HIV-ASSOCIATED OPPORTUNISTIC FUNGAL INFECTIONS: A GUIDE TO USING THE CLINICAL MICROBIOLOGY LABORATORY

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This review aims to provide a guide for clinicians to using the clinical microbiology laboratory for management of common HIV-associated opportunistic fungal infections, e.g. mucosal candidiasis, cryptococcosis, *Pneumocystis jirovecii* pneumonia (PCP), histoplasmosis, etc. Laboratory tests provide valuable guidance at various stages of management of HIV-infected patients with fungal infections: (*i*) establishing a diagnosis, (*ii*) guiding appropriate antifungal therapy in selected circumstances, (*iii*) providing laboratory prognostic markers, (*iv*) monitoring response to therapy; and (*v*) detecting relapses. However, the laboratory is not always able to provide reliable answers to clinically relevant questions, and these limitations must be considered in the interpretation of test results.

The HIV pandemic has contributed greatly to the emergence of opportunistic fungal pathogens and increased incidence of opportunistic fungal infections over the last two decades;[1] the morbidity and mortality impact is especially pronounced in sub-Saharan Africa. A systematic review of African cohort studies and selected cross-sectional studies in HIV-infected patients reported incidence rate ranges for three common fungal opportunistic infections: oral candidiasis (3.8 - 43.8 cases/100 person-years-observation (PYO)), cryptococcosis (0.3 - 4 cases/100 PYO) and *Pneumocystis jirovecii* pneumonia (PCP) (0 - 0.5 cases/100 PYO).[2] Cryptococcosis caused 44% of deaths in a cohort of HIV-infected South African miners and was the third commonest infectious cause for hospitalisation.[3] South African adult and paediatric studies have shown that PCP accounts for 20 - 52% of community-acquired pneumonia in hospitalised HIV-infected patients.[4] This review aims to provide a guide for clinicians to using the clinical microbiology laboratory for the management of common HIV-associated opportunistic fungal infections. Close interaction with the local laboratory is recommended to facilitate awareness of the spectrum of laboratory tests on offer (Table I), to guide appropriate specimen collection and to assist with interpretation of laboratory test results.

MUCOSAL CANDIDIASIS

Candidiasis almost always presents as mucocutaneous disease in HIV-infected patients; the disseminated form of disease only occurs in the presence of additional predisposing factors.[5] Oropharyngeal candidiasis (OPC) is one of the commonest HIV-associated opportunistic fungal infections, occurring at CD4+ T-cell counts as high as 300 cells/µl. OPC is usually diagnosed clinically by visualisation of the typical white pseudomembranous (Fig. I) or hypertrophic forms. Laboratory diagnosis, by examination of mucosal scrapings, is useful for less typical forms, e.g. erythematous OPC. Oesophageal candidiasis (OC) occurs at lower CD4+ T-cell counts (<100 cells/µl). The diagnosis is usually made clinically (dysphagia or odynophagia in the presence/absence of OPC). Endoscopic examination and mucosal biopsy (or brushings/scrapings) to confirm the diagnosis is reserved for failure of empiric therapy or for severe disease requiring hospitalisation.[6] Vulvovaginal candidiasis (VVC) in HIV-infected women occurs at all CD4+ T-cell counts with manifestations that are similar to those in immunocompetent women. Laboratory diagnosis may be considered in cases where complicated vulvovaginitis (infection with *Candida* species other than *C. albicans*) is possible, e.g. prior chronic use of fluconazole in HIV-infected women.[7] Antifungal susceptibility testing (AST), although well standardised for a number of organism-drug combinations with validated interpretive breakpoints,[8] is not routinely indicated for mucosal candidiasis. AST may be requested in treatment-refractory cases,[9] if the laboratory has the capacity to perform testing using a standardised method.

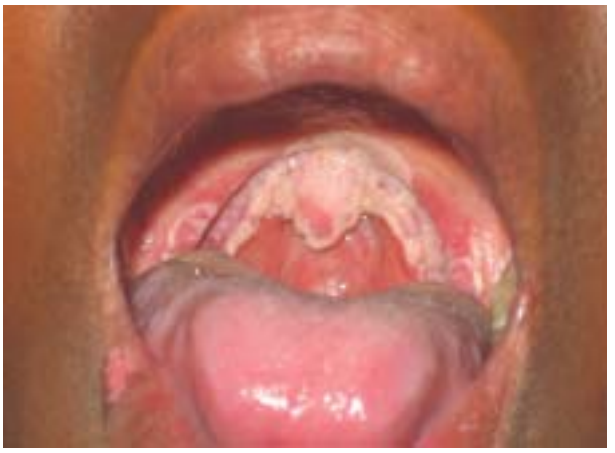

Fig. 1. Pseudomembranous oropharyngeal candidiasis in an HIV-infected adult patient (courtesy Dr Kerrigan McCarthy).

TABLE I. DIAGNOSIS OF COMMON HIV-ASSOCIATED OPPORTUNISTIC FUNGAL INFECTIONS

Fungus	Common site/s of infection	When is it appropriate to use the laboratory for diagnosis?	Clinical specimen	Appropriate laboratory test	Comments
Candida species	Oropharyneal, oesophageal and vulvovaginal mucosa	Atypical forms	Mucosal scrapings, brushings or biopsy	Microscopy	Provides a rapid presumptive diagnosis
				Culture	Provides confirmation of diagnosis and species identification which may guide choice of antifungal therapy within days
Cryptococcus species	Meninges and brain	All suspected cases	Cerebrospinal fluid	Microscopy (India ink)	Provides a rapid presumptive diagnosis. Sensitivity and specificity is good in a high prevalence setting (compared with culture)
				Culture	Provides confirmation of diagnosis but may be slow (up to 2 weeks in some cases)
				Antigen test	Rapid and is highly sensitive and specific (compared with culture) Not universally available in South African laboratories
			Blood (or serum)	Culture	Provides confirmation of diagnosis but is slow (up to 2 weeks)
				Antigen test	Rapid and highly sensitive and specific (compared with culture) Not universally available in South African laboratories
Pneumocystis jirovecii	Pulmonary	All suspected cases	Induced sputum, bronchoalveolar lavage, lung biopsy, etc.	Direct immunofluorescence microscopy	Sensitivity improves as the test is performed on more invasively obtained respiratory tract specimens Not universally available in South African laboratories
Histoplasma capsulatum	Disseminated (skin, blood, bone marrow, etc.)	All suspected cases	Skin biopsy, blood, bone marrow biopsy, etc.	Microscopy	Provides a rapid presumptive diagnosis
				Culture	Provides confirmation of diagnosis but is very slow (up to 6 weeks)
			Urine or serum	Antigen test	Rapid and has excellent sensitivity and specificity – especially with urine specimens Not available in South African laboratories at present
Dermatophyte infections	Superficial (cutaneous)	Atypical forms	Skin scrapings, nail and hair clippings	Microscopy	Provides a rapid presumptive diagnosis
				Culture	Provides diagnostic confirmation but is slow (weeks)

CRYPTOCOCCOSIS

Acquired immune deficiency syndrome (AIDS)-associated cryptococcosis usually presents at low CD4+ T-cell counts (<100 cells/µl); the median CD4+ T-cell count at time of initial diagnosis in a Ugandan cohort study was 17 cells/µl.[10] Although the clinical manifestations of symptomatic incident disease are varied, meningoencephalitis is the commonest presentation. Ninety-seven per cent of laboratory-confirmed incident cases, detected by population-based surveillance for cryptococcosis in Gauteng, presented with symptoms or signs of meningoencephalitis.[11] However, an autopsy study of South African miners has shown that cryptococcal pneumonia may be more common than previously thought and under-diagnosed in life.[12] Cerebrospinal fluid (CSF), submitted to a laboratory for diagnosis of a symptomatic incident episode, may be subjected to a number of tests which provide differing levels of diagnostic sensitivity and specificity compared with the reference standard method (culture), which provides a definitive diagnosis. These tests are widely utilised because culture of *Cryptococcus* species is not universally available in South African laboratories as a diagnostic tool. Most cryptococcal isolates will be detected on standard fungal and bacterial media within 48 - 72 hours, but longer periods (up to 2 weeks) may be required to produce visible colonies, particularly if the fungal burden is low (early disease or prior antifungal therapy).[13] The sensitivity of the inexpensive India ink staining technique (Fig. 2) compared with culture has been estimated to be 98% in an antiretroviral therapy (ART)-naïve South African population (compared with a somewhat lower sensitivity (79 - 88%) in developed countries in the pre-ART era[14,15]); this may be related to the late presentation of patients with a high CSF fungal burden and/or experienced laboratory personnel.[11] The cryptococcal antigen (CrAg) test is available in two formats: latex agglutination assay and enzyme-linked immunosorbent assay (ELISA). The more widely available latex agglutination test (Fig. 3), usually performed on either CSF or serum, has excellent sensitivity (93 - 100%) and specificity (93 - 98%) compared with culture for diagnosis of incident cryptococcosis.[16] Rare false-negative[17-21] and false-positive[22-33] CrAg test results have been reported. CrAg specificity may be improved when titres ≥8 are reported as positive by the laboratory.[34] It may be most cost-effective to reserve use of the CrAg test for diagnosis of incident episodes where the India ink test is negative despite a compatible clinical and laboratory picture (CSF CrAg) or if a lumbar puncture is contraindicated (serum CrAg).[35] The CrAg test can provide semi-quantitative results; titres of >1 024 at baseline (which correlate with baseline CSF cryptococcal colony-forming units) have been associated with increased risk of death at 10 weeks.[36,37] Many South African laboratories provide only qualitative results for the CrAg test. Culture of blood, using standard automated blood culturing systems, e.g. BacT/Alert (Biomerieux Inc., Durham, NC), may provide additional evidence for the initial diagnosis of cryptococcosis. However, the sensitivity (73%) is somewhat lower than CSF culture.[11] Screening HIV-infected patients at risk for cryptococcal infection with the serum CrAg test remains an unresolved issue, especially related to management of patients with isolated positive antigenaemia.[38] Whereas there is some evidence for screening in high-prevalence settings,[34,39,40] no benefit has been shown in low-prevalence settings.[41,42] Monitoring CrAg titres following diagnosis of incident disease is not recommended.[43-45] Diagnostic challenges become more pronounced in patients receiving ART.[46] The interpretation of the serum CrAg test result in patients who become CrAg test negative after antifungal treatment, discontinue secondary antifungal prophylaxis and revert to CrAg test positivity is unclear.[47] Similarly, the utility of the CrAg test in detecting immune reconstitution inflammatory syndrome (IRIS)-associated culture-negative episodes of cryptococcosis remains uncertain.[48] AST of *Cryptococcus* species is complex; however, a reference method has been developed.[8] Commercial tests, e.g. E-test (AB Biodisk, Solna, Sweden), produce minimum inhibitory concentration (MIC) results with reasonable concordance to the reference method.[49] However, when the E-test is used to predict fluconazole MICs, 1 - 2-fold higher MICs are produced compared with the reference CLSI method (Beth Arthington-Skaggs – personal communication). It is important to note that interpretive breakpoints, which allow

Fig. 2. India ink preparation of cerebrospinal fluid from a patient with cryptococcal meningitis showing a budding yeast cell of Cryptococcus neoformans *surrounded by the characteristic large capsule (courtesy Professor David Ellis, Kaminski's Digital Image Library, http://www.mycology.adelaide.edu.au).*

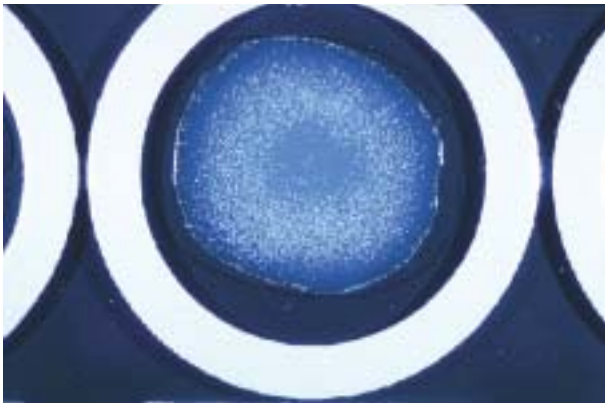

Fig. 3. Cryptococcal latex antigen test showing a positive agglutination reaction (courtesy Professor David Ellis, Kaminski's Digital Image Library, http://www.mycology.adelaide.edu.au).

the MIC to be categorised as 'susceptible' or 'resistant', are not clearly defined owing to limited clinical outcome data.[50,51] Given that 99% of MIC results of amphotericin B in a global survey clustered below 1 µg/ml,[52] it should be evident that testing amphotericin B is of little value in routine practice. There may be a limited role for investigation of fluconazole 'resistance' in recurrent cryptococcal episodes where the laboratory has the capacity to perform a well-standardised quality-controlled test on both incident and recurrent isolates in parallel.[35] Interpretation of these AST results requires expert input.

PNEUMOCYSTIS JIROVECII PNEUMONIA

PCP, caused by the recently reclassified human fungal pathogen *P. jirovecii*, rarely occurs in patients with CD4+ T-cell counts >250 cells/µl.[53] The diagnosis of PCP in patients with AIDS is facilitated by the relatively higher organism burden in the lungs compared with non-AIDS patients; however, diagnosis may be impeded by lack of clinical suspicion, concurrent use of prophylactic drugs and polymicrobial lung infection in patients with AIDS.[54] *P. jirovecii* cannot be cultured *in vitro*. Laboratory diagnosis hinges on microscopic examination of the fungus using techniques which reveal the cyst, trophic or both forms. The cyst form may be visualised using the Gomori methenamine silver, toluidine blue O, cresyl echt violet or calcofluor white stains; trophic forms are visualised by modified Papanicolaou, Gram-Weigert or Wright-Giemsa stains. Monoclonal antibody techniques (e.g. immunofluorescence assay (IFA)), which can detect both forms, are now widely used because of improved sensitivity and specificity compared with conventional stains in induced sputum samples.[54] Optimal respiratory tract specimen collection facilitates diagnosis: the sensitivity and specificity of the IFA was determined to be 90.6% and 100% respectively for sputum specimens and 96.3% and 100% respectively for bronchoalveolar lavage specimens in one study.[55] Induced sputum, which is less invasively obtained than a bronchoscopically obtained specimen yet is superior to expectorated sputum, is recommended as a first-line specimen to diagnose PCP in patients with AIDS.[54,56] Clinicians often treat for PCP empirically in symptomatic patients at risk of infection; however, widespread laboratory test availability and improved laboratory turnaround time should facilitate use of laboratory diagnostic tests. Use of the polymerase chain reaction (PCR) to amplify fungal DNA directly from clinical specimens remains an investigational tool, which is not currently widely available. PCR is more sensitive than conventional methods and may be able to detect colonisation by the organism in asymptomatic HIV-infected patients.[57] PCR may also be used to investigate genetic mutations which potentially confer resistance to trimethoprim-sulfamethoxazole.[58]

HISTOPLASMOSIS

Histoplasmosis, an AIDS-defining illness since 1987,[59] is caused by the dimorphic fungus *Histoplasma capsulatum*. Only the 'classic' var. *capsulatum* form has been described in South Africa.[60] The progressive disseminated form of histoplasmosis (PDH), the commonest manifestation in AIDS patients, occurs at CD4+ T-cell counts <50 cells/µl[53] and may mimic disseminated tuberculosis.[61] Culture provides a definitive diagnosis (Fig. 4); however, *H. capsulatum* is a slow-growing pathogen requiring up to 6 weeks.[62] Various specimens may be submitted for culture: (*i*) specimens which can be obtained relatively non-invasively, e.g. biopsies of skin/mucosal lesions, blood or urine, should be submitted in all cases; and (*ii*) deeper tissues, e.g. bone marrow, may be submitted, if indicated, to improve the diagnostic yield. It is important to note that appropriate blood culturing systems are required to detect *H. capsulatum*, e.g. Isolator lysis-centrifugation system (Wampole Laboratories, Cranbury, NJ). Automated blood culturing systems like BACTEC (Becton Dickinson, Sparks, Md) and BacT/Alert (Biomerieux Inc., Durham, NC) do not yield growth reliably.[63] The high organism load in AIDS patients facilitates a high sensitivity (urine 95%, serum 86%) and specificity (99%) of the *H. capsulatum* antigen test (MiraVista laboratories, Indianapolis, USA) for diagnosis of incident disease. This antigen test is extremely useful to monitor response to therapy and detect relapses; titres correspond closely to the clinical course of disease.[64] The *H. capsulatum* antigen test is available to South African clinicians through MiraVista laboratories (Indianapolis, USA) (Joe Wheat – personal communication); however, the high cost of the test, international shipping of the specimen and delayed turnaround time may be prohibitive from a clinical diagnostic standpoint. Serological diagnostic tests have limited sensitivity (up to a quarter are negative) in immunocompromised patients and are not widely available. Many clinicians rely on histopathological methods to provide a tentative diagnosis; however, it is important to realise that sensitivity is low (<50%), false positives may be reported because it may be difficult to distinguish other yeasts or staining artifacts from *H. capsulatum*, and persistence of non-

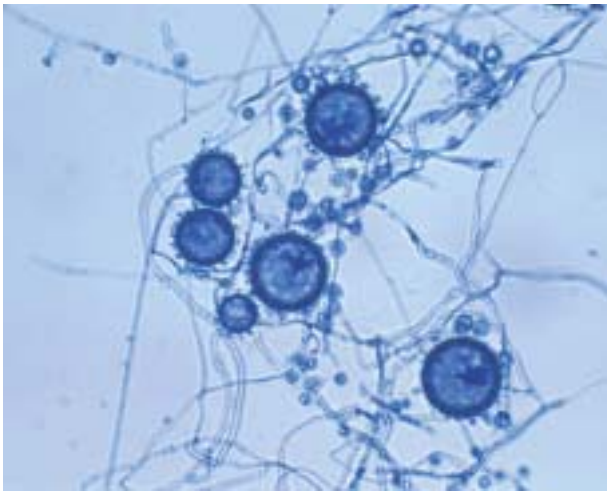

Fig. 4. Microscopic view of the mycelial form of Histoplasma capsulatum *showing the characteristic tuberculate macroconidia formed on short condiophores (courtesy Professor David Ellis, Kaminski's Digital Image Library, http://www.mycology.adelaide.edu.au).*

viable *H. capsulatum* yeasts in tissue for prolonged periods after acute infection may complicate the diagnosis.[64]

OTHER FUNGAL OPPORTUNISTIC INFECTIONS

Dermatophyte infections have not increased in incidence in HIV-infected patients,[65] but may present with: (*i*) a more widespread body distribution, e.g. tinea capitis in adults (Fig. 5); (*ii*) more disfiguring lesions, e.g. hyperkeratotic lesions; (*iii*) atypical lesions, e.g. 'anergic' tinea corporis lacking typical central clearing and defined elevated borders; and (*iv*) poor clinical response to therapy.[66] The laboratory can assist with the definitive diagnosis of atypical or non-responsive dermatophytosis, if keratinised tissue samples are provided, e.g. skin scrapings, nail or hair clippings. Invasive aspergillosis (IA) is rare in patients with AIDS, usually requiring the presence of additional immunocompromising factors, e.g. neutropenia or high-dose corticosteroids, to occur.[5] Diagnosis of IA is definitively established by demonstration of hyphae in tissue in the presence of clinically compatible disease. Colonisation of the airways may be a 'red herring' and limits the usefulness of respiratory tract specimen culture for diagnostic purposes. Disseminated sporotrichosis, presenting as cutaneous, osteoarticular, pulmonary or meningeal disease, has been infrequently diagnosed and reported in South African AIDS patients.[65] A presumptive diagnosis of sporotrichosis may be established by visualisation of the typical 'cigar-shaped' yeasts in tissue specimens; culture of the slow-growing fungus from tissue or body fluids will confirm the diagnosis. Endemic and tropical mycoses, e.g. blastomycosis and eumyectoma, have not increased over time parallel to HIV incidence rates in South Africa.[65] Mycoses that are AIDS-defining in other regions of the world, e.g. *Penicillium marneffei* in South East Asia, have not been reported in South African AIDS patients.[65]

CONCLUSIONS

Laboratory tests can provide valuable guidance at various stages of management of HIV-infected patients with fungal infections: establishing a diagnosis, guiding appropriate antifungal therapy in selected circumstances, providing laboratory prognostic markers, monitoring response to therapy and detecting relapses. However, there are important limitations that require consideration. Current laboratory methods are not always able to provide a definitive diagnosis owing to limited sensitivity and specificity. Tests that provide a qualitative result are not always able to distinguish fungal colonisation from disease. Antifungal susceptibility testing, although standardised for some pathogenic fungi, provides complex challenges for the laboratory with regard to performance and interpretation; the laboratory is not easily able to provide a 'susceptible' or 'resistant' answer to guide management.[67]

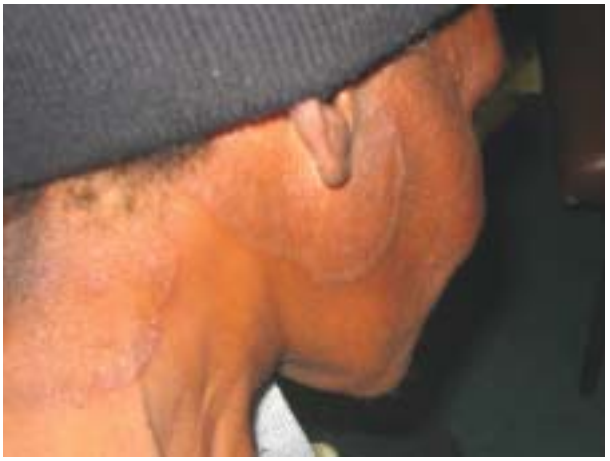

Fig. 5. Superficial dermatophyte infection of the skin in an adult HIV-infected patient (courtesy Dr Kerrigan McCarthy).

Conflicts of interest

None to declare.

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