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

The Evolving Landscape of Diagnostics for Invasive Fungal Infections in Lung Transplant Recipients

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Abstract

Purpose of Review The objective of this paper is to review the armamentarium of tests available for diagnosis of invasive fungal infections (IFI) in lung transplant recipients (LTs), focusing on developments over the last 5 years.

Recent Findings The use of fungal biomarkers is increasing, especially *Aspergillus* galactomannan, which now has an established role in diagnosis and prevention of invasive aspergillosis. Molecular diagnostics are increasingly being applied to tissue and other specimens to assist identification of fungi. Functional imaging has an evolving role, improving diagnostic precision and time to diagnosis.

Summary While demonstration of fungi in tissue obtained biopsy remains the gold standard for diagnosis of IFI in LTs, this is not always possible. There are now a host of biomarkers, molecular, and imaging techniques available that are less invasive and allow earlier diagnosis of IFIs.

Keywords Lung transplant · Fungal · Fungus · Diagnostics

Introduction

Thirty years have passed since the first long-term successful lung transplantation. While survival following lung transplantation has increased over time [1, 2], infections, including invasive fungal infections (IFIs), remain a significant source of morbidity and mortality. During the first 12 months post-transplant, the cumulative incidence of IFI ranges from 3.8 to 16.0% [3–5, 6••, 7]. Although most lung transplant recipients (LTs) with IFI are alive or demonstrate response to antifungal therapy at 12 weeks, overall mortality remains high at 12.9–17.0% [3, 8]; with the highest mortality of 50% associated with mucormycosis [9].

The pathophysiology of IFIs in LT is complex and multifactorial. In the early post-transplant period, breaches in the epithelial barrier, as occurs with surgical incisions, indwelling vascular access and extra-corporeal membrane oxygenation (ECMO) catheters predispose to invasive candidiasis, which most frequently occurs early post-transplant [10, 11]. Within the allograft, factors that predispose to IFI include ischemic injury to the bronchial anastomosis (BA) and distal airways; altered epithelial integrity, ciliary function, and mucus production; denervation which impairs the cough reflex; and necrosis or stenosis of the BA [12–14]. The risk of IFI is increased with the use of T cell–depleting agents to treat allograft rejection [15] and the presence of hypogammaglobulinemia [16]. Cytomegalovirus (CMV) viremia impairs the function of cytotoxic T cell, neutrophil, and macrophage [17] and is associated with an increased risk of IFI in LT [18]. Prerequisite to the development of IFI is of course exposure to the fungus. While all LTs are at risk of ubiquitous fungi such as *Aspergillus*, incidence will vary depending on location, and endemic fungi such as *Cryptococcus*, *Blastomiyces*, *Coccidioides*, and *Histoplasma* must be considered in certain regions. IFI is often preceded by colonization; however, fungal colonization does not always lead to IFI and may be transient [19–21]. Distinguishing

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between colonization and invasive infection remains a challenge for clinicians when a fungus is isolated from a clinical specimen from a LT, but necessary to avoid over-/under-treatment and unnecessary anti-fungal toxicities.

This review will provide an overview of the diagnostic approaches, focusing on recent developments including non-culture-based diagnostic assays. We will predominantly focus on pulmonary IFI, which is the most common IFI site in LTs [3, 10] and concentrate on developments in diagnostics aimed at *Aspergillus, Candida, Cryptococcus, Mucormycosis*, and *Pneumocystis.* Endemic mycoses will not be discussed but remain important etiologies of IFI in LTs patients in these specific regions.

Approach to Diagnosis of Invasive Fungal Infections in Lung Transplant Recipients

The presentation of IFIs in LTs depends on the pathogen and time post-transplant. Invasive candidiasis tends to occur early post-transplant and can present as surgical site infections, vascular access catheter–related infections, and candidemia [4, 10, 11, 22]. Invasive aspergillosis (IA) and other invasive mold infections occur at any time post-transplant. The pulmonary parenchyma and the airways including the BA are the most common sites of infection; however, the central nervous system (CNS), sinuses, and other sites including the eyes, pleural space, skin, and vertebrae may be involved [3, 10, 23]. Dissemination occurs in 10% of IA in LTs [23].

During the early post-transplant period in particular, routine bronchoscopies allow direct visualization and sampling of the airways. Beyond this, the modalities for fungal surveillance for IFIs in LTs are limited. Traditional diagnostic techniques such as microscopy and culture rely on obtaining a clinical specimen. The BA is readily visualized via bronchoscopy, but there is a risk of anastomotic dehiscence associated with biopsy, particularly in the setting of ischemia, recent treatment of rejection and airway infection, especially *Aspergillus* [24, 25]. The overall risk of pneumothorax or significant bleeding from a transbronchial biopsy in LTs is low [26], but the tolerance of such complications needs to be considered in light of the individual patient's condition. The diagnostic yield of any biopsy must be weighed against potential adverse events.

Therefore, a greater emphasis of non-culture-based diagnostics has occurred with the application of biomarkers and molecular diagnostics to specimens obtained via less invasive procedures including blood, exhaled breath condensate (EBC), and bronchoalveolar lavage (BAL) fluid. The various tools for diagnosing IFIs in LTs are demonstrated in Table 1.

Diagnostic Criteria

Given the complex nature of IFIs, the European Organisation for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group Education and Research Consortium (MSGERC) have provided standardized definitions for IFI diagnosis for the purposes of research [27••]. These definitions were updated in 2019 to incorporate advances in the field with a further update of the specific criteria for *Pneumocystis jirovecii* published in 2021 [28•]. The EORTC/MSGERC document outlines three categories, proven, probable, and possible IFI. "Proven" IFI requires the demonstration of the presence of a fungus from a normally sterile site by histopathology, culture, or tissue nucleic acid diagnosis and is not dependent on the immune status of the host. Cryptococcal antigen positivity in the blood or cerebrospinal fluid is the only fungal biomarker that fulfills the mycologic evidence criterion for "proven IFI." The diagnosis of "probable" IFI is specific to immunocompromised hosts (ICH) and requires the presence of at least one clinical feature and mycologic evidence in the setting of specific host factors, depending on the fungus concerned. The category of "possible IFI" is less well defined and infrequently used in the research setting. Whereas the EORTC/MSGERC criteria are not specific for LTs, the International Society for Heart and Lung Transplantation (ISHLT) 2010 consensus statement provides syndrome-based definitions, specific for LTs, for fungal pneumonia, tracheobronchitis, BA infection, and pulmonary colonization [29]. Of note, these definitions are also intended for use in the research rather than for strict application to the clinical setting. In practice, the diagnosis of IFI in LTs can be difficult and the decision to treat must consider the nuances of the host, results of diagnostic tests, and potential toxicities of treatment.

Fungal Microscopy and Culture

Microscopy

Despite recent advances in molecular and other diagnostics, demonstration of fungi in histopathologic examination of tissue and culture remains the gold standard for IFI diagnosis. In the microbiology laboratory, microscopy is performed on the primary clinical specimen, including sputum, BAL or pleural fluid and biopsy tissue, or culture isolates, to aid in the identification of possible fungal isolates. Although important in determining the isolate present, this result per se does not differentiate fungal colonization from invasion.

Table 1 Radiologic and mycologic diagnostic modalities for invasive pulmonary fungal infections in lung transplant recipients

DIAGNOSTIC MODALITY / SAMPLE SOURCE				
Imaging	Blood/Serum	EBC	Sputum, BAL fluid, bronchial brush	Lung biopsy
Least invasive →				→ Most invasive
PROVEN - **EORTC/MSGERC Criteria:** Diagnosis requires the fulfillment of at least one of the diagnostic criteria listed in the row below, independent of clinical presentation - **ISHLT Criteria:** Diagnosis requires histologic evidence or fungal culture from sterile tissue +/- characteristic CT chest or chest xray (not detailed here) findings, independent of clinical presentation				
	Blood culture yielding a mold or yeast (*Cryptococcus, Candida*) **Cryptococcal antigen**		**Immunofluorescence or conventional staining** - Demonstration of *Pneumocystis*	**Microscopic analysis** Histopathologic, cytopathologic or direct examination of respiratory tract biopsy - Hyphae or melanized yeast form (molds) - Encapsulated budding yeasts (Cryptococcus) - Pseudohyphae/true hyphae (Candida) **Tissue nucleic acid** - Amplification of fungal DNA by PCR + DNA sequencing when molds/yeast seen in formalin-fixed, paraffin-embedded tissue **Immunofluorescence or conventional staining** - Demonstration of *Pneumocystis*
PROBABLE - **EORTC/MSGERC:** Diagnosis requires compatible signs/symptoms (not detailed in this table) plus fulfillment of at lease one of the diagnostic criteria listed in the row below - **ISHLT:** Diagnosis requires compatible signs/symptoms with consistent radiology AND positive BAL/blood fungal culture OR at least TWO positive sputum cultures/PCRs demonstrating fungal organisms (excluding *Candida* sp.) OR positive BAL Galactomannan in the setting of negative/no histologic evidence				
Computed tomography (chest) - At least one of: dense, well-circumscribed lesion(s) +/- halo sign, air crescent sign, cavity, wedge-shaped and segmental/lobar consolidation - Any of the above plus reverse halo sign	**Beta-D-Glucan (Fungitell)** [1] -≥80ng/L in 2 consecutive serum samples (Candida and Pneumocystis) **Galactomannan** – Single serum/plasma ≥1.0 (≥0.7 if BAL fluid GM ≥0.8) **Aspergillus PCR** - Detected in plasma/serum/blood on 2 occasions (1 if also detected in BAL fluid)		**Fungal microscopy and culture** - Microscopic detection of fungal elements - Recovery of any mold or *Cryptococcus* by culture **Galactomannan** - BAL fluid ≥1.0 (≥0.8 if serum GM ≥0.7) **Aspergillus PCR** - Detected in BAL fluid with 2 or more duplicate PCR tests positive (1 if also detected in plasma/serum/blood) **Pneumocystis PCR** - Detection of *Pneumocystis jirovecii* DNA by quantitative PCR	
INVESTIGATIONAL/ADJUNCTIVE DIAGNOSTICS				
Computed tomography combined with - FDG-PET - Monoclonal antibodies labelled with PET tracer		**Galactomannan** **Multiplex PCR** **VOC**	**Pentraxin-related protein 3** **JF5 lateral flow assay**	

The diagnostic tests are arranged horizontally by source of specimen and vertically by the EORTC/MSGERC criterion for proven and probable IFI. The ISHLT definitions for proven and probable IFIs are included; however, the diagnostic criteria cross over EORTC/MSGERC categories

BAI bronchial anastomotic infection, *BAL* bronchoalveolar lavage, *CT* computed tomography, *EBC* exhaled breath condensate; *EORTC* European Organisation for Research and Treatment of Cancer, *MSGERC* Mycoses Study Group Education and Research Consortium, *PCR* polymerase chain reaction, *FDG-PET* (18)F-fluorodeoxyglucose positron emission tomography, *VOC* volatile organic compounds

[1]Must exclude alternative etiologies of positive BDG

Color key

Green	Aspergillus
Blue	Pneumocystis
Red	Candida
Orange	*Cryptococcus*
Purple	Multiple fungal species

Culture

An advantage of fungal culture is the ability to speciate and perform antifungal susceptibility testing; however, results can take up to 4 weeks and requires skilled laboratory facilities and personnel. The sensitivity of culture is affected by the source of the sample. In patients with invasive pulmonary aspergillosis (IPA), the sensitivity of culture ranges from 8 to 34% for sputum, 45 to 63% for BAL [30, 31•], and is highest for lung biopsy, particularly when guided by imaging [32•]. In LTs with proven or probable IPA, fungal culture are negative in 40% [33•]. While the fungal agents of mucormycosis grow rapidly under appropriate conditions, their delicate hyphae may be damaged during biopsy and specimen preparation, resulting in negative culture results [34].

Matrix-Assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF)

MALDI-TOF MS is a method to assist microbial identification from a culture specimen, which is fixed in a crystalline matrix on a target plate, bombarded by a laser resulting in vaporization and ionization and then accelerated using a high voltage. The molecular weight of the ions is determined by their time-of-flight to a detector, and a peptide mass fingerprint is generated and compared to a reference library for species identification. The uptake of MALDI-TOF in mycology has been slower than for bacteriology as fungi are biologically more complex than bacteria and can exist in different states (hyphae and/or conidia), complicating identification [35]. Yeasts including *Candida*, *Cryptococcus*, and *Pichia* can be readily identified by MALDI-TOF using standard procedures as used for bacteria [36, 37]. Although inherently more challenging to identify using this method, reference libraries for filamentous fungi have expanded in recent years [38–40] and MALDI-TOF MS has been shown to correctly identify 95.4% of filamentous fungi to the species level [41]. MALDI-TOF requires a culture isolate and cannot be applied to a primary specimen such as BAL fluid (BALF) or biopsy; thus, there is a delay to identification when compared with PCR. Reference libraries must be continuously expanded to maintain the usefulness of MADLI-TOF.

Fungal Biomarkers

The role of fungal biomarkers in the diagnosis of IFI is evolving. While biomarkers may be used to diagnose IFI, they are unable to provide antifungal susceptibilities or species identification.

Galactomannan (GM)

Galactomannan (GM) is a soluble polysaccharide present in the cell wall of *Aspergillus* that is released during growth [42]. Commercial enzyme-linked immunosorbent assays (ELISA) detect GM using a rat monoclonal antibody (MAb) EB-A2 commercial enzyme-linked immunosorbent assay (ELISA) and is an EORTC/MSGERC mycologic criterion for “probable IFI.” In LTs, GM has been evaluated in BALF, EBC, and serum for both diagnosis of IA and in the setting of pre-emptive monitoring.

The utility of BAL GM in LTs for diagnosis has been assessed in several studies. In LTs with invasive pulmonary aspergillosis (IPA), BAL GM has a specificity of 95% at an index cut-off value of ≥ 0.5 and 98% at ≥ 1.0, with a sensitivity of 60% at both cut-off values [43]. The sensitivity of BAL GM increases when the index cut-off value is increased to ≥ 1.5 [44]. Complicating interpretation of BAL GM is the lack of standardization of BALF sampling with considerable variation in method of collection including the volume of fluid instilled and number of sites sampled. Nevertheless, BAL GM continues to have a role in IPA prevention. Husain et al. demonstrated the effectiveness of a pre-emptive strategy using routine surveillance bronchoscopies with BAL fungal culture and GM for LTs not receiving antifungal prophylaxis. They reported $< 1\%$ probability of IPA in LTs with negative BAL GM (cut-off index ≤ 1.0) and negative BAL *Aspergillus* culture [6••].

Bronchoscopy is not always feasible and EBC GM has been evaluated. This method involves exhaled vapor being condensed into a liquid for testing. Bhimji et al. compared EBC GM to BALF GM in 444 matched specimens from LTs and patients with hematologic malignancies. Although GM was detectable in EBC, they were unable to correlate EBC optical density index (ODI) values and IA in LTs [45•]. EBC GM remains a promising diagnostic tool, which merits further assessment including defining cut-off values, which may require adjustment based on the proportion of alveolar air in the sample [46]. Inherent to the method of airway sampling, a positive EBC GM result cannot delineate the site (upper vs lower airway) or extent (colonization vs invasive) of disease.

The presence of GM in the serum may reflect fungal invasion [47]. The relative utility of the test depends on several factors including the population being tested, the number of consecutive positive tests obtained, the index observed, and whether the patient is on antifungal prophylaxis. In a prospective study of 70 LTs, Husain et al. performed twice weekly serum GM screening in LTs during their index and subsequent hospitalizations within 18 months post-transplant [48]. Of LTs, 17.1% developed IPA during the study period. While serum GM was detected (cut-off ≥ 0.5 value) in 29% of LTs with IPA, it was not detected in any cases of *Aspergillus* tracheobronchitis [48], where disease

is localized to the airway. In a subsequent meta-analysis of 27 studies, serum GM was found to be less useful in solid organ transplant (SOT) recipients when compared to patients with a hematologic malignancy [49] and should rarely be tested in LTs.

The *Aspergillus* GM lateral flow assay (GM-LFA) is a rapid immunochromatographic test that uses two MAbs including ME-A5 Mab, which binds to a similar epitope as EB-A2 and an undisclosed MAb to detect GM in serum and BALF. As a point of care test, it can provide a result in less than an hour and does require minimal laboratory support. The GM-LFA was assessed on BALF of 20 LTs with proven/probable IPA. At an ODI cutoff of ≥ 0.5, the sensitivity and specificity of GM-LFA were 40% and 80%, respectively. By increasing the ODI cut-off to ≥ 1.0, the sensitivity remained unchanged but the specificity increased to 95% [50•]

A limitation of the use of GM for the diagnosis of IA is the potential for false positives due to the presence of other fungi (*Penicillium* sp., *Paecilomyces* sp., *Cryptococcus* sp., *Paracoccidioides brasiliensis, Histoplasma capsulatum*) [43, 51]. False positives were previously seen with the use of piperacillin-tazobactam [52], as *Penicillium,* which also contains GM in its cell wall [53], was used for its production; however, this is less of an issue with newer formulations [54].

Cryptococcal Antigen (CrAg)

The CrAg can be detected in whole blood, serum, plasma, or CSF using latex agglutination, lateral flow assays (LFA), or enzyme immunoassay. It is one of the most useful fungal biomarkers with sensitivity and specificity of more than 95% for cryptococcosis [55, 56], which exceeds that of culture, although not specifically evaluated in LTs. The CrAg cannot differentiate between *Cryptococcus neoformans* and *Cryptococcus* gattii, with the former being more common in immunocompromised hosts. False negatives may be seen and are often due to a prozone effect at higher antigen titers, a phenomenon which is not seen when newer, semi-quantitative assays are used [57]. False positives/cross-reactivity can be seen in the presence of IFI due to *Trichosporon* sp., which are uncommon pathogens in LT [58]. Although the CrAg titer correlates with the fungal burden at diagnosis [59], it cannot be used to monitor treatment response as there is no relationship between the rate clearance of the cryptococcal capsule polysaccharide and the killing of yeast by antifungals [60, 61]

Beta-D-Glucan (BDG)

BDG is a cell wall component of many fungi including *Aspergillus*, *Candida*, and *Pneumocystis* but not *Cryptococcus* and *Mucorales.* BDG has a limited role in the diagnosis of IFI in LTs. Several different assays are in use, each with different cut-offs. A recent Cochrane analysis of BDG in ICH and critically ill people documented significant heterogeneity amongst studies, which prevented a formal meta-analysis [62]. The utility of BAL BDG has been assessed in LTs for the diagnosis of IPA and found to have limited utility due to poor sensitivity (71–80%) and specificity (38–81%) [63–65] and thus not recommended. BDG is present in the wall of the cystic but not trophic form of *P. jirovecii* [66•]. In a recent meta-analysis found, the pooled sensitivity and specificity of serum BDG testing for PJP were 91% and 79%, respectively [66•]. The overall sensitivity was higher in patients with HIV (94%) compared to non-HIV patients (86%). The authors of this meta-analysis concluded that BDG was only useful to exclude PJP when the pre-test probability was low [66•], which is likely the best use of this test in LTs. Further limitations on the use of BDG in in LTs is the propensity of false positives due to cross reactivity with components of some beta-lactam antibiotics, hemodialysis filters, and immunoglobulins [67], which are often used post-transplant.

Molecular Diagnostics

The advantage of molecular fungal diagnostics is the potential for higher sensitivity and more rapid diagnosis when compared to microscopy and culture. When used on tissue biopsies, fungal PCR and/or sequencing allows for species identification and is a mycologic criterion for "proven IFI" when consistent fungi are seen on histopathology of the same tissue biopsy. For other specimen types, fungal DNA detection by PCR is a "probable" mycologic criterion, reflecting the inability of PCR to differentiate between IFI and fungal colonization or environmental contamination There are many commercial fungal PCRs available, which have either broad range (panfungal) or species-specific targets. Below is a brief summary of the relevant PCRs available for fungal diagnostics with many lacking data specific in LTs.

Panfungal PCR

Panfungal PCR assays utilize universal fungal primers with targets that include at least one of the D1/D2 regions of the 28 s gene and internal transcribed spacers 1 and 2 (ITS1 and ITS2). While the specificity of panfungal PCR is high across the spectrum of specimen types from BAL to formalin-fixed tissue paraffin embedded tissue, the greatest sensitivity is reported for sterile site specimens, such as tissue and blood [68•, 69•]. The diagnostic yield is improved when tissue is obtained by open biopsy [70] and when corresponding histopathology is consistent with IFI [69•]. With the ability to detect broad range of fungal

pathogens, another benefit of panfungal PCR testing is the potential to detect rare fungi. This is particularly important for LTs many of whom remain on antifungal prophylaxis long-term, which may result in perturbations of the pulmonary mycobiome and increased susceptibility to atypical or emerging fungal pathogens.

Multiplex PCR

Multiplex PCRs differ from panfungal PCR in that they contain a panel of specific fungal primers in one reaction and can be considered a more targeted test with negative results only excluding those pathogens examined for. Importantly, this assay would exclude common contaminants, e.g., *Penicillium* species that may be detected using a ITS-based approach. Although it can be used on all specimen types, it has mostly been used to confirm fungal pathogens on biopsy specimens. In a proof-of-concept study, Bhimji et al. compared testing of EBC with the Luminex multiplex xTAG fungal ASR assay to conventional BAL GM and fungal culture. While fungal DNA was detected in EBC, the sensitivity and specificity were 38.9% and 97.6% compared to BAL fungal culture and 15.6% and 100% compared to BAL GM [71]. The low sensitivity compared to other diagnostics limits the current clinical usefulness of the multiplex fungal PCR assay for this sample type. However, given the non-invasive nature of EBC collection, it is an appealing specimen type and requires further assessment to establish its role, if any, in diagnosis of colonization and/or IFI in LTs.

PCR for Aspergillus

Several PCR tests for *Aspergillus* spp. with different primers and PCR formats have been developed; however, there are few studies specifically in LTs. The sensitivity of pan-*Aspergillus* PCR performed on BALF in LTs with IPA is higher than GM (80–100% vs 60–88%) with a similar specificity (74–93% for PCR and 71–89% for GM) [33•, 72]. In LTs with *Aspergillus* colonization, *Aspergillus* is more likely to be detected on PCR than by GM or culture; thus, cautious interpretation is required to avoid over-treatment [33•]. In both the screening and diagnostic settings, a combination of tests improves the diagnostic yield; however, the additive benefit of *Aspergillus* PCR to GM and culture in LTs requires further evaluation, particularly considering the cost of the test and limited availability.

Some commercial *Aspergillus* PCRs include targets for resistance associated mutations, for example, in the CYP15A gene, which allow for rapid genotypic identification of resistance, permitting earlier modification of antifungal therapy [73•, 74]. In LTs, this may be of greatest utility when fungal colonization or infection is identified in the peri-transplant period, to guide post-transplant antifungal choice, especially in cystic fibrosis patients where prior antifungal exposure has been associated with development of antifungal resistance [75].

PCR for Pneumocystis jirovecii

PCR for the detection of *Pneumocystis* is becoming increasingly common due to improved sensitivity when compared with microscopy [76], reduced time to result and laboratory limitations such as lack of personnel skilled in microscopy. BALF is the preferred specimen for *Pneumocystis* detection to maximize sensitivity and reduce contamination from upper respiratory tract colonization [77]; however, induced sputum is an appropriate alternative. Several meta-analyses, none specifically in LTs, have demonstrated that the sensitivity of *Pneumocystis* PCR is 97–99% and specificity 90–94% [78–80]. Quantitative assays perform better than qualitative assays [78, 79] although no “burden” cut-offs have not been established for differentiation between colonization and infection and hence are not included in diagnostic criteria [28•]. Irrespective of these testing interpretation uncertainties, lifelong prophylaxis is currently recommended as the incidence, and significance of asymptomatic *Pneumocystis* colonization in LTs has not been established [81••]. Quantitative *Pneumocystis* PCR on BALF, in addition to imaging and clinical features, can be interpreted in conjunction with serum BDG testing to differentiate between *Pneumocystis* colonization and infection [82].

Molecular Tests for Candida

Molecular assays available for *Candida* have been mostly applied to blood samples to improve sensitivity and time to diagnosis, compared to culture, particularly when fungal burden is low [83]. Assays generally target the five most common *Candida* sp. (*C. albicans, C. glabrata, C. parapsilosis* complex, *C. tropicalis*, and *C. krusei*). In a meta-analysis of 54 studies, PCR sensitivity for blood specimens for proven/probable candidiasis was 95% and specificity 92%, with specificity reducing in the setting of *Candida* colonization [84].

The T2 Magnetic Resonance (T2MR) assay is an all-in-one automated system that lyses red blood cells, amplifies *Candida* DNA, and then detects amplified product by amplicon-induced agglomeration of supermagnetic particles. The time to result is 3–5 h and T2MR has a negative predictive value for candidemia of 99% [85]. It is not designed for use on non-blood samples, where *Candida*

colonization and contamination would impact the specificity. Evaluation of these assays in deep-seated intra-thoracic invasive candidiasis is lacking, and the impact of antifungal prophylaxis on sensitivity has not been assessed; thus, their role in diagnosis of IC in the early post-transplant period requires further evaluation.

PCR for Mucorales

Mucorales can be identified to the species level using a variety of different targets (ribosomal 18S, 28S and internal transcribed spacer, FTR1 gene, cytochrome B) [86] either by panfungal or Mucorales-specific PCR. This can help to overcome some of the challenges associated with diagnosis including lack of typical morphologic criteria for some species and when the organism fails to grow on culture but is visualized on microscopy. When applied to BALF from immunocompromised patients, primarily those with hematologic malignancy and pulmonary infiltrates, Mucorales PCR followed by high-resolution melt analysis demonstrated a sensitivity of 100% and specificity of 93% [87]. The utility of PCR for detection of Mucorales in LTs, where the fungal burden and incidence may be lower, has not been studied.

Imaging Modalities

Computerized Tomography (CT)

CT is the usual initial imaging modality in the diagnostic work-up of IFI in LTs, and the sensitivity is greater than that of plain radiographs [88]. Classic CT findings of IPA such as the halo sign, air-crescent sign, and well-defined nodular lesions are less commonly seen in non-neutropenic patients, including LTs. IPA may present as ground-glass opacities, consolidation, cavitation, and tree-in-bud opacities [23, 89]. Multiple lesions and pleural effusions are more commonly seen with pulmonary mucormycosis than IPA, the former classically presents as consolidation or as a nodule/mass surrounded by a reverse halo [90, 91]. CT of the chest is useful for diagnosis of *Pneumocystis* pneumonia, where plain radiographs may be normal. Findings of *Pneumocystis* are not specific to LTs and typically include bilateral ground-glass opacities and interstitial infiltrates and may progress to pulmonary nodules, consolidation and/or pleural effusions [92–94].

Magnetic Resonance Imaging (MRI)

magnetic resonance imaging is useful for detecting cns ifis [95], but has a limited role in pulmonary ifi due to poor air-tissue contrast due to the lack of detectable protons in air-filled spaces [96].

Functional Imaging

Imaging modalities that combine anatomic depiction with specific functionality are a potential way to improve the diagnostic sensitivity of imaging for IFI. The addition of positron emission tomography (PET) with fluorodeoxyglucose (FDG) to CT improves the sensitivity, across the spectrum of IFI, compared with CT alone, allows detection of occult dissemination and can be used to guide duration of therapy [97–103]. The use of PET-CT using radiolabelled siderophores, such as iron-scavenging siderophores labelled with ^{68} Ga or ^{89}Zr for detecting IA, is gaining attention but lacks specificity [104•]. Monoclonal antibodies against fungal pathogens, including *Aspergillus, Scedosporium, Fusarium*, and *Lomentospora*, labelled with a PET tracer combined with MRI or CT may improve sensitivity and specificity but require further evaluation. The functional imaging modalities described have not been specifically evaluated in LTs.

Novel and Emerging Diagnostic Tests

Monoclonal Antibodies

JF5 is a monoclonal antibody that binds to an extracellular glycoprotein secreted during growth by *Aspergillus* sp. and can be detected by lateral flow assay (LFA). In a recent meta-analysis, BALF JF5 LFA performed better than serum for detection of IA with a pooled sensitivity and specificity in BALF of 86 and 93%, respectively [105]. LTs have been included in mixed cohort studies but JF5 LFA has not been specifically evaluated in LTs [106]. Limitations of the test relevant to LTs include reduced sensitivity in the setting of antifungal use [106], and cross-reactivity with *Penicillium* sp. [107].

Pentraxin-Related Protein 3

Pentraxin 3 (PTX3) is a TNF-inducible gene 14 protein of the pentraxin superfamily, which includes serum amyloid P-component and C-reactive protein. It is produced locally by a variety of cells in response to inflammatory cytokines produced by inflamed or injured tissue. For example, in renal allografts with acute rejection–increased PTX3 expression, which decreases with treatment of rejection, has been demonstrated [108]. In patients with IFI, circulating PTX3 levels are elevated and subsequently normalized with antifungal treatment [109]. Kabbani et al.

examined PTX3 levels in a retrospective study of 322 BALF samples from LTs, which included 15 events of IPA, 38 instances of *Aspergillus* colonization, and 17 cases of GM positive/*Aspergillus* culture negative [110•]. PTX3 levels were significantly higher in those with IPA, compared to those with *Aspergillus* colonization and healthy controls [110•]. PTX3 is not specific to IPA and elevated levels are seen with other etiologies of pneumonia [110•, 111]. PTX3 may be a useful adjunct when there is uncertainty about the presence or absence of invasive diseases in the setting of other BAL indicators of the presence of *Aspergillus*.

Volatile Organic Compounds (VOCs)

VOCs are low molecular mass organic substances that vaporize at room temperature [112]. Human breath contains > 3000 VOCs, the composition of which is altered in various disease states including infection [113]. During growth, fungi produce a specific VOC "signature." The role of VOCs in pathogenicity of fungi is unclear, but they may have a role in diagnosis. The detection of exogenous fungal metabolites in the breath of hematology SOT patients with IPA has been described [114, 115]. In a small study of cystic fibrosis patients colonized with *A. fumigatus*, distinctive breath print patterns were demonstrated in 89% [116]. Early experience with VOCs is promising; however, in order to be clinically useful in LTs, a database of fungal molecular VOC "fingerprints" is required and the sensitivity/specificity of VOCs for detecting IFI and colonization in LTs examined, including the potential to differentiate between the two states, for which there is currently no data.

Conclusions

Over the past three decades, the field of lung transplantation has progressed significantly in terms of improved survival and the complexity of the patients being offered transplantation. However, IFI continues to threaten morbidity and mortality in LTs and the optimum strategy for prevention has not been established [117–119]. While biopsy, microscopy, and culture remain the gold standard for diagnosis, this approach is invasive and relatively slow to yield results. We expect the field to progress with greater use of non-invasive specimens and a combination of highly specific molecular tests to rapidly speciate fungi and provide genotypic susceptibilities and biomarkers and imaging to assist differentiation between colonization and invasive disease. To achieve these advances in precision diagnostics, large-scale studies of IFI diagnostics in LTs are required.

Declarations

Conflict of Interest TM and SVH do not have any conflicts to disclose.

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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