media such as chromogenic agar allow the separation of similar-looking colonies and the direct identification of *Candida* species (13). Matrix-assisted laser desorption ionization–time of flight mass spectrometry has become a standard tool for the accurate, rapid, and economical identification of pathogens in the clinical diagnostics laboratory (14).

Microscopic examination of a primarily sterile site can determine whether or not the infection is due to a fungus and differentiate between fungal colonization and IFI (13, 15). Microscopy, however, cannot determine the specific cause of infection. While Gram stain lacks sensitivity, fluorescent brighteners (Calcofluor white, or Blankophor), which bind to chitin in the fungal cell wall, are a rapid means of scanning samples for fungal hyphae, and enhance morphology assessment (16).

Interpretation depends on the type of sample investigated (13, 15). Yeasts obtained from non-sterile body sites like oropharynx or airways may be part of the normal flora or may cause infection. Hence, the history and physical examination are of utmost importance to help determine if the recovered yeast represents colonization or is causing IFI. Particular appearances may be highly characteristic of

certain infections, such as India Ink in cerebrospinal fluid, which can identify *Cryptococcus* spp. The microscopic detection of typical budding yeast cells, pseudohyphae, and/or true hyphae in samples obtained from otherwise sterile sites is indicative of fungal infections.

3.1.2. Serology including antigen-based tests

Non-culture-based methods are increasingly used in clinical practice for the management of patients at high risk of fungal infection and can help reduce the time to diagnosis and allow for timely initiation of antifungal treatment. Antibody based techniques are based on detecting circulating antigens in different body fluids. Enzyme-linked immunosorbent assay (ELISA) kits for detection of *Candida* mannan antigen are commercially available to detect Candida in serum samples for the diagnosis of invasive candidiasis (Platelia *Candida* Antigen, Biorad, Marnes-la-Coquette, France). When used in combination with anti-*Candida* mannan antibodies (Platelia *Candida* Antibody, Biorad, Marnes-la-Coquette, France), this combination of serology tests has demonstrated good sensitivity (83%) and specificity (86%) (17). However, the sensitivity of both mannan and anti-mannan vary for different Candida species, with lower sensitivity for *C. parapsilosis* and *C. krusei* (17). Of note, the number of studies evaluating these assays is limited, and whether or not performance of mannan/anti-mannan is impacted by antifungal agents remains unknown.

The presence of 1,3-β-d-glucan (BDG) in serum can be used to diagnose some fungal infections (including *Candida* but not *Cryptococcus*). Since it is present in the cell wall of several fungal species (18), a positive result is not specific for invasive candidiasis. While the vast majority of studies to date evaluated the Fungitell assay (Associates of Cape Cod Diagnostics; MA, USA), other commercial test are available which may show similar performance; however, optimal universal cut off values for non-Fungitell tests are still lacking (19, 20). Sensitivity and specificity for diagnosing invasive candidiasis are both around 80% (21, 22), but false positive results have been described (23), in particular in conditions associated with fungal translocation in the gut such as sepsis or advanced liver cirrhosis (24, 25). BDG results should therefore be carefully evaluated and always interpreted with other clinical data. Serum BDG may be a useful tool for diagnosing bIFI; however, similar to other diagnostic tests, reduced sensitivities have been observed in the presence of antifungal prophylaxis or treatment (26, 27).

Cryptococcus antigen can be detected by a lateral flow assay (LFA; CrAg Immuno-Mycologics [IMMY], Norman, OK, USA), or via latex-agglutination (CryptoPlus assay, Biorad, Marnes-la-Coquette, France). The LFA has high sensitivity (98–100%) and specificity (97–100%) in serum, plasma, cerebrospinal fluid and urine and is the recommended biomarker for the diagnosis of cryptococcosis (28). This assay has been extensively evaluated and it has been included in the "Essential in vitro diagnostic list" of the WHO (29) and thus is recommended by the WHO for the screening and diagnosis of patients at risk for cryptococcal infection (30). An ELISA kit is also available but is less commonly used due to a comparative performance with the LFA and the advantage of the LFA being a true point-of-care test (POCT).

3.1.3. Nucleic-acid based assays/others

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Using molecular tools, it is possible to diagnose and identify yeasts directly from clinical samples (including blood, serum, plasma, other sterile fluid, bronchoalveolar lavage and tissues) and to rapidly identify the species attributed for positive blood cultures during bloodstream infections.

A large number of commercial and in-house targeted (simplex or multiplex) PCR assays with specific primers for various genetic sequences (18S rDNA, 28S rDNA, 5.8S rDNA, internal transcribed spacer regions and mitochondrial DNA) have been developed (31, 32), including for infections from *C. auris* and its relatives, *C. haemulonii, C. duobushaemulonii* and *C. pseudohaemulonii* (33).

Depending on the assay, multiplex panels or pan-fungal panels are available. In a meta-analysis of 54 studies with almost 5,000 patients tested by blood-based PCR, pooled sensitivity and specificity for proven or probable invasive candidiasis vs. at-risk controls were 95% and 92%, respectively (34). PCR plus blood culture, *Candida* PCR and, to a lesser extent, BDG testing, significantly enhanced the performance of PCR alone for the diagnosis of invasive candidiasis (27). A recent and fully automated assay combining ITS2 region amplification and T2 magnetic resonance, the T2Candida Panel (T2Biosystems, Wilmington, MA, USA), has been developed. This assay detects three groups of *Candida* (*C. albicans*/*C. tropicalis, C. glabrata*/*C. krusei*/*C. bracarensis*, and *C. parapsilosis*/*C. orthopsilosis*/*C. metapsilosis*) in EDTA blood samples within 5 hours and proved efficient for the diagnosis of candidemia and of intra-abdominal candidiasis (35-37).

While blood cultures lack sensitivity, they still represent the diagnostic gold standard for candidemia and molecular blood culture identification (BCID) panels provide precise and rapid identification of cultured pathogens. Results are obtained with minimal hands-on time compared to conventional methods like chromogenic media and biochemical identification, proteomic identification using MALDI-TOF MS or fluorescence in situ hybridization (FISH) assays. Two BCID kits offer different fungal panels: The GenMark Dx ePlex (Carlsbad, CA, USA) fungal pathogen panel (BCID-FP) rapidly detects 15 fungal targets (*C. albicans, C. auris, C. dubliniensis, C. famata, C. glabrata, C. guilliermondii, C. kefyr, C. krusei, C. lusitaniae, C. parapsilosis, C. tropicalis, Cryptococcus neoformans*/*gattii, Fusarium spp.*, and *Rhodotorula spp*.)(38) and the Biofire FilmArray BCID-FP (Biomérieux, Marcy-l'Etoile, France) (*C. albicans, C. auris, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis* and *Cryptococcus neoformans*/*gattii*)(39).

3.1.4. Consensus recommendation

The cornerstone for diagnosing a breakthrough yeast infection relies on obtaining the isolate, most commonly with conventional culture methods. Prior antifungal treatment may confer a selection pressure for drug-resistant isolates. If a bIFI is diagnosed, the objective is to adapt the antifungal therapy to the species identified and to the in vitro susceptibility profile. The main steps of the diagnosis are the following:

- Direct examination of sterile samples is recommended for the proof of infection given the potential effect of antifungal therapy on fungal culture sensitivity. However, given limited sensitivity, a negative direct examination does not exclude infection.

- Once an isolate is grown, identification should be performed. Particularly in case of positive blood culture, molecular blood culture identification (BCID) panels provide precise and rapid identification.

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- Antifungal susceptibility testing should be performed on invasive isolates to evaluate the activity of alternative drugs.

- Non-culture methods of detection (serology and/or PCR) can be considered but the impact of antifungal therapy on their sensitivity has not been well-enough studied. Specificity is also a concern, especially with non-sterile samples, because highly sensitive molecular techniques can also reflect the presence of commensal yeasts.

3.2. Diagnosis of Breakthrough Infections caused by Molds

3.2.1. Conventional Diagnostics

To establish a diagnosis of "proven" invasive mold infection per consensus definitions by the European Organization for Research and Treatment of Cancer (EORTC) and the MSGERC (40), histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy must show hyphae with evidence of associated tissue damage, recovery of mold by culture from a normally sterile site, or a positive blood culture with compatible signs and symptoms of infection. Although microscopy and culture are the traditional cornerstones for the diagnosis of invasive mold infection, most diagnoses are not made in this manner and the diagnosis is determined to be "probable" or "possible" per EORTC/MSGERC criteria.

Culture-based approaches have the potential to detect the causative fungal pathogen and antifungal resistance and are the gold standard for investigating bIFI by Mucorales, *Fusarium* spp., *Lomentospora* spp., and other rare molds for which reliable antigens and other diagnosis are not available (41-44). However, it is not always possible to attempt to obtain tissue biopsy for culture from a sterile site due to the risk of excessive bleeding or clinical contraindications, especially in patients with cytopenias. In addition, culture-based approaches lack sensitivity. Positive blood culture is seen in fusariosis and lomentosporiosis (43, 45), but rare in invasive aspergillosis (IA) (46). Most cases of IA in immunocompromised patients are not proven by EORTC/MSGERC criteria (i.e. culture from sterile site or biopsy evidence of invasion): for example, in one large survey of HSCT recipients, only 11.5% of patients with IA met criteria for proven infection (47). The sensitivity of culture is imperfect, ranging from 30% - 60% from bronchoalveolar lavage fluid (BALF) (48), and is lower for diagnosing bIFI in patients taking antifungals. In a study of 53 patients with diagnosed IFI, of which 34/53 (64%) were on mold-active antifungal prophylaxis, 16/53 (30%) were diagnosed with "proven" infection, with a sensitivity of culture from BALF of only 3/16 (18.8%) (8). Similar low sensitivities of culture have been described in other studies for patients on old-active antifungals (49-51). Thus, the diagnosis of bIFI in many cases may be missed if diagnosis is relied on conventional diagnostics such as culture-based methods alone. In addition to limited sensitivity, culture-based diagnostics involving non-sterile samples suffer from limited specificity: positive culture results can represent fungal colonization which can lead to misdiagnosis and overtreatment (52).

3.2.2. Antigen based diagnostics

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Although Galactomannan (GM) detection plays a crucial role for the diagnosis of invasive aspergillosis, several studies have shown that systematically screening for GM in blood for the detection of bIFI in patients receiving either posaconazole or micafungin during high-risk episodes is not useful due to the low prevalence of infection and the associated low positive predictive value of a positive test result (53, 54). As a consequence, the 2017 ESCMID–ECMM–European Respiratory Society (ERS) guidelines for the diagnosis and management of *Aspergillus* disease provides a recommendation against the use of serum GM screening in patients on mold-active prophylaxis (55). However, antigen-based diagnostics remain critical to manage suspected fungal disease in these patients. Several studies demonstrated a better performance of GM detection in BAL versus blood whereas BDG testing only provides reliable results in blood (56). BAL GM testing may be particularly warranted in non-neutropenic patients, which often show an airway invasive growth pattern of IA, and therefore rarely produce positive serum GM results (57, 58). In a homogenous cohort of acute myeloid leukemia patients during induction chemotherapy, increasing the posaconazole concentration was shown to decrease the sensitivity of serum GM assay. In general, the sensitivity of serum GM assay to detect probable and proven IA is 81.8%, but none of patients with IA and posaconazole concentration ≥ 0.5 mg/L had a positive serum GM test (59). Slightly reduced sensitivities in the presence of mold-active antifungals has also been described for the BAL GM: at a cut-off of 0.5 optical density index (ODI), Eigl and colleagues showed a 71% sensitivity for probable/proven IA in those on antifungals versus 95% in those without antifungals (60, 61). Sensitivity for diagnosing breakthrough IA in patients on antifungal prophylaxis dropped to 52% in that study when using a 1.0 ODI cutoff (61). Therefore, using a lower cut-off of 0.5 ODI from BALF for diagnosing breakthrough IA may be preferable and was also recommended in another study (60). Combining several antigen detection assays or antigen detection tests with PCR has shown convincing diagnostic potential for the diagnosis of breakthrough mold infections (50, 62, 63). Data on the performance of the new lateral flow tests for the diagnosis of breakthrough IA, such as the AspLFD (OLM Diagnostics, Newcastle upon Tyne, UK; herein LFD) and the sōna Aspergillus galactomannan LFA (IMMY, Norman, OK, USA; herein LFA), are limited. For the LFD prototype test, sensitivity in BALF was only 52% in those receiving mold-active antifungals versus 86% in those not receiving mold-active antifungals (61), with a similar impact shown for serum LFD results in an animal model (26). For the LFA, so far limited data has shown no significant impact of mold-active antifungals on efficacy (51, 64-66). Thus, both the LFA and the LFD are attractive options for the diagnosis of breakthrough infections in BALF and also serum, especially when used in combination with other biomarkers. Mold-active prophylaxis is affecting the epidemiology of invasive mycoses resulting in a shift towards less common entities such as fusariosis. In a retrospective cohort (2004-2017) from a tertiary hospital in Madrid, Spain, all (n=7) cases of breakthrough invasive fusariosis were characterized by positive BDG tests in blood (67), and GM testing has also been shown to be useful for diagnosing fusariosis (68).

3.2.3. Nucleic-acid based assays/others

Aspergillus PCR has demonstrated high sensitivity and high negative predictive value in severely immunocompromised patients in settings where antifungal prophylaxis is not used (69, 70). PCR is also an important diagnostic test for mucormycosis (71, 72). However, the performance of PCR from blood decreases significantly in patients receiving mold active antifungal agents as shown in a recent

review (49, 73, 74). While mold-active prophylaxis seems to affect *Aspergillus* PCR on BALF less than blood, reduced diagnostic performance has also been described from BALF (75). Given the reduced sensitivity of all diagnostic tests in the presence of mold-active antifungal prophylaxis or treatment, the combination of multiple diagnostic tests is warranted. Immunological markers may also be utilized as combination partners, and particularly high serum IL-8 levels (>300 pg/mL) have been shown to be highly specific for IA (50, 76-78), and have shown high sensitivity and specificity when combined with BALF LFD or BALF *Aspergillus* PCR (50). Larger multicenter studies are currently in progress to validate these findings. The most secreted siderophore of *A. fumigatus* is triacetylfusarinine C (TAFC), which is produced only by actively growing cells, is not present in conidia, and can be detected in urine, BALF and blood (79-81). TAFC can be detected by mass spectrometry and has shown excellent performance as a biomarker for breakthrough IA in urine samples, when normalized to urine creatinine, with similar performance to those reported for GM determination in serum and BALF (79). In BALF, TAFC was shown to be an independent biomarker for IA, improving the performance of BALF GM for detection of IA when used in combination (80). These results warrant further exploration of this promising new biomarker. Other potential approaches for mycological detection of IA include the detection of volatile organic compounds in exhaled air (82), and Bis(methylthio)gliotoxin, an inactive derivative of gliotoxin (83).

3.2.4. Consensus recommendation

The diagnosis of breakthrough mold infections is challenging, as all diagnostic tests have reduced sensitivity in samples obtained during treatment or prophylaxis with mold active antifungals.

- Despite reduced sensitivities, antigen based diagnostics such as GM (in BALF and serum) and BDG (in serum only) or newer assays such as the LFA and LFD (both in BALF or serum) have an important role for diagnosing breakthrough IA when the degree of clinical suspicion is high, because the sensitivity of fungal culture may be even further reduced.

- While we do not recommend using these tests for screening in patients on mold active prophylaxis or treatment, a combination of multiple antigen-based diagnostics, conventional diagnostics, PCR-based assays, and novel diagnostic markers can help diagnose breakthrough mold infections. Particularly combination of GM with one or more other tests, such as the LFA, LFD or PCR-based assays shows promise for diagnosis of breakthrough IA in case of clinical suspicion, with positivity of one of those tests indicating breakthrough IA, even when others result negative.

- Many of the available antigen based diagnostics such as GM or the LFA and the LFD tests are specific for IA and very few other mold infections such as fusariosis, therefore negative test results do not automatically rule out a breakthrough mold infection, but instead should raise the suspicion for mucormycosis or another rare mold as a potential causative pathogen.

- Conventional diagnostics – in particular fungal culture – are essential for susceptibility testing and for diagnosing mold infections other than IA such as mucormycosis, fusariosis, scedosporiosis, lomentosporiosis, and infections caused by other rare molds such as *Scopulariosis*, *Rasamsonia*, or basidiomycetes, which are normally only detected by fungal culture with subsequent ITS sequencing or MALDI-TOF MS (84) of the isolate for species identification. Importantly, a negative fungal culture does not rule out a breakthrough invasive mold infection, given the very low sensitivity of culture in this setting.

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3.3. Diagnosis of Breakthrough Infections due to Endemic Mycoses

3.3.1. Conventional Diagnostics

Diagnosis of the endemic mycoses (*Blastomyces*, *Coccidioides*, *Emergomyces*, *Histoplasma*, *Paracoccidioides*, *Sporothrix* spp., and *Talaromyces marneffei* (formerly *Penicillium marneffei*) is confirmed by histopathologic or direct microscopy of specimens from an affected site. Samples obtained by bronchoscopy are most frequently examined following pneumonia or when suspicious lesions are identified on radiographic imaging. However, biopsy results of affected sites or cerebrospinal fluid are also frequently helpful if cultures or typical *in vivo* findings of these fungi are observed (40).

Culture provides confirmation of infection and allows for susceptibility testing or identification to the species level, although the clinical correlation of susceptibility results to clinical outcomes has not been definitively demonstrated for the endemic mycoses. However, *in vitro* MICs do suggest resistance likely occurs (85), may develop on therapy (86), and may be increasing in frequency (87, 88). With attempts at culture isolation, biosafety is an important consideration when handling these organisms, and laboratories should incorporate national guidance and regulations into their processes and practices to ensure the safety of laboratory staff.

3.3.2. Serology

Serologic testing is widely used for the diagnosis and care of patients with coccidioidomycosis. In this group, serology has been found to be helpful diagnostically, but also correlates with patient symptoms and is useful to follow prognostically as a biomarker of infection. Relapse of infection is typically accompanied by a rise in the complement fixation (CF) antibody titer (89). Serology of blastomycosis is less helpful due to the lower sensitivity and specificity of testing (90-92). Serologic testing for histoplasmosis is most useful for patients with chronic pulmonary disease and may not be helpful in those with severe immunosuppression (93-95). In contrast, paracoccidioidomycosis serologies exhibit high sensitivity and specificity (96, 97). Sporotrichosis serologic testing is infrequently used due to the lack of a commercial assay, while the sensitivity of antibody testing for talaromycosis ranges from 30-80% likely due to the highly immunosuppressed state (e.g. advanced HIV disease) of most affected patients (98, 99).

It is important to recall that in the immunosuppressed patient population the endemic mycoses, particularly *Coccidioides* or *Histoplasma*, may recur years after initial infection, and serology may not be positive or may have aberrant kinetics compared to immunocompetent hosts(89, 100, 101).

Prior to initiating immunosuppressive therapy, it is often prudent to evaluate a patient's past travel history to determine the individual risk for endemic mycoses. For those with a suggestive history, serologic testing can be performed to ascertain the potential need or to guide prophylaxis/treatment practices so as to avoid potential breakthrough infection at a later time point.

Antigen testing for *Blastomyces* spp is commercially available and has a reported sensitivity of 85- 93% and a specificity of 79-99% (102-106). Test positivity in *Coccidioides* depends upon the degree of host immunosuppression and is largely unhelpful in the immunocompetent. In highly immunosuppressed patients, antigenuria has been detected in up to 70% of patients (107). *Histoplasma* antigen assays are most useful in patients who have disseminated histoplasmosis and acute pulmonary histoplasmosis, but are less useful in localized pulmonary infection and chronic cavitary pulmonary histoplasmosis (108, 109). Antigen detection for paracoccidioidomycosis has been investigated although is not commercially available (110), and has not been evaluated for sporotrichosis. Antigen detection in talaromycosis is highly accurate and is well suited for patients with advanced immunosuppression and a high blood fungal burden (111). However, it is not widely available.

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3.3.2. Nucleic-acid based assays/others

Nucleic acid amplification using polymerase chain reaction (PCR) tests are not commercially available for the endemic mycoses, but detection of DNA in clinical specimens has been evaluated for: *Blastomyces* (sensitivity 60-86%) (112-114), *Coccidioides* (~50%) (115, 116), *Histoplasma* (18-65%) (95, 117), *Paracoccidioides* (91-100%) (101, 118) *Sporothrix* (83-92%) (119-121), and *Talaromyces* (70-86%) (122).

The use of BDG for the diagnosis of endemic mycosis is problematic due to the lack of specificity and the poor positive predictive value and although have been evaluated in limited fashion, are generally unhelpful in the diagnosis or management of endemic mycoses (123, 124).

3.3.3. Consensus recommendation

Specific recommendations for diagnosis of breakthrough endemic mycoses include:

- Whenever possible, diagnosis of bIFI caused by endemic mycoses should be confirmed by obtaining affected tissue for examination by direct microscopy, histopathology, and fungal culture.

- More nuanced approaches are required for individual diseases that are suspected based on the relevant clinical picture and exposure history. In acute disease in immunocompromised patients, histoplasmosis and talaromycosis can both be diagnosed with antigen tests, although the latter assay is not widely available.

- Antibody tests for coccidioidomycosis, paracoccidioidomycosis, and acute and chronic histoplasmosis should be considered, but antibody tests for histoplasmosis are not recommended in patients with immunosuppression or those with cystic fibrosis. Serology for other endemic mycoses (i.e. blastomycosis, sporotrichosis, emergomycosis) have limited sensitivities and specificities or are not commercially available.

4. Discussion

The diagnosis of bIFIs remain challenging, with limited sensitivities of most available fungal diagnostics. With these consensus recommendations we intend to support the design of future clinical trials in the field of clinical mycology.

The diagnosis of breakthrough yeast infections and the endemic mycoses should rely on the isolation of the causative pathogen, such as by conventional culture methods, although the yield is often reduced in patients on antifungal prophylaxis, making diagnosis of bIFI even more challenging. Other non-culture methods such as BDG and PCR for the diagnosis of yeast infection and serologic tests for acute histoplasmosis and coccidioidomycosis can be considered, but the effect of antifungal therapy likely decreases the yield of these tests, although this has not been well-studied.

Conversely, antigen-based assays such as GM, the LFD, and LFA have an important role in the diagnosis of breakthrough invasive mold infections, although antifungal therapy may reduce the sensitivity of these assays and a combination of multiple antigen-based diagnostics, along with conventional culture and PCR-based assays, may further increase the diagnostic yield. Breakthrough infections occurring under antifungal prophylaxis mostly require combinations of multiple tests and biomarkers in order to achieve an acceptable sensitivity. Optimally, diagnostic approaches for fungal infections should be initiated before initiation of antifungal treatment, however in the real world this is often not possible.

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More effective, simpler, and cheaper diagnostic tests are needed with more rapid turnaround time to diagnose bIFIs, particularly non-*Aspergillus* mold infections and endemic mycosis. Given that antifungal therapy can decrease the diagnostic yield of conventional culture and a number of the serologic and PCR-based assays discussed, improved diagnostics, particularly for bIFIs, are needed. While these definitions represent the status of published literature, future studies are needed to fill important gaps.

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References

1. Jenks JD, Cornely OA, Chen SC, Thompson GR, 3rd, Hoenigl M. Breakthrough Invasive Fungal Infections: Who is at risk? Mycoses. 2020.

2. Lee WS, Hsieh TC, Ou TY, Teng SO, Chen FL, Wang FD. Breakthrough disseminated cryptococcosis during micafungin therapy. J Microbiol Immunol Infect. 2015;48(4):456-8.

3. Cornely OA, Hoenigl M, Lass-Flörl C, Chen SC, Kontoyiannis DP, Morrissey CO, et al. Defining breakthrough invasive fungal infection-Position paper of the mycoses study group education and research consortium and the European Confederation of Medical Mycology. Mycoses. 2019;62(9):716-29.

4. Jenks JD, Spiess B, Buchheidt D, Hoenigl M. (New) Methods for Detection of Aspergillus fumigatus Resistance in Clinical Samples. Curr Fungal Infect Rep. 2019;13(3):129-36.

5. Buchheidt D, Reinwald M, Hoenigl M, Hofmann WK, Spiess B, Boch T. The evolving landscape of new diagnostic tests for invasive aspergillosis in hematology patients: strengths and weaknesses. Curr Opin Infect Dis. 2017;30(6):539-44.

6. Hoenigl M, Prattes J, Neumeister P, Wölfler A, Krause R. Real-world challenges and unmet needs in the diagnosis and treatment of suspected invasive pulmonary aspergillosis in patients with haematological diseases: An illustrative case study. Mycoses. 2018;61(3):201-5.

7. Eigl S, Prattes J, Reinwald M, Thornton CR, Reischies F, Spiess B, et al. Influence of mould-active antifungal treatment on the performance of the Aspergillus-specific bronchoalveolar lavage fluid lateral-flow device test. Int J Antimicrob Agents. 2015;46(4):401-5.

8. Eigl S, Hoenigl M, Spiess B, Heldt S, Prattes J, Neumeister P, et al. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. Medical mycology. 2017;55(5):528-34.

9. Hoenigl M, Seeber K, Koidl C, Buzina W, Wölfler A, Duettmann W, et al. Sensitivity of galactomannan enzyme immunoassay for diagnosing breakthrough invasive aspergillosis under antifungal prophylaxis and empirical therapy. Mycoses. 2013;56(4):471-6.

10. Cornely OA, Lass-Florl C, Lagrou K, Arsic-Arsenijevic V, Hoenigl M. Improving outcome of fungal diseases - Guiding experts and patients towards excellence. Mycoses. 2017;60(7):420-5.

11. Hoenigl M, Gangneux JP, Segal E, Alanio A, Chakrabarti A, Chen SC, et al. Global guidelines and initiatives from the European Confederation of Medical Mycology to improve patient care and research worldwide: New leadership is about working together. Mycoses. 2018.

12. Wickes BL, Romanelli AM. Diagnostic Mycology: Xtreme Challenges. J Clin Microbiol. 2020;58(4).

13. Cuenca-Estrella M, Verweij PE, Arendrup MC, Arikan-Akdagli S, Bille J, Donnelly JP, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2012;18 Suppl 7:9-18.

14. Mery A, Sendid B, Francois N, Cornu M, Poissy J, Guerardel Y, et al. Application of Mass Spectrometry Technology to Early Diagnosis of Invasive Fungal Infections. Journal of clinical microbiology. 2016;54(11):2786-97.

15. Lass-Flörl C. How to make a fast diagnosis in invasive aspergillosis. Medical mycology. 2019;57(Supplement_2):S155-s60.

16. Clancy CJ, Nguyen MH. Rapid diagnosis of invasive candidiasis: ready for prime-time? Curr Opin Infect Dis. 2019;32(6):546-52.

17. Mikulska M, Calandra T, Sanguinetti M, Poulain D, Viscoli C, Third European Conference on Infections in Leukemia G. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. Critical Care (London, England). 2010;14(6):R222.

18. Ostrosky-Zeichner L, Alexander BD, Kett DH, Vazquez J, Pappas PG, Saeki F, et al. Multicenter clinical evaluation of the (1-->3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005;41(5):654-9.

19. Marchetti O, Lamoth F, Mikulska M, Viscoli C, Verweij P, Bretagne S. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. Bone marrow transplantation. 2012;47(6):846-54.

20. Friedrich R, Rappold E, Bogdan C, Held J. Comparative Analysis of the Wako β-Glucan Test and the Fungitell Assay for Diagnosis of Candidemia and Pneumocystis jirovecii Pneumonia. J Clin Microbiol. 2018;56(9).

21. Onishi A, Sugiyama D, Kogata Y, Saegusa J, Sugimoto T, Kawano S, et al. Diagnostic accuracy of serum 1,3-β-D-glucan for pneumocystis jiroveci pneumonia, invasive candidiasis, and invasive aspergillosis: systematic review and meta-analysis. J Clin Microbiol. 2012;50(1):7-15.

22. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, Michalopoulos A, Rafailidis PI, Falagas ME. beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;52(6):750-70.

23. Liss B, Cornely OA, Hoffmann D, Dimitriou V, Wisplinghoff H. 1,3-ss-D-glucan concentrations in blood products predict false positive post-transfusion results. Mycoses. 2015.

24. Hoenigl M. Fungal Translocation: A driving force behind the Occurrence of non-AIDS Events? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019.

25. Yang AM, Inamine T, Hochrath K, Chen P, Wang L, Llorente C, et al. Intestinal fungi contribute to development of alcoholic liver disease. The Journal of clinical investigation. 2017.

26. Wiederhold NP, Najvar LK, Bocanegra R, Kirkpatrick WR, Patterson TF, Thornton CR. Interlaboratory and interstudy reproducibility of a novel lateral-flow device and influence of antifungal therapy on detection of invasive pulmonary aspergillosis. Journal of clinical microbiology. 2013;51(2):459-65.

27. Nguyen MH, Wissel MC, Shields RK, Salomoni MA, Hao B, Press EG, et al. Performance of Candida real-time polymerase chain reaction, beta-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54(9):1240-8.

28. Vidal JE, Boulware DR. LATERAL FLOW ASSAY FOR CRYPTOCOCCAL ANTIGEN: AN IMPORTANT ADVANCE TO IMPROVE THE CONTINUUM OF HIV CARE AND REDUCE CRYPTOCOCCAL MENINGITIS-RELATED MORTALITY. Rev Inst Med Trop Sao Paulo. 2015;57 Suppl 19(Suppl 19):38-45.

29. First WHO Model List of Essential In Vitro Diagnostics. Geneva: World Health Organization; 2019.

30. WHO Guidelines Approved by the Guidelines Review Committee. Guidelines for The Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-Infected Adults, Adolescents and Children: Supplement to the 2016 Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Geneva: World Health Organization

© World Health Organization 2018.; 2018.

31. Camp I, Spettel K, Willinger B. Molecular Methods for the Diagnosis of Invasive Candidiasis. J Fungi (Basel). 2020;6(3).

32. Clancy CJ, Nguyen MH. Non-Culture Diagnostics for Invasive Candidiasis: Promise and Unintended Consequences. J Fungi (Basel). 2018;4(1).

33. Arastehfar A, Fang W, Daneshnia F, Al-Hatmi AM, Liao W, Pan W, et al. Novel multiplex real-time quantitative PCR detecting system approach for direct detection of Candida auris and its relatives in spiked serum samples. Future Microbiol. 2019;14:33-45.

34. Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. J Clin Microbiol. 2011;49(2):665-70.

35. Zurl C, Prattes J, Zollner-Schwetz I, Valentin T, Rabensteiner J, Wunsch S, et al. T2Candida magnetic resonance in patients with invasive candidiasis: Strengths and limitations. Medical mycology. 2020;58(5):632-8.

36. Clancy CJ, Pappas PG, Vazquez J, Judson MA, Kontoyiannis DP, Thompson GR, 3rd, et al. Detecting Infections Rapidly and Easily for Candidemia Trial, Part 2 (DIRECT2): A Prospective, Multicenter Study of the T2Candida Panel. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;66(11):1678-86.

37. Lamoth F, Clancy CJ, Tissot F, Squires K, Eggimann P, Flückiger U, et al. Performance of the T2Candida Panel for the Diagnosis of Intra-abdominal Candidiasis. Open Forum Infect Dis. 2020;7(3):ofaa075.

38. Zhang G, Hu C, Luo L, Fang F, Chen Y, Li J, et al. Clinical features and short-term outcomes of 221 patients with COVID-19 in Wuhan, China. J Clin Virol. 2020;127:104364.

39. Simor AE, Porter V, Mubareka S, Chouinard M, Katz K, Vermeiren C, et al. Rapid Identification of Candida Species from Positive Blood Cultures by Use of the FilmArray Blood Culture Identification Panel. J Clin Microbiol. 2018;56(12).

40. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019.

41. Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger B, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. The Lancet Infectious diseases. 2019.

42. Jenks JD, Seidel D, Cornely OA, Chen S, van Hal S, Kauffman C, et al. Voriconazole plus terbinafine combination antifungal therapy for invasive Lomentospora prolificans infections: analysis of 41 patients from the FungiScope® registry 2008-2019. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2020:S1198-743X(20)30037-9.

43. Jenks JD, Seidel D, Cornely OA, Chen S, van Hal S, Kauffman C, et al. Clinical Characteristics and Outcomes of invasive Lomentospora prolificans Infections: Analysis of Patients in the FungiScope® Registry. Mycoses. 2020:10.1111/myc.13067.

44. Jenks J, Reed SL, Seidel D, Koehler P, Cornely OA, Mehta SR, et al. Rare Mold Infections Caused by Mucorales, Lomentospora Prolificans and Fusarium, San Diego: The Role of Antifungal Combination Therapy. Int J Antimicrob Agents. 2018.

45. Nucci M, Marr KA, Vehreschild MJ, de Souza CA, Velasco E, Cappellano P, et al. Improvement in the outcome of invasive fusariosis in the last decade. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2013.

46. Kontoyiannis DP, Sumoza D, Tarrand J, Bodey GP, Storey R, Raad, II. Significance of aspergillemia in patients with cancer: a 10-year study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2000;31(1):188-9.

47. Neofytos D, Horn D, Anaissie E, Steinbach W, Olyaei A, Fishman J, et al. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;48(3):265-73.

48. Hage CA, Carmona EM, Epelbaum O, Evans SE, Gabe LM, Haydour Q, et al. Microbiological Laboratory Testing in the Diagnosis of Fungal Infections in Pulmonary and Critical Care Practice. An Official American Thoracic Society Clinical Practice Guideline. American journal of respiratory and critical care medicine. 2019;200(5):535-50.

49. Eigl S, Hoenigl M, Spiess B, Heldt S, Prattes J, Neumeister P, et al. Galactomannan testing and Aspergillus PCR in same-day bronchoalveolar lavage and blood samples for diagnosis of invasive aspergillosis. Medical mycology. 2017;55(5):528-34.

50. Heldt S, Prattes J, Eigl S, Spiess B, Flick H, Rabensteiner J, et al. Diagnosis of Invasive Aspergillosis in Hematological Malignancy Patients: Performance of Cytokines, Asp LFD, and Aspergillus PCR in Same Day Blood and Bronchoalveolar Lavage Samples. The Journal of infection. 2018.

51. Jenks JD, Mehta SR, Taplitz R, Law N, Reed SL, Hoenigl M. Bronchoalveolar lavage Aspergillus Galactomannan lateral flow assay versus Aspergillus-specific lateral flow device test for diagnosis of invasive pulmonary Aspergillosis in patients with hematological malignancies. The Journal of infection. 2019;78(3):249-59.

52. Paterson DL, Singh N. Invasive aspergillosis in transplant recipients. Medicine. 1999;78(2):123-38.

53. Duarte RF, Sanchez-Ortega I, Cuesta I, Arnan M, Patino B, Fernandez de Sevilla A, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;59(12):1696-702.

54. Vena A, Bouza E, Alvarez-Uria A, Gayoso J, Martin-Rabadan P, Cajuste F, et al. The misleading effect of serum galactomannan testing in high-risk haematology patients receiving prophylaxis with micafungin. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2017;23(12):1000.e1-.e4.

55. Ullmann A, Aguado JM, Arikan S, Denning D, Groll A, Lagrou K, et al. Executive Summary of the 2017 ESCMID-ECMM Guideline for the Diagnosis and Management of Aspergillus Disease. Clin Microb Infect. 2018;in press.

56. Reischies FM, Prattes J, Pruller F, Eigl S, List A, Wolfler A, et al. Prognostic potential of 1,3-beta-d-glucan levels in bronchoalveolar lavage fluid samples. The Journal of infection. 2016;72(1):29-35.

57. Verweij PE, Gangneux J-P, Bassetti M, Brüggemann RJM, Cornely OA, Koehler P, et al. Diagnosing COVID-19-associated pulmonary aspergillosis. The Lancet Microbe.

58. Bergeron A, Porcher R, Sulahian A, de Bazelaire C, Chagnon K, Raffoux E, et al. The strategy for the diagnosis of invasive pulmonary aspergillosis should depend on both the underlying condition and the leukocyte count of patients with hematologic malignancies. Blood. 2012;119(8):1831-7; quiz 956.

59. Calmettes C, Gabriel F, Blanchard E, Servant V, Bouchet S, Kabore N, et al. Breakthrough invasive aspergillosis and diagnostic accuracy of serum galactomannan enzyme immune assay during acute myeloid leukemia induction chemotherapy with posaconazole prophylaxis. Oncotarget. 2018;9(42):26724-36.

60. Reinwald M, Spiess B, Heinz WJ, Vehreschild JJ, Lass-Florl C, Kiehl M, et al. Diagnosing pulmonary aspergillosis in patients with hematological malignancies: a multicenter prospective evaluation of an Aspergillus PCR assay and a galactomannan ELISA in bronchoalveolar lavage samples. European journal of haematology. 2012;89(2):120-7.

61. Eigl S, Prattes J, Reinwald M, Thornton CR, Reischies F, Spiess B, et al. Influence of mould-active antifungal treatment on the performance of the Aspergillus-specific bronchoalveolar lavage fluid lateral-flow device test. International journal of antimicrobial agents. 2015.

62. Boch T, Spiess B, Cornely OA, Vehreschild JJ, Rath PM, Steinmann J, et al. Diagnosis of invasive fungal infections in haematological patients by combined use of galactomannan, 1,3-beta-D-glucan, Aspergillus PCR, multifungal DNA-microarray, and Aspergillus azole resistance PCRs in blood and bronchoalveolar lavage samples: results of a prospective multicentre study. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2016;22(10):862-8.

63. Hoenigl M, Prattes J, Spiess B, Wagner J, Prueller F, Raggam RB, et al. Performance of galactomannan, beta-d-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. Journal of clinical microbiology. 2014;52(6):2039-45.

64. Jenks JD, Salzer HJF, Hoenigl M. Improving the rates of Aspergillus detection: an update on current diagnostic strategies. Expert review of anti-infective therapy. 2019;17(1):39-50.

65. Jenks JD, Mehta SR, Taplitz R, Aslam S, Reed SL, Hoenigl M. Point-of-care diagnosis of invasive aspergillosis in non-neutropenic patients: Aspergillus Galactomannan Lateral Flow Assay versus Aspergillus-specific Lateral Flow Device test in bronchoalveolar lavage. Mycoses. 2019;62(3):230-6.

66. Mercier T, Dunbar A, de Kort E, Schauwvlieghe A, Reynders M, Guldentops E, et al. Lateral flow assays for diagnosing invasive pulmonary aspergillosis in adult hematology patients: A comparative multicenter study. Medical mycology. 2019:myz079.

67. Fernández-Cruz A, Semiglia MA, Guinea J, Martínez-Jiménez MDC, Escribano P, Kwon M, et al. A retrospective cohort of invasive fusariosis in the era of antimould prophylaxis. Medical mycology. 2020;58(3):300-9.

68. Nucci M, Carlesse F, Cappellano P, Varon AG, Seber A, Garnica M, et al. Earlier diagnosis of invasive fusariosis with Aspergillus serum galactomannan testing. PloS one. 2014;9(1):e87784.

69. White PL, Wingard JR, Bretagne S, Loffler J, Patterson TF, Slavin MA, et al. Aspergillus Polymerase Chain Reaction: Systematic Review of Evidence for Clinical Use in Comparison With Antigen Testing. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;61(8):1293-303.

70. Arastehfar A, Carvalho A, van de Veerdonk FL, Jenks JD, Koehler P, Krause R, et al. COVID-19 Associated Pulmonary Aspergillosis (CAPA)-From Immunology to Treatment. J Fungi (Basel). 2020;6(2).

71. Baldin C, Soliman SSM, Jeon HH, Alkhazraji S, Gebremariam T, Gu Y, et al. PCR-Based Approach Targeting Mucorales-Specific Gene Family for Diagnosis of Mucormycosis. Journal of Clinical Microbiology. 2018;56(10):e00746-18.

72. Rocchi S, Scherer E, Mengoli C, Alanio A, Botterel F, Bougnoux ME, et al. Interlaboratory evaluation of Mucorales PCR assays for testing serum specimens: A study by the fungal PCR Initiative and the Modimucor study group. Medical mycology. 2020.

73. Egger M, Jenks JD, Hoenigl M, Prattes J. Blood Aspergillus PCR: The Good, the Bad, and the Ugly. Journal of fungi (Basel, Switzerland). 2020;6(1):E18.

74. Springer J, Lackner M, Nachbaur D, Girschikofsky M, Risslegger B, Mutschlechner W, et al. Prospective multicentre PCR-based Aspergillus DNA screening in high-risk patients with and without primary antifungal

mould prophylaxis. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2016;22(1):80-6.

75. Reinwald M, Hummel M, Kovalevskaya E, Spiess B, Heinz WJ, Vehreschild JJ, et al. Therapy with antifungals decreases the diagnostic performance of PCR for diagnosing invasive aspergillosis in bronchoalveolar lavage samples of patients with haematological malignancies. The Journal of antimicrobial chemotherapy. 2012;67(9):2260-7.

76. Heldt S, Eigl S, Prattes J, Flick H, Rabensteiner J, Neumeister P, et al. Levels of IL-6, IL-8, IL-10 and IL-17A in serum and IL-8 in bronchoalveolar lavage fluid are elevated in haematological patients with invasive pulmonary aspergillosis. ECCMID 2017. 2017:Poster #P0989.

77. Rawlings SA, Heldt S, Prattes J, Eigl S, Jenks JD, Flick H, et al. Using Interleukin 6 and 8 in Blood and Bronchoalveolar Lavage Fluid to Predict Survival in Hematological Malignancy Patients With Suspected Pulmonary Mold Infection. Frontiers in immunology. 2019;10:1798.

78. Jenks JD, Rawlings SA, Garcia-Vidal C, Koehler P, Mercier T, Prattes J, et al. Immune Parameters for Diagnosis and Treatment Monitoring in Invasive Mold Infection. Journal of fungi (Basel, Switzerland). 2019;5(4):E116.

79. Hoenigl M, Orasch T, Faserl K, Prattes J, Loeffler J, Springer J, et al. Triacetylfusarinine C: A urine biomarker for diagnosis of invasive aspergillosis. The Journal of infection. 2019;78(2):150-7.

80. Orasch T, Prattes J, Faserl K, Eigl S, Duettmann W, Lindner H, et al. Bronchoalveolar lavage triacetylfusarinine C (TAFC) determination for diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies. The Journal of infection. 2017.

81. Haas H. Fungal siderophore metabolism with a focus on Aspergillus fumigatus. Natural product reports. 2014;31(10):1266-76.

82. Koo S, Thomas HR, Daniels SD, Lynch RC, Fortier SM, Shea MM, et al. A breath fungal secondary metabolite signature to diagnose invasive aspergillosis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;59(12):1733-40.

83. Vidal-Garcia M, Redrado S, Domingo MP, Marquina P, Colmenarejo C, Meis JF, et al. Production of the Invasive Aspergillosis Biomarker Bis(methylthio)gliotoxin Within the Genus Aspergillus: In Vitro and in Vivo Metabolite Quantification and Genomic Analysis. Frontiers in microbiology. 2018;9:1246.

84. Normand AC, Becker P, Gabriel F, Cassagne C, Accoceberry I, Gari-Toussaint M, et al. Validation of a New Web Application for Identification of Fungi by Use of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. Journal of clinical microbiology. 2017;55(9):2661-70.

85. Thompson GR, 3rd, Barker BM, Wiederhold NP. Large-Scale Evaluation of In Vitro Amphotericin B, Triazole, and Echinocandin Activity against Coccidioides Species from U.S. Institutions. Antimicrob Agents Chemother. 2017;61(4).

86. Brilhante RSN, Guedes GMM, Silva M, Castelo-Branco D, Cordeiro RA, Sidrim JJC, et al. A proposal for antifungal epidemiological cut-off values against Histoplasma capsulatum var. capsulatum based on the susceptibility of isolates from HIV-infected patients with disseminated histoplasmosis in Northeast Brazil. Int J Antimicrob Agents. 2018;52(2):272-7.

87. Rodrigues AM, de Hoog GS, de Cassia Pires D, Brihante RS, Sidrim JJ, Gadelha MF, et al. Genetic diversity and antifungal susceptibility profiles in causative agents of sporotrichosis. BMC Infect Dis. 2014;14:219.

88. Borba-Santos LP, Rodrigues AM, Gagini TB, Fernandes GF, Castro R, de Camargo ZP, et al. Susceptibility of Sporothrix brasiliensis isolates to amphotericin B, azoles, and terbinafine. Med Mycol. 2015;53(2):178-88.

89. McHardy IH, Dinh BN, Waldman S, Stewart E, Bays D, Pappagianis D, et al. Coccidioidomycosis Complement Fixation Titer Trends in the Age of Antifungals. J Clin Microbiol. 2018;56(12).

90. Turner S, Kaufman L, Jalbert M. Diagnostic assessment of an enzyme-linked immunosorbent assay for human and canine blastomycosis. J Clin Microbiol. 1986;23(2):294-7.

91. Klein BS, Kuritsky JN, Chappell WA, Kaufman L, Green J, Davies SF, et al. Comparison of the enzyme immunoassay, immunodiffusion, and complement fixation tests in detecting antibody in human serum to the A antigen of Blastomyces dermatitidis. Am Rev Respir Dis. 1986;133(1):144-8.

92. Klein BS, Vergeront JM, Kaufman L, Bradsher RW, Kumar UN, Mathai G, et al. Serological tests for blastomycosis: assessments during a large point-source outbreak in Wisconsin. J Infect Dis. 1987;155(2):262-8.

93. Leimann BC, Pizzini CV, Muniz MM, Albuquerque PC, Monteiro PC, Reis RS, et al. Histoplasmosis in a Brazilian center: clinical forms and laboratory tests. Rev Iberoam Micol. 2005;22(3):141-6.

94. Falci DR, Hoffmann ER, Paskulin DD, Pasqualotto AC. Progressive disseminated histoplasmosis: a systematic review on the performance of non-culture-based diagnostic tests. Braz J Infect Dis. 2017;21(1):7-11.

95. Dantas KC, Freitas RS, da Silva MV, Criado PR, Luiz ODC, Vicentini AP. Comparison of diagnostic methods to detect Histoplasma capsulatum in serum and blood samples from AIDS patients. PLoS One. 2018;13(1):e0190408.

96. de Camargo ZP. Serology of paracoccidioidomycosis. Mycopathologia. 2008;165(4-5):289-302.

97. Perenha-Viana MC, Gonzales IA, Brockelt SR, Machado LN, Svidzinski TI. Serological diagnosis of paracoccidioidomycosis through a Western blot technique. Clin Vaccine Immunol. 2012;19(4):616-9.

98. Wang YF, Cai JP, Wang YD, Dong H, Hao W, Jiang LX, et al. Immunoassays based on Penicillium marneffei Mp1p derived from Pichia pastoris expression system for diagnosis of penicilliosis. PLoS One. 2011;6(12):e28796.

99. Cao L, Chen DL, Lee C, Chan CM, Chan KM, Vanittanakom N, et al. Detection of specific antibodies to an antigenic mannoprotein for diagnosis of Penicillium marneffei penicilliosis. J Clin Microbiol. 1998;36(10):3028-31.

100. Jenks JD, Reed SL, Hoenigl M. Risk factors and outcomes of culture-proven acute Coccidioides spp. infection in San Diego, California, United States. Mycoses. 2020.

101. Buitrago MJ, Bernal-Martinez L, Castelli MV, Rodriguez-Tudela JL, Cuenca-Estrella M. Histoplasmosis and paracoccidioidomycosis in a non-endemic area: a review of cases and diagnosis. J Travel Med. 2011;18(1):26-33.

102. Durkin M, Witt J, Lemonte A, Wheat B, Connolly P. Antigen assay with the potential to aid in diagnosis of blastomycosis. J Clin Microbiol. 2004;42(10):4873-5.

103. Bariola JR, Hage CA, Durkin M, Bensadoun E, Gubbins PO, Wheat LJ, et al. Detection of Blastomyces dermatitidis antigen in patients with newly diagnosed blastomycosis. Diagn Microbiol Infect Dis. 2011;69(2):187-91.

104. Connolly P, Hage CA, Bariola JR, Bensadoun E, Rodgers M, Bradsher RW, Jr., et al. Blastomyces dermatitidis antigen detection by quantitative enzyme immunoassay. Clin Vaccine Immunol. 2012;19(1):53-6.

105. Frost HM, Novicki TJ. Blastomyces Antigen Detection for Diagnosis and Management of Blastomycosis. J Clin Microbiol. 2015;53(11):3660-2.

106. Wheat J, Wheat H, Connolly P, Kleiman M, Supparatpinyo K, Nelson K, et al. Cross-reactivity in Histoplasma capsulatum variety capsulatum antigen assays of urine samples from patients with endemic mycoses. Clin Infect Dis. 1997;24(6):1169-71.

107. Durkin M, Connolly P, Kuberski T, Myers R, Kubak BM, Bruckner D, et al. Diagnosis of coccidioidomycosis with use of the Coccidioides antigen enzyme immunoassay. Clin Infect Dis. 2008;47(8):e69-73.

108. Libert D, Procop GW, Ansari MQ. Histoplasma Urinary Antigen Testing Obviates the Need for Coincident Serum Antigen Testing. Am J Clin Pathol. 2018;149(4):362-8.

109. Wheat LJ, Connolly-Stringfield P, Kohler RB, Frame PT, Gupta MR. Histoplasma capsulatum polysaccharide antigen detection in diagnosis and management of disseminated histoplasmosis in patients with acquired immunodeficiency syndrome. Am J Med. 1989;87(4):396-400.

110. Moreira ALE, Oliveira MAP, Silva LOS, Inácio MM, Bailão AM, Parente-Rocha JA, et al. Immunoproteomic Approach of Extracellular Antigens From Paracoccidioides Species Reveals Exclusive B-Cell Epitopes. Frontiers in microbiology. 2019;10:2968.

111. Thu TC, JFW; Hien HTA; et al, editor Clinical Performance of the Mp1p Immunoassay for Rapid Diagnosis of Talaromyces marneffei Infection. Conference of Retroviruses and Opportunistic Infections; 2017; Seattle, WA, USA.

112. Bialek R, Cirera AC, Herrmann T, Aepinus C, Shearn-Bochsler VI, Legendre AM. Nested PCR assays for detection of Blastomyces dermatitidis DNA in paraffin-embedded canine tissue. J Clin Microbiol. 2003;41(1):205-8.

113. Sidamonidze K, Peck MK, Perez M, Baumgardner D, Smith G, Chaturvedi V, et al. Real-time PCR assay for identification of Blastomyces dermatitidis in culture and in tissue. J Clin Microbiol. 2012;50(5):1783-6.

114. Babady NE, Buckwalter SP, Hall L, Le Febre KM, Binnicker MJ, Wengenack NL. Detection of Blastomyces dermatitidis and Histoplasma capsulatum from culture isolates and clinical specimens by use of real-time PCR. J Clin Microbiol. 2011;49(9):3204-8.

115. Vucicevic D, Blair JE, Binnicker MJ, McCullough AE, Kusne S, Vikram HR, et al. The utility of Coccidioides polymerase chain reaction testing in the clinical setting. Mycopathologia. 2010;170(5):345-51.

116. Thompson GR, Sharma S, Bays DJ, Pruitt R, Engelthaler DM, Bowers J, et al. Coccidioidomycosis: adenosine deaminase levels, serologic parameters, culture results, and polymerase chain reaction testing in pleural fluid. Chest. 2013;143(3):776-81.

117. Vasconcellos I, Dalla Lana DF, Pasqualotto AC. The Role of Molecular Tests in the Diagnosis of Disseminated Histoplasmosis. J Fungi (Basel). 2019;6(1).

118. Rocha-Silva F, Maria de Figueiredo S, Rutren La Santrer EF, Machado AS, Fernandes B, Assuncao CB, et al. Paracoccidioidomycosis: Detection of Paracoccidioides brasiliensis genome in biological samples by quantitative chain reaction polymerase (qPCR). Microb Pathog. 2018;121:359-62.

119. Liu X, Zhang Z, Hou B, Wang D, Sun T, Li F, et al. Rapid identification of Sporothrix schenckii in biopsy tissue by PCR. J Eur Acad Dermatol Venereol. 2013;27(12):1491-7.

120. Hu S, Chung WH, Hung SI, Ho HC, Wang ZW, Chen CH, et al. Detection of Sporothrix schenckii in clinical samples by a nested PCR assay. J Clin Microbiol. 2003;41(4):1414-8.

121. Bernardes-Engemann AR, de Lima Barros M, Zeitune T, Russi DC, Orofino-Costa R, Lopes-Bezerra LM. Validation of a serodiagnostic test for sporotrichosis: a follow-up study of patients related to the Rio de Janeiro zoonotic outbreak. Med Mycol. 2015;53(1):28-33.

122. Hien HTA, Thanh TT, Thu NTM, Nguyen A, Thanh NT, Lan NPH, et al. Development and evaluation of a real-time polymerase chain reaction assay for the rapid detection of Talaromyces marneffei MP1 gene in human plasma. Mycoses. 2016;59(12):773-80.

123. Girouard G, Lachance C, Pelletier R. Observations on (1-3)-beta-D-glucan detection as a diagnostic tool in endemic mycosis caused by Histoplasma or Blastomyces. Journal of medical microbiology. 2007;56(Pt 7):1001-2.

124. Thompson GR, 3rd, Bays DJ, Johnson SM, Cohen SH, Pappagianis D, Finkelman MA. Serum (1->3)-beta-D-glucan measurement in coccidioidomycosis. J Clin Microbiol. 2012;50(9):3060-2.