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ADVANCES IN DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS (O MORRISSEY, SECTION EDITOR)

Update on the Diagnosis of Candidemia and Invasive Candidiasis

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Abstract

Purpose of Review This review summarizes the fungal diagnostic tests and clinical prediction rules available for use in patients at risk for developing invasive candidiasis (IC). The advantages and limitations of each method is described based on available literature.

Recent Findings Available studies show elevated sensitivity and specificity of novel diagnostic tests such as T2Candida and fungal sequencing. They still remain to be incorporated into routine clinical practice.

Summary The use B-D glucan, a major fungal wall component, is a sensitive tool to diagnose IC when consecutive positive results are found. Consecutively negative results allow discontinuation of empirical antifungal therapy, contributing to stewardship programs. T2Candida combines PCR and magnetic resonance to identify IC that is culture negative and monitor post-treatment candidemia clearance. Prediction rules using clinical risk factors for IC allows identification of patients likely to benefit from prophylaxis or early treatment.

Keywords Candidemia · Invasive candidiasis · Fungal diagnosis · Non-culture diagnosis · Fungal PCR · Fungal biomarkers

Introduction

Invasive candidiasis (IC) is a spectrum of disease that comprises candidemia (i.e., *Candida* bloodstream infection) and deep-seated infection arising either from hematogenous dissemination or direct inoculation of *Candida* into the abdominal or pleural cavities [1••]. IC is the most common fungal infection in hospitalized patients, ranking in the top four most common etiologic agents of health-care associated bloodstream infection (BSI) and causing up to 18–22% of episodes [2, 3]. Incidence rates vary between 9.5 and 15 cases per 100,000 persons in population-based studies [4, 5]. Approximately 50% of the candidemia episodes occur among ICU patients. The presence of central vascular catheters, recent surgery (particularly abdominal surgery with anastomotic leakages), the administration of broad-spectrum antibiotic therapy, and the extremes of age constitute major risk factors for IC. A comprehensive list of risk factors is described in Table 1.

At least 17 *Candida* species can cause human disease, but more than 95% of IC are caused by: *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *and C. krusei* [6]. Globally, *C. albicans* is the most prevalent species and accounts for more than 50% of the episodes. The distribution of non-albicans *Candida* spp. has significant geographical variations [1••]. *C. glabrata* is the second cause of candidemia in the USA, northern Europe, and Canada. This pathogen is more common among older patients (> 60 years) with cancer and solid organ transplant. *C. parapsilosis* and *C. tropicalis* are major pathogens in Latin America. *C. parapsilosis* forms biofilms in catheters causing central line infection and outbreaks. *C. tropicalis* is frequently found among patients with hematologic malignancies or stem cell transplant. *C. krusei* is less frequent and occurs in patients with severe immunodeficiency and on prolonged azole therapy [7]. Recently, the multidrug pathogen *C. auris* has emerged as a major threat worldwide [8••].

Candida spp. are commensals in healthy human skin, mucosal surfaces, and gastrointestinal and genitourinary tracts.

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Table 1 Risk factors for invasive candidiasis

Critical illness
Long term ICU stay
Mechanical ventilation
Abdominal surgery (trauma, perforation, repeat laparotomies, anastomotic leakages)
Acute necrotizing pancreatitis
Hematologic (particularly mucositis, graft versus host disease, and profound neutropenia)
Solid neoplasia
Stem cell and solid organ transplantation
Neonates (preterm, low weight, ICU stay)
Older age
Broad spectrum antibiotics
Central vascular catheters, left ventricular-assist device
Total parenteral nutrition
Dialysis (particularly hemodialysis)
Corticosteroids and other immunosuppresants
Cancer chemotherapy
Candida colonization (colonization index > 0.5)
Diabetes
IV drug use

The use of broad-spectrum antibiotics along with immunosuppression resulting from chemotherapy, steroids, or innate immune system impairment leads to *Candida* overgrowth and increased colonization. When a breach in mucosal barriers or skin occurs as is the case for gastrointestinal surgery, invasive procedures, intravascular catheters or mucositis, *Candida* spp. gain access to the bloodstream directly or indirectly after gastrointestinal translocation or ascending pyelonephritis. The degree of iatrogenic immunosuppression results in end organ invasion to the spleen, liver, brain, kidneys, or heart [6]. There are no specific clinical signs and symptoms, merely unexplained fever, sepsis, and systemic inflammatory response syndrome. Early diagnosis is essential since a 12-h delay in antifungal therapy can double mortality [9, 10]. Unfortunately, diagnosing IC is challenging. The current review will describe the characteristics of the different culture and non-culture techniques available for this purpose.

Direct Detection

For direct detection, specimens of blood or tissue from normally sterile sites are cultured or sent to histopathology for microscopic examination.

Culture

The reference standard for IC diagnosis is culture from sterile sites: blood, peritoneal fluid, pleural fluid, etc. Culture enables species identification and susceptibility testing; however, they are insensitive tools. The overall sensitivity of blood cultures is 50% (range 21–71%) in studies of autopsy proven IC [11••]. The median *Candida* concentration in a first positive blood culture is 1 colony-forming unit (CFU)/ml, although 26–65% of positive blood cultures have < 1 CFU/ml. Subjects with a *C. parapsilosis* intravascular source of IC and pediatric patients are more likely to have high organism burden, compared with adults, subjects with *C. glabrata* and extravascular sources which usually have lower burden [12, 13]. *Candida* cells are rapidly eliminated from the bloodstream during the first days of treatment [14].

The median time for blood culture positivity is 2–3 days; but growth can take as long as 8 days.

C. glabrata and *C. parapsilosis* are associated with longer and shorter times to positivity, respectively, probably due to organism burden. The use of lysis centrifugation blood cultures and fungal selective media can increase the detection rate of fungemia and decreases the recovery time of yeasts [15–17].

For non-candidemic IC, the gold standard tests are culture samples collected for culture form infected sites under sterile procedure. The sensitivity is 42% (range 30–61%), and it is limited by the need of surgery or invasive procedures [18]. Tissue or cytology specimens should undergo microscopy and histopathologic examination, using special staining for fungi (e.g., periodic acid-Schiff or Grocott-Gomori methenamine silver) or a fluorescent agent to identify yeasts [1••].

Identification Through Automatized Methods

Species level identification for *Candida* species causing IC is necessary. The interpretation of MICs performed for susceptibility depends on the species. The recent emergence of *C. auris* is another reason to identify *Candida* to the species level.

Species identification can usually be obtained at least 48 h before susceptibility testing results are available. For this purpose, several commercial yeast identification systems are available, such as Vitek-2, BD Phoenix, API-20, MicroScan, and MALDI-TOF [19]. These techniques have a turnaround time of 48–72 h since they require subcultures of specimens. Of note, many commercial automatized methods misidentify *C. auris as C. haemulonii*, *Rhodotorula*, *Saccharomyces cerevisiae*, or other *Candida* sp. Correct identification of C. auris requires the use of MALDI-TOF with updated or "research only" libraries, PCR, or sequencing [19].

Indirect Detection

Antibody Essays

The earliest nonculture diagnostic methods were serum assays for *Candida* antigens and anti-*Candida* antibodies. Mannan and antimannan IgG tests [Platelia Candida Ag-Plus and Ab-Plus Bio-Rad (Marnes-la-Coquette, France and Serio GmbH, Wurzburg, Germany, respectively)] and *C. albicans* germ tube antibody (CAGTA) assays (Vircell kit and VirClia IgG Monotest, Vircell, Grenada, Spain) are used in Europe but are not approved by the Food and Drug Administration (FDA) [11••]. The sensitivity and specificity of mannan and antimannan were 58 and 93% and 59% and 86%, respectively in a meta-analysis of 14 studies. Sensitivity and specificity of a combined mannan/antimannan assay was 83% and 96% respectively. The best performance was seen in patients with *C. albicans*, *C. glabrata*, and *C. tropicalis* infection. Significant heterogeneity among studies included in this meta-analysis was observed [20].

B-D Glucan (BDG) Detection

BDG is an abundant cell wall polysaccharide found in most fungi with the exception of the Mucorales, Cryptococci, and *Blastomyces dermatitidis*. The Fungitell assay (Associates of Cape Cop, Inc., East Falmouth, MA, USA) is the only FDA-approved assay. It is a quantitative, chromogenic immunoassay (EIA) designed to detect BDG using lysed horseshoe crab (Lumulis Polyphemus) amebocytes. These cells initiate the coagulation cascade in the presence of BDG in serum samples [21]. Different BDG assays have distinct performances because they differ in the B-glucan standards used, specimen pretreatment methods, and kit lysates. Several meta-analysis showed the pooled sensitivity and specificity of BDG testing in patients with proven and probable invasive fungal diseases was 80% (95%CI 77–82) and 82% (95%CI 81–83%), respectively. The best diagnostic accuracy has been described for a cutoff value of > 80 pg/mL area under the curve (AUC) of 0.92 or > 60 pg/mL with the European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) EORTC/MSG criteria as the reference standard. Marked heterogeneity among included studies was seen [22••, 23, 24].

The greatest limitation of this test is the large number of conditions associated with false-positive results, such as: albumin infusions, human blood products such as immunoglobulin, coagulation factors, plasma and protein factors, gauze packing, intravenous amoxicillin-clavulanic acid and piperacillin tazobactam, severe mucositis, enteral nutrition, disruption of the GI tract, and systemic bacterial infections. All these factors are highly prevalent in patients at risk for candidiasis [11••]. Also, the one-time use of 96-well trays limits the frequency of batch testing due to elevated cost.

BDG accuracy can be improved when sequential positive results are found: Hanson et al. found ≥ 2 sequential positive results had 100% sensitivity, 75% specificity, and 100% negative predictive value for IC in non-neutropenic critically ill patients [25]. Ellis et al. found 86.8% sensitivity, 81.3% specificity, and 86.5% negative predictive value in patients with neutropenic fever [26]. In the subgroup of patients with deep-seated candidiasis without candidemia, BDG had a sensitivity and specificity of 56–76.7% and 57–92.9% respectively [27–29].

BDG levels correlate with IC prognosis: A baseline BDG level < 416 pg/mL may predict a favorable outcome in patients with IC, with 89% positive predictive value. Patients with a successful treatment outcome B-D glucan levels on serial measurements tend to have negative slope, while serial measurements in patients with treatment failure tend to have a positive slope [30, 31].

Also, BDG has been used as an antifungal stewardship tool, allowing early discontinuation of empirical echinocandin therapy in high-risk ICU patients with consecutively negative results [32•, 33].

T2-*Candida* Detection Panel

T2*Candida* detects *Candida* on whole blood using an automated platform that lyses blood cells, *Candida* cells, and debris by mechanical bead-beating and amplifies DNA using pan-Candida primers for the ribosomal DNA intervening transcribed spacer regions (ITS2) [34]. Additionally, the amplified PCR product induces agglomeration of supra-magnetic nanoparticles, making changes in the T2 relaxation time that are detectable by magnetic resonance. Two hundred fifty blood culture samples from patients referred for a blood culture per routine standard of care were manually supplemented with clinically relevant titers of the 5 *Candida* species targeted by T2Candida: *C. albicans*, *C.glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei.* Overall, sensitivity was 91.1% (95%CI 86.9–94.2%), and specificity was found to be 99.4% (95%CI 99.1–99.6%). The limit of detection was 1 CFU/ml for *C. tropicalis* and *C. krusei*, 2 CFU/mL for *C. albicans and C. glabrata*, and 3 CFU/mL for *C. parapsilosis*. The mean time for species identification is 4.4 h ± 1 h compared to 129.9 ± 26.3 h for the blood culture [35].

The DIRECT2 study enrolled 152 patients after diagnostic blood cultures were reported as positive for *Candida*. When companion blood cultures were positive, T2Candida had a sensitivity of 89%. Of note, 74% of patients received > 1 dose of an antifungal agent prior to obtaining the T2Candida sample. T2Candida may improve the management of candidemia by shortening times to *Candida* detection and species identification compared to blood cultures. A positive T2Candida

with a negative blood culture was found in 24% cases, because it detects nonviable, growth inhibited, or latent *Candida*. Also, T2Candida is likely to diagnose at least some cases of candidemia that would be missed by cultures during preemptive or empirical antifungal treatment. The anticipated positive predictive value (PPV) if T2Candida is measured in any hospitalized patient is 15%; however, in patients at greater risk of candidemia (i.e., those identified through prediction rules), the PPV is > 80%. Of note, the T2Candida instrument may return invalid results due to internal control failures, which can occur in 7–9%. Also, Candidemia caused by species not included in this assay, may go unidentified [36].

T2Candida also outperformed blood cultures in monitoring post-treatment candidemia clearance. The STAMP trial showed T2Candida can detect ongoing fungemia in 33% patients with negative follow-up blood culture [37].

PCR Assays

Multiple commercial and in-house tests have been investigated. Different methodologies, assay, and study design heterogeneity complicate PCR data interpretation. A meta-analysis including 4694 patients showed a pooled sensitivity and specificity of 95% and 92%, respectively. Improved sensitivity was observed when assays with limits of detection < 10 UFC/ml, whole blood rather than serum, and panfungal primers were used [38]. The sensitivity of PCR for intraabdominal candidiasis ranges from 86 to 91% with specificity between 33 and 97% [27, 28, 39]. No PCR assay has been validated or FDA approved. In addition, the turn-around-time varies depending on each assay.

Prediction Rules

Several prediction rules have been designed to identify patients at the highest risk of developing IC who would most likely benefit from prophylaxis or early antifungal therapy (Table 2). The candida score includes total parenteral nutrition, surgery, multifocal *Candida* colonization, and severe sepsis. The main limitation is that establishing colonization status requires culture incubation for 48 h. The *Candida* colonization index requires systematic culturing of non-sterile sites and requires at least 3 out of 5 cultures from different sites with *Candida* growth, making the process labor intensive, long and adding laboratory costs [40–42].

Hermsen et al. validated the performance of the Paphitou and Ostrosky Zeichner rules using a contemporary set of cases and controls from the Nebraska Medical Center. The overall incidence was 2.3%. Sensitivity and specificity for the Paphitou rule was 40% and 80%, respectively, while the Ostrosky-Zeichner rule had a sensitivity of 70% and specificity 60%. The authors developed and additional prediction rule which had the highest AUC, with a sensitivity and specificity of 84.1% and 60.2%, a positive predictive value of 4.7%, and a negative predictive value of 99.4% [43•, 44, 45].

Implementing predictive rules has limitations: each rule was generated and validated in a single center population, and when attempted to validate in a multicenter study, the rules tend to lose sensitivity and specificity. Also, the diversity of patients in specific ICU settings may require individual predictive rule validation. The value of the rules are likely to be in determining patients who would be less likely to benefit from prophylaxis or early treatment, and to discontinue antifungal prophylaxis in the evolution of an antifungal stewardship program [46].

The Future: Whole-Genome Sequencing

Fungal sequencing assays target one or more regions in the multilocus ribosomal RNA (rRNA) genomic locus such as the 18S rRNA, D1, and D2 regions of 28 s rRNA, 5.8S rRNA, and internal transcribed spacers 1 and 2 (ITS1 and ITS2). There is a paucity of studies describing its performance under routine clinical practice [47, 48]. Gomez et al. evaluated the

Table 2 Clinical prediction systems for invasive candidiasis

Rule	Risk factors	Sensitivity	Specificity	PPV	NPV
Paphitou	Hemodyalisis TPN, DM broad spectrum antibiotics	40	91	4.8	98.3
Candida score	TPN, surgery, multifocal colonization, severe sepsis	77	66	13.8	97.7
Ostrosky-Zeichner1	CVC or broad-spectrum antibiotics PLUS 1 of the following: pancreatitis, major surgery, TPN, immunosuppresants, steroids, or dialysis	34	90	10	97
Ostrosky- Zeichner2	Mechanical ventilation and central venous catheter and broad spectrum antibiotics PLUS one of the following: major surgery, pancreatitis, steroids or immunosuppresants, TPN, dialysis	50	83	10	97
Hermsen	Broad spectrum antibiotics, CVC, TPN, steroids, abdominal surgery, pre-ICU length of stay	84	60	4.7	99.4

TPN total parenteral nutrition, *DM* diabetes mellitus, *CVC* central venous catheter, *ICU* intensive care unit

performance of targeted fungal sequencing for diagnosis of IFD in patients with known and suspected IFD. Stored specimens with another positive or negative reference method were used to evaluate sensitivity or specificity. The authors found sensitivity and specificity of fungal sequencing of 96.6% (87.4–99.4%) and 98.2% (89.4–99.9%), respectively. Fungal sequencing was tested in fresh tissue, body fluids, and formalin-fixed paraffin-embedded material. Fungal pathogens including *Candida* were identified in 71.3% of patients classified as proven IFD per the EORTC/MSG criteria. The diagnostic yield of fungal sequencing was 66.6% in body fluids and 67.9% in fresh tissue. The results of fungal sequencing triggered modification of empirical antifungal therapy in 54% with a median time to modification of 2 days [49••].

While DNA sequencing is used by many investigators and many reference laboratories, it is not widely commercialized due to the large capital investment and expertise needed to run a sequence-based assay system. A rapidly developing alternative to rDNA sequencing is whole-genome sequencing (WGS). However, even with falling costs, this technology is still too expensive, complicated, and too slow for routine use in most clinical microbiology laboratories [50]. Sequencing has the potential for false results, as a consequence of inadvertent errors causing sample contamination with environmental DNA during pre-analytical and analytical steps. Contamination with commensals and environmental fungi can result in false-positive results. Also, public databases such as GenBank may contain erroneous or incomplete entries, leading to misidentification of rare fungi. Finally, ITS2 and D2 do not allow species-level identification for all fungi [49••, 51].

Conclusion

Cultures are the reference standard for diagnosing IC; however, they have limited sensitivity and average turn-around-times of 2–3 days. The main advantage is they allow susceptibility testing to guide antifungal therapy. Currently, several non-culture methods exist, including biomarkers, PCR, and T2Candida. The most widely used biomarker is B-D-glucan, when measured in serum it may reach sensitivity and specificity of 80% and 82%, respectively. Several conditions occurring in critically ill patients may lead to false-positive results. Serial measurement of serum B-D glucan improves diagnostic yield and allows antifungal discontinuation in patients receiving empirical antifungal therapy with consecutive negative results. The combination of mannan-antimannan has a comparable performance but is only available in Europe. T2Candida is a novel assay that combines PCR with magnetic resonance in a single test. It detects simultaneously the five most common *Candida* species. This test detects 24% additional cases compared to blood cultures and is more accurate to identify candidemia clearance following antifungal treatment. The highest positive predictive value is found when high-risk patients are tested. Although PCR may be a sensitive and specific test for invasive candidiasis, the lack of standardization limits its use. Finally, fungal sequencing directly from body fluids or tissue is a useful, sensitive technique with promising results. High cost, long turn-around-times, and reduced availability need to be addressed in the near future.

Compliance with Ethical Standards

Conflict of Interest Maria Gonzalez-Lara reports personal fees and other from Pfizer, and personal fees from Grupo Biotoscana outside the submitted work. Luis Ostrosky-Zeichner reports personal fees and other from Pfizer; personal fees from Merck, Scynexis, Astellas, Gilead, The Medicines Company, Cidara, Aradigm, and Bayer outside the submitted work.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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