ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 11, Issue, 08, pp.6098-6101, August, 2019*

**DOI: https://doi.org/10.24941/ijcr.35703.08.2019**

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

## RESEARCH ARTICLE

# DIAGNOSTIC CHALLENGES IN FUNGAL INFECTIONS IN IMMUNOCOMPROMISED INFECTED INDIVIDUALS

***Somya Sinha**

Indira Gandhi Institute of Medical Science, India

**ARTICLE INFO**

*Article History:*
Received 12th May, 2019
Received in revised form
16th June, 2019
Accepted 20th July, 2019
Published online 31st August, 2019

*Key Words:*

AIDS, HIV, IFT,
PCR, FISH.

**Corresponding author:* **Somya Sinha**

**ABSTRACT**

There has been an enormous increase in the frequency and severity of fungal infection inrecent years. This increase has been driven ina large part by two factors. First, the global AIDS epidemic has fostered the emergence of life-threatening infections by the opportunistic fungi Cryptococcus neoformans and Pneumocystisjiroveci and by other fungi such as Histoplasma capsulatum and Penicilliummarneffei. These infections occur most oftenin resource-limited countries in Africa, SouthAmerica, and Southeast Asia. Second, advancesin medical care and treatment have led to increases in the number of opportunistic infections in patients who are immunocompromised drugs or chemotherapy, or who are infected by way of treatment with immunosuppressive agents. This review lays emphasis on the older and newer methods for fast diagnosis of fungi so that the benefits of modern antifungal treatment are availed and not misused.



***Citation: Somya Sinha,* 2019.** "Diagnostic challenges in fungal infections in immunocompromised infected individuals", *International Journal of Current Research*, 11, (08), 6098-6101.

## INTRODUCTION

In recent years, fungal infections are on the rise due to various predisposing factors such as long term administration of antibiotics, use of steroids, pulmonary tuberculosis, immunosuppressive drugs and HIV infection (Chen *et al.,* 2013). *Candida* species are one of the potentially pathogenic fungal agents in patients with broncho-pulmonary disease. They are associated with secondary infections in tuberculosis patients (Pfaller and Castanheira, 2016). When host resistance is lowered, these unrecognized opportunistic fungi may affect the progress of disease or may even become fatal1 (World Health Organization, 2014). Hence, there is need to consider the possible importance of these saprophytic organisms when they are found repeatedly and evidently from the site of the lesion. *Candida albicans* (*C.albicans*) was considered the most important pathogen causing secondary infection inpulmonary tuberculosis. *C.albicans* stimulated growth of *M. tuberculosis* of reduced viability (Yu, 2011). Diagnosis of invasive fungal disease (IFD) is challenging because current diagnostic method slack sensitivity and specificity, or take too long to yield a result to be clinically useful. Such limitations have consequences; delayed diagnosis leads to delayed treatment. Speed to diagnosis is a key risk factor in patient outcomes (Barnes 2008). Diagnosis of fungal infection is further complicated by problematic developments in the field of medical mycology due to loss of senior mycologist and the impact of AIDS in causing monumental rise I various opportunistic fungal infections (Chen *et al.,* 2013).

These patients are susceptible to infections from fungi rarelyseen, or never reported as a human pathogen, which can cause identification problems for even the most experienced mycologists. Whereas mycologists in the past needed to be ableto identify _50 commonly encountered fungi, and _300 total fungi that were pathogenic for humans, the number of potential fungal pathogens is likely many times what is described in textbooks, and will continue to grow as the severely immunosuppressed patient population continues to grow (Ajello and Hay 1998). Diagnosis of fungal infection has relied primarily on methods such as direct microscopic examination of clinical samples, histopathology, and culture. Such approaches are dependent on personnel with relatively high levels of specific mycology training. The growth in the number of fungi that clinical mycologists must identify has forced investigators to develop and apply new methods for fungal identification that go beyond classical phenotypic methods. As a consequence, there is an increased emphasis on the use of molecular methods and antigen detection as surrogates for culture in diagnosis of fungal infection.(1 and 3)

### Culture, Direct Microscopy, and Histopathology

Culture, direct microscopy, and histopathology have been the foundation for diagnosis of fungal infection for many decades. Microscopy, histopathology, and use of fungal-specific stains play important roles in diagnosis of infection by C. neoformans, P. jirovecci, Candida spp., Aspergillusspp., H. capsulatum, Blastomyces dermatitidis, Coccidioides immitis,

Sporothrix schenckii, Paracoccidioides brasiliensis, and the Mucorales. Sensitivity of microscopy for diagnosis of fungal infection varies with the individual agent, the source and quality of the specimen, and the skills and experience of the laboratorian. Finally, diagnosis of invasive fungal infection by direct microscopy and histopathology may require the use of biopsies of deep tissues, which poses a risk to those patients who are most susceptible to invasive disease. Culture from a clinical sample is the gold standard for diagnosis of fungal infection. Culture has the advantage of yielding the specific etiological agent if positive. Moreover, culture allows for susceptibility testing. However, use of culture for diagnosis of IFD has significant limitations. Culture may take many days to a result with several of the filamentous fungi. In the case of disseminated candidiasis, blood culture (Chen *et al.,* 2013) may miss _50% of patients with documented disease (Fraser *et al.,* 1992; Ostrosky-Zeichnerand Pappas 2006; Ostrosky-Zeichner 2012), may only become positive late in infection (Ellepola and Morrison 2005), and typically takes 24–72 h for identification of Candida in aclinical sample too long for early treatment. Positive blood culture is rare in invasive aspergillosis and is most often owing to environmental contamination (Kontoyiannis *et al.,* 2000). Recovery of H. capsulatum from sputum of patients with acute pulmonary histoplasmosisranges from 10% to 15%; however, in cavitaryhistoplasmosis, sputum cultures are positive inup to 60% of patients (Deepe 2010).

In patients with pulmonary blastomycosis, sputum culture or culture of specimens obtained by bronchoscopy has a high yield (86% per patient for sputum culture and 92% for broncoscopy) (Chapman and Sullivan 2010). Culture of *Coccidioides* spp. is complicated by the biosafety hazard associated with culture of the mycelial form. Making and Excluding the Diagnosis of *Pneumocystis* Pneumonia in AIDS *Pneumocystis* pneumonia (PCP) in AIDS is often diagnosed empirically based on a sub acute onset of cough; breathlessness out of proportion to abnormalities seen on chest radiographs; and subtle, bilateral changes seen on chest radiographs, in the context of a low CD4 cell count (Figure 3). Co-trimoxazole (trimethoprim/sulfamethoxazole, Bactrim, Septrin) is the most effective agent for prevention and therapy of PCP. A low dose is effective for prophylaxis, but a 3-week course of high and potentially toxic doses is required for effective therapy. The differential diagnosis of PCP is broader in children because bacterial pneumonia is more common among them. If a precise diagnosis could be achieved in most cases of PCP, much of the inappropriate use of co-trimoxazole could be prevented. Currently, bronchoscopy and microscope examination of bronchoalveolar lavage fluid is the most common definitive means of establishing a diagnosis of PCP; this method has a sensitivity of 75%–90%, depending on the microscopy technique (Global Action Fund for Fungal Infections, 2015; Arendrup, 2010). *P. jirovecii* fungus is nonculturable in routine laboratories; in Europe, it is commonly molecularly detected using PCR, which has a sensitivity of 95%–99% (Neofytos *et al.,* 2009). *Pneumocystis* PCR performed on expectorated sputum is also effective for detecting *P. jirovecii* fungus (Kontoyiannis *et al.,* 2010), but this method is infrequently used. For children who are breathless, PCR of nasopharyngeal aspirates is currently the only realistic means of establishing a diagnosis. 1,3 β-D-glucan is detectable in the serum of nearly all patients with PCP (Tarrand *et al.,* 2005); if a sample is negative, the infection can be ruled out (Pickering *et al.,* 2005). Misdiagnosis of Smear-Negative Pulmonary Tuberculosis as Tuberculosis: Tuberculosis is one of the diseases that cause high morbidity and mortality in the world, particularly in developing country (Bansod, 2008). One –third of the human population is infected with mycobacterium tuberculosis and every year about two million persons die of it (Vannberg, 2008). The disease is treated with antibiotics on immunosuppressive agent which predisposes tuberculosis patients to immunocompromised and so susceptible to fungal infections (phukan, 2000). The fungal *Candida spp* colonise the oral cavity as commensals but becomes pathogenic in immunocompromised individuals (Pattons, 2002). Deep Candidal infection rarely occurs in healthy host. This situation may be increased in tuberculosis patients whose natural immune system is directly affected by the use of immune-suppressive drugs (Mukadi, 1993) (World Health Organization, 2014; Arendrup, 2010; Neofytos *et al.,* 2009). Smear-negative pulmonary tuberculosis (TB) is a problematic area for clinicians and policymakers. Post-TB sequelae are common, are poorly studied, and may be mistaken for active, recurrent TB (Pickering *et al.,* 2005). An apparent under recognized issue for patients with smear-negative TB is chronic pulmonary aspergillosis (CPA), which can mimic the signs and symptoms of TB. In 544 patients in the United Kingdom who had previously received treatment for TB with a residual cavity, precipitating antibodies to *Aspergillus fumigatus* developed in 24.6% at 2 years and in 34.0% at 5 years. Within 2 years, aspergilloma, a late stage of CPA, developed in 78 (58%) of the 134 patients with precipitating antibody to *A. fumigatus* (Saccente and Woods, 2010). Few prospective studies have been conducted on CPA after treatment for TB, so the incidence of such cases cannot be stated with certainty; conservatively, however, a rate of ≈10% among survivors of pulmonary TB is likely and a global prevalence of ≈1.2.The use of new, highly sensitive, DNA detection assays (e.g., Xpert MTB/RIF) directly on respiratory specimens has transformed the rapidity of detecting positive samples, but there remain millions of unwell, smear-negative, PCR-negative patients. Some of these patients have relapsed after anti-TB therapy, and CPA has developed subsequent to cured TB. Among HIV-positive persons, those with smear-negative TB test results have a higher death rate than those with smear-positive results (Kontoyiannis *et al.,* 2010; Pickering *et al.,* 2005), probably because many do not have TB at all. It is increasingly recognized that many of these patients are chronically infected with *Aspergillus* spp., resulting in CPA that is largely undiagnosed and untreated.

**Serology for fungi**

A recentmeta-analysis indicated that-glucan assay performed on serum has a sensitivity and specificity of 94.8% and 86.3%, respectively, for the diagnosis of *Pneumocystis* pneumonia (Pickering *et al.,* 2005; Saccente and Woods, 2010), while a large retrospective cohort showed that a positive-glucan test correlates well with BAL fluid fungal loads. Therefore, _-glucanassay can be an excellent screening tool to rule out the disease inat-risk populations, while additional confirmatory tests are necessary because of the high rate of false-positive results. *Cryptococcus* spp. are known to affect primarily immunocompromised individuals, such as people with HIV infection, with the exception of *Cryptococcus gattii*, which is notorious for its ability to cause disease in immunocompetent patients. The main characteristic of all *Cryptococcus* spp., which is the basis for the majority of current diagnostic tests, is

the polysaccharide capsule, which contains the glucuronoxylomannan antigen. Cryptococcalmeningitis, the most common presentation of cryptococcal disease, is diagnosed primarily with CSF cultures, which grow creamcoloredmucoid colonies within 3 to 7 days. The most accurate screening method, however, is the cryptococcal antigen test. The test has a high sensitivity and specificity when performed with CSF (97% and 93 to 100%, respectively), while it also has the advantage that it can be performed on serum, with acceptable sensitivity (87%), when CSF isnot available (Hage *et al.,* 2011). False-positive findings have been reported in cases of *Trichosporon* sp., *Capnocytophaga* sp., or *Stomatococcus* sp. invasive infections (Arendrup, 2010). The dimorphic fungi *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis* share many similar characteristics in morphology and the clinical picture of the infections that they can cause. Indeed, antibody tests that use complement fixation (the most common) or immunodiffusion are available for *Histoplasma* spp., although their sensitivities are not ideal (75% for disseminated cases and 66.7% for acute pulmonary histoplasmosis) (100). Similarly, in the case of blastomycosis, antibody tests have low sensitivities, although immunodiffusionis more sensitive and specific than complement fixation (Arendrup *et al.,* 2014). Importantly, cross-reactivity of these antigen tests seems to be a problem, and although they are specific when tested against nonfungal pathogens, they cannot differentiate between *H. capsulatum*, *B. dermatitidis*, and *C. immitis*, despite the fact that the antigen level is generally higher in cases of disseminated histoplasmosis than in cases of other endemic mycoses (Pickering *et al.,* 2015). Each of the nonmolecular assays (cultures, histopathology, and biomarker assays) provides a piece of information to aid clinicians with diagnosing fungal infections. Taking into consideration that IFIs are difficult to diagnose and that any delay in treatment initiation could lead to a steep rise in mortality rates, newer diagnostic assays with high negative predictive values, such as-glucan or galactomannan assay, should be evaluated in clinical decision algorithms for the ability to serve dual purpose.

## MATERIALS AND METHODS

Molecular methods, the most important of which is PCR, are used every day in routine clinical practice and have replaced traditional diagnostic procedures for a variety of human infections (Global Action Fund for Fungal Infections, 2015). Their simplicity, ease of use, and short turnaround time are among their most important advantages over traditional techniques. PCR is one of the oldest and most widely used molecular methods in fungal diagnostics. A major drawback of all traditional PCR techniques initially developed as potential fungal diagnostic tests is that they do not quantify the amount of amplified DNA. Fungal organisms, and especially molds, have strong cell walls that are particularly difficult to lyse, thus requiring complex and cumbersome methods for DNA isolation (Hummel *et al.,* 2010). Examples of lysis techniques utilized are enzymatic digestion processes that often rely on use of toxic chemicals, such as phenol-chloroform, mechanical disruption using glass beads, and sonication (Badiee *et al.,* 2012). In an effort to overcome this barrier, automated extraction methods have been developed that are able to decrease the time for sample processing and lessen the possibility of errors (Kawazu *et al.,* 2004; Hummel *et al.,* 2010). Another problem associated with fungal PCR is the potential for contamination. Fungi are ubiquitous in the environment and can easily contaminate surfaces and materials used in all steps of fungal PCR, including commercially available reagents and collection tubes (Kawazu *et al.,* 2004). Therefore, careful precautions and highly experienced personnel are necessary to avoid false-positive findings associated with contaminants. Furthermore, without international standards, it is difficult to assess the agreement of quantitative data from different tests and thus to determine the clinical significance of various levels of fungal DNA. Finally, the choice of primers is another important factor that could alter the diagnostic performance of PCR tests for IFIs. For invasive candidiasis. Multiple studies have evaluated the performance of PCR tests for the diagnosis of invasive *Candida* infections in patient populations (World Health Organization, 2015; McMullan *et al.,* 2012). Despite the promising reports of detection of *Candida* spp. By PCR, much effort should be made to standardize the method and decrease the inconsistencies between different tests. An important and ongoing debate is focused on the choice of specimen type on which to conduct the PCR test. Indeed, serum, whole blood, and plasma have all been used for *Candida* sp. An alternative approach is to use PCR to identify *Candida* spp. directly from blood culture bottles (15) using MALDITOF and multiplex – PCR techniques which significantly decrease the time for species identification from apositive blood culture to 96 h. In fact, an early study showed that a multiplex real-time PCR assay was able to identify the isolated *Candida* spp. in less than 2 h, and the results were 100% concordant with results of nonmolecular methods (Badiee *et al.,* 2012).

### New diagnostic methods

Fluorescence *in situ* hybridization (FISH) is a technique that uses fluorescent probes to identify target areas on the genomes of microbial pathogens in human samples, which can then be detected by fluorescence microscopy. This method has been used as an adjunct to culture or PCR and has been proven to have. Nucleic acid sequence-based amplification (NASBA) is a method very similar to PCR but differs in the sense that it amplifiesm RNA by using an RNA polymerase instead of DNA, and it is isothermal. High accuracy for the identification of *Candida* sp. infections from blood culture bottles (20).They reported a threshold for detection of 1 CFU per 100 microlitre of whole blood.

### Conclusion

It is undoubtedly true that current gold standards for IFI diagnosis are lacking in both sensitivity and rapidity, thus delaying treatment and undermining survival of patients at risk. This underscores the need for the development of faster and more accurate diagnostic tests. Although novel serologic and molecular methods for detection and identification of fungal pathogens have been developed and are showing the potential to replace traditional diagnostic assays, inconsistencies between different approaches limit their reproducibility and prohibit large-scale clinical implementation. Thus, much effort should be made to standardize these techniques and ensure their reliability in order to significantly improve our ability to detect and treat fungal pathogens in an effective and timely manner. With continued emergence of new methods, we are reminded that

fungal diagnostics is still in its infancy, with much room for improvement and refinement (Fan *et al.,* 2013).

## REFERENCES


Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O, ESCMID EFISG Study Group and ECMM. 2014. ESCMID/ECMM joint clinical guideline for the diagnosis and management of rare invasive yeast infections. Clin. Microbiol. Infect. 20(Suppl 3):76–98. http://dx.doi.org/10.1111/1469-0691.12360.

Arendrup MC. 2010. Epidemiology of invasive candidiasis. Curr Opin Crit Care. 16:445–52. http://dx.doi.org/10.1097/MCC.0b013e32833e84d2.36.

Arendrup MC. 2010. Epidemiology of invasive candidiasis. *Curr Opin Crit Care,* 16:445–52. http://dx.doi.org/10.1097/MCC.0b013e32833e84d2

Badiee P, Alborzi A, Karimi M, Pourabbas B, Haddadi P, Mardaneh J, Moieni M. 2012. Diagnostic potential of nested PCR, galactomannan EIA, and beta-D-glucan for invasive aspergillosis in pediatric patients. J. Infect. Dev. Ctries. 6:352–357. http://dx.doi.org/10.3855/jidc.2110.

Chen Y, Wang H, Kantarjian H, Cortes J. 2013. Trends in chronic myeloid leukemia incidence and survival in the United States from 1975 to 2009. Leuk. Lymphoma 54:1411–1417. http://dx.doi.org/10.3109/10428194.2012. 745525.

Ellis M, Al-Ramadi B, Bernsen R, Kristensen J, Alizadeh H, Hedstrom U. 2009. Prospective evaluation of mannan and anti-mannan antibodies for diagnosis of invasive Candida infections in patients with neutropenic fever. *J. Med. Microbiol.,* 58:606–615. http://dx.doi.org/10.1099/jmm.0.006452-0.

Fan L-C, Lu HW, Cheng K-B, Li HP, Xu JF. Evaluation of PCR in bronchoalveolar lavage fluid for diagnosis of *Pneumocystis jirovecii* pneumonia: a bivariate meta-analysis and systematic review. PLoS One. 2013;8:e73099. http://dx.doi.org/10.1371/journal.pone.0073099

Global Action Fund for Fungal Infections, 2015. Roadmap–executive summary. Improving outcomes for patients with fungal infections across the world: a road map for the next decade. http://www.gaffi.org/roadmap.

Global Action Fund for Fungal Infections. Roadmap–executive summary. Improving outcomes for patients with fungal infections across the world: a road map for the next decade. May 2015 [cited 2015 May 10]. http://www.gaffi.org/roadmap/

Hage CA, Kirsch EJ, Stump TE, Kauffman CA, Goldman M, Connolly P, Johnson PC, Wheat LJ, Baddley JW. 2011. Histoplasma antigen clearance during treatment of histoplasmosis in patients with AIDS determined by a quantitative antigen enzyme immunoassay. *Clin. Vaccine Immunol.,* 18:661–666. http://dx.doi.org/10.1128/CVI.00389-10.

Hummel M, Spiess B, Cornely OA, Dittmer M, Morz H, Buchheidt D. 2010. *Aspergillus* PCR testing: results from a prospective PCR study within the AmBiLoad trial. Eur. J. Haematol. 85:164–169. http://dx.doi .org/10.1111/j.1600-0609.2010.01452.x.

Kawazu M, Kanda Y, Nannya Y, Aoki K, Kurokawa M, Chiba S, Motokura T, Hirai H, Ogawa S. 2004. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1¡3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J. Clin. Microbiol.,* 42:2733–2741. http://dx.doi.org./10.1128/JCM.42.6.2733-2741.2004.

Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ, Ito J, Andes DR, Baddley JW, Brown JM, Brumble LM, Freifeld AG, Hadley S, Herwaldt LA, Kauffman CA, Knapp K, Lyon GM, Morrison VA, Papanicolaou G, Patterson TF, Perl TM, Schuster MG, Walker R, Wannemuehler KA, Wingard JR, Chiller TM, Pappas PG. 2010. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin. Infect. Dis.,* 50:1091–1100. http://dx.doi. org/10.1086/651263.

McMullan BJ, Halliday C, Sorrell TC, Judd D, Sleiman S, Marriott D, Olma T, Chen SC. 2012. Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory. PLoS One 7:e49541. http://dx.doi.org/10.1371/journal.pone.0049541.

Neofytos D, Horn D, Anaissie E, Steinbach W, Olyaei A, Fishman J, Pfaller M, Chang C, Webster K, Marr K. 2009. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. *Clin. Infect. Dis.,* 48:265–273. http: //dx.doi.org/10.1086/595846.

Pfaller MA, Castanheira M. 2016. Nosocomial candidiasis: antifungal stewardship and the importance of rapid diagnosis. *Med Mycol.,* 2016; 54:1–22.

Pickering JW, Sant HW, Bowles CAP, Roberts WL, Woods GL. 2005. Evaluation of a (1¡3)-beta-D-glucan assay for diagnosis of invasive fungal infections. *J. Clin. Microbiol.,* 43:5957–5962. http://dx.doi.org/10.1128/JCM.43.12.5957-5962.2005.

Saccente M, Woods GL. 2010. Clinical and laboratory update on blastomycosis. *Clin. Microbiol. Rev.,* 23:367–381. http://dx.doi.org/10.1128 /CMR.00056-09.

Tarrand JJ, Han XY, Kontoyiannis DP, May GS. 2005. Aspergillus hyphae in infected tissue: evidence of physiologic adaptation and effect on culture recovery. *J. Clin. Microbiol.,* 43:382–386. http://dx.doi.org/10.1128/JCM.43.1.382-386.2005.

World Health Organization. Antimicrobial resistance global report on surveillance 2014 [cited 2016 Apr 30]. http://www.who.int/drugresistance/documents/AMR_report_Web_slide_set.pdf

World Health Organization. Global action plan on antimicrobial resistance. 2015 [cited 2016 Feb 16]. http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf

Yu VL. 2011. Guidelines for hospital-acquired pneumonia and health-care-associated pneumonia: a vulnerability, a pitfall, and a fatal flaw. *Lancet Infect Dis.,* 11:248–52. http://dx.doi.org/10.1016/S1473-3099 (11)70005-6


*******