Current status of serious fungal infections in Nigeria

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy in the Faculty of Faculty of Biology, Medicine and Health.

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Abbreviations

ABPA Allergic broncho-pulmonary Aspergillosis
AAFB Acid alcohol fast facilli
AIDS Acquired immune deficiency syndrome
AmB Amphotericin B
ART Antiretroviral therapy
BAL Broncho-alveolar lavage
BD β-D glucan
CCPA Chronic cavitary pulmonary aspergillosis
CD4 Cluster of differentiation 4
CF Cystic fibrosis
CFPA Chronic fibrosing pulmonary aspergillosis
CIE Count Immuno-electrophoreisis
CMV Cytomegalovirus
CNPA Chronic necrotizing pulmonary aspergillosis
CNS Central nervous system
COPD Chronic obstructive pulmonary disease
CPA Chronic pulmonary aspergillosis
CrAg Cryptococcal antigen
CRP C-reactive protein
CXR Chest X-ray
DD Double diffusion
Dx Diagnosis
EDTA Ethylenediaminetetraacetic acid
EIA Enzyme Immunoassay
ELISA Enzyme linked immunosorbant assay
EORTC European Organization for Research and Treatment of Cancer
ESR Erythrocyte sedimentation rate
FEIA Flouroenzyme immunoassay
GDP Gross domestic product
GM Galactomannan
GvHD Graft versus host disease
LUTH Lagos University Teaching Hospital
<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>HA</td>
<td>Haemagglutination</td>
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<tr>
<td>HAI</td>
<td>Hospital acquired infection</td>
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<tr>
<td>Hcc</td>
<td><em>Histoplasma capsulatum</em> var. <em>capsulatum</em></td>
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<tr>
<td>Hcd</td>
<td><em>Histoplasma capsulatum</em> var. <em>duboisii</em></td>
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<td>HCWs</td>
<td>Health care workers</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>HSCT</td>
<td>Hematopoietic stem-cell transplants</td>
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<tr>
<td>IA</td>
<td>Invasive aspergillosis</td>
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<tr>
<td>IC</td>
<td>Immunocompromised</td>
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<td>ICI</td>
<td>Invasive <em>Candida</em> infections</td>
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<tr>
<td>ICU</td>
<td>Intensive care unit</td>
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<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IRIS</td>
<td>Immune reconstitution inflammatory syndrome</td>
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<tr>
<td>JCRC</td>
<td>Joint clinical research centre</td>
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<tr>
<td>LFD</td>
<td>Lateral flow device</td>
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<td>MRC</td>
<td>Medical Research Council</td>
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<td>NIMR</td>
<td>Nigeria Institute for Medical Research</td>
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<tr>
<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<tr>
<td>NPA</td>
<td>Nasopharyngeal aspirate</td>
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<td>OI</td>
<td>Opportunistic infection</td>
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<tr>
<td>PEPFAR</td>
<td>US President Emergency Plan for AIDS Relief</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>RAST</td>
<td>Radioallergen absorbent test</td>
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<td>SAFS</td>
<td>Severe asthma with fungal sensitization</td>
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<td>SCT</td>
<td>Stem cell transplant</td>
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<td>TB</td>
<td>Tuberculosis</td>
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<tr>
<td>TIMM</td>
<td>Trends in Medical Mycology</td>
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<td>UHSM</td>
<td>University Hospital of South Manchester</td>
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<tr>
<td>UK</td>
<td>United Kingdom</td>
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<td>USA</td>
<td>United States of America</td>
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Abstract
Fungal infections are ignored by social and political communities. However, they are estimated to affect more than a billion people, resulting in approximately 11.5 million life-threatening infections in the ‘at risk’ population and more than 1.5 million deaths annually. Though there have been huge advances in diagnostics and antifungal drug development over the past two decades, however, resource limited settings have not benefited from these advances. The aim of this research was to determine the burden of serious fungal infections in Nigerians with the appropriate underlying diseases.

This epidemiological research was conducted across four study populations. Study 1; HIV-infected patients with CD4+ counts <250 cells/mm³, irrespective of their ART status, a CrAg lateral flow assay was used for detecting cryptococcal antigenaemia (n=214). Study 2; a cross-sectional multicentre survey of TB patients being managed for smear negative or treatment failure TB irrespective of their HIV status (n=208). Study 3; a multicentre histoplasmin skin sensitivity survey amongst healthy HIV-infected and non-HIV infected participants; intradermally; induration ≥5 mm was considered to be histoplasmin positive (n=750). Study 4; a prospective cohort study of critically ill patients in a Nigerian ICU (n=71). Two retrospective studies to analyse the clinical picture of serious fungal infections in two at risk populations (HIV/AIDS and neonatal intensive care babies) in Nigerians was also conducted (n=7034; n=2712 respectively).

Results revealed an overall seroprevalence of cryptococcal antigenemia of 8.9% with 6 (9.8%) in those with CD4+ cell counts <100 cells/mm³, 4 (5.0%) in the 100-200 group and 9 (12.3%) in 200-250 cells/mm³ group; a CPA prevalence of 8.7% (6.5% had HIV infection and 14.5% were HIV-negative) and a prior subclinical histoplasmosis of 4.4%. The ICU study revealed a 45% healthcare associated infection rate representing an incidence rate of 79/1000 patient-days in the ICU. The retrospective studies revealed a 2.3% rate of neonatal ICI with a case fatality rate of 18.5%. In the 12 years retrospective study 18% had a fungal OI with 88% of patients having initiated ART.

In conclusion, serious fungal infections do occur in the at risk population in Nigeria and they constitute a significant public health challenge. Our findings demonstrate that there has been an underestimation of the burden of the problem in Nigerians. There is a dire need to design guidelines for the management of fungal infections in at risk population.
Declaration

The author has not submitted any portion of the work referred to in the thesis in support of an application for another degree or qualification of this or any other university or other institute of learning. In papers 1, 2, 3, 4, and 7, I was the first author; I designed the studies under the supervision of Prof Denning and Prof Richardson, performed laboratory processes, analysed the data with Dr Foden under the supervision of Prof Denning, and wrote the manuscripts. The adult ICU study which resulted in paper 5 ‘Incidence, Clinical Outcome and Risk Factors of Intensive Care Unit Infections in the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria’ was used by Dr Anthony Iwuafor for his Part 2 dissertation at the National Postgraduate College of Medicine of Nigeria in 2014, I was the co-supervisor of the work with Prof Ogunsola. I was fully involved in the design and conceptualisation of the study, laboratory processes, data collation, data analysis, writing and revision of the manuscript. Paper 6 is a retrospective study and I was fully involved in the design and conceptualisation of the study, data analysis, writing and revision of the manuscript; however, I am the second author because Dr Ezenwa graciously requested to be the first author due to her departmental policy for promotion.

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The author
Dr Rita Okeoghene OLADELE MBBS, MSc, FMCPath (Nig). The author graduated from The University of Benin, Medical School in 1997. She was awarded the fellowship of the National Postgraduate Medical College of Nigeria in the Faculty of Pathology (specialising in medical microbiology) in 2009. Her dissertation for the fellowship was on ‘Candidaemia in critically patients admitted at University College Hospital, Ibadan’. She obtained her MSc in Medical Microbiology from the University of Lagos, Nigeria in 2014. Her thesis for the MSc was ‘Onychomycosis in patients attending dermatology outpatient clinic at Lagos University Teaching Hospital’. In 2013, she was invited to attend the preceptorship program supported by Pfizer pharmaceuticals and organised by Professor Malcolm Richardson in the Mycology Reference Centre under the auspices of the University Hospital South Manchester Academy. There she met Prof Denning, who became her role model and mentor. He motivated her to do an estimation of the burden of serious fungal infections in Nigeria, which formed the basis of this PhD work.

She works as a clinical microbiologist at the Lagos University Teaching Hospital, Lagos, Nigeria and as a Lecturer in Department of Medical Microbiology, College of Medicine, University of Lagos, Lagos, Nigeria. She has published several research papers, including those in this thesis.
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I would like to first and foremost thank my supervisor, Prof Denning for his mentorship and excellent guidance in planning this project. He is an impossible act to follow but I intend to try. David, you are my HERO.

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Mrs Nwosu, Laboratory Scientist at APIN lab, LUTH, for your tireless contribution to the success of the work.

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The management of University of Lagos, Nigeria; for making this PhD program a reality.
Finally I owe significant thanks to my family, for your tireless support and accommodation in my endless pursuit of ‘academic grandeur’! God bless you all.
Thesis structure
This thesis is submitted in the alternative (journal) format. This format was selected as the completed epidemiological research took the form of seven separate studies, five of which have been published; one is in press and the last one is being written up to be submitted for publication in due course. It has however been presented as an oral presentation in an international conference (The EMBO meeting 2016; Capetown, South Africa) last year. The alternative format is therefore suitable.

Part of the literature review section has been accepted for publication as a review article in the Journal for Healthcare for the Poor and Underserved. Also part of it has been submitted as a commissioned review article to the Journal of Fungi. The rest is a further review of the literature relating to the serious fungi infections with emphasis on the challenges with diagnosis and management in resource limited settings. The main goal of this study was to investigate the epidemiology of serious fungal infections in Nigerians. However, we first determined the existing current status of these diseases in Nigeria by doing a ‘systematic’ review of the literature on the most commonly reported endemic mycosis from Nigeria – Histoplasmosis. We also conducted two retrospective studies using laboratory and clinical data of two at risk groups (HIV-infected attending PEPFAR clinic in Lagos and critically ill neonates in LUTH); one was a 12years retrospective study (PEPFAR patients) and the other a four year retrospective study of critically ill neonates. Four prospective studies were designed, conducted and completed.

The first study in this thesis demonstrated the seroprevalence of cryptococcal antigen in both anriretroviral therapy (ART) naïve and ART experienced patients. This is paper one and it has been published in Open forum Infectious Diseases. PMID: 27186581

The second study (also paper two) revealed that chronic pulmonary aspergillosis (CPA) was the cause of smear negative TB and or treatment failure in patients being managed for pulmonary tuberculosis in Nigeria. This study population was comprised of both HIV-infected and non HIV-infected patients, it was a multicenter study. We developed a predictive model for CPA in Nigerian patients from this study. This study has been published in International Journal of Tuberculosis and Lung diseases. PMID: 28826456

Study three (paper three) was a ‘systematic’ review of the existing literature on histoplasmosis in Africa; to determine if this was an emerging disease or a neglected
disease. This paper has been accepted for publication in PlosOne Neglected Tropical Diseases.

The forth study (paper four) was a histoplasmin skin sensitivity survey in Nigeria. The study population was both HIV infected patients attending a PEPFAR clinic with CD4 count ≥350 cells/mm regardless of their ART status and healthy persons from the community (whose HIV status are unknown) from six centres across five geopolitical zones of Nigeria. We demonstrated the prevalence of histoplasmin skin sensitivity and the factors associated with it in Nigeria. This paper has been submitted to PlosOne and is under review.

Study five (paper five) was an intensive care unit (ICU) study, where we identified the microbiological profile of infections in a Nigerian ICU and the clinical outcomes of these critically ill patients. This paper has been in published in PlosOne. PMID: 27776162

The sixth study (paper six), was a retrospective study was a retrospective descriptive study of all cases of culture-proven invasive Candida infection (ICI) in high risk neonates admitted to the neonatal intensive care unit (NICU) over a 4-year period. The study participants were identified from microbiology records of all neonates with a positive Candida culture. This paper has been published in Nigerian Postgraduate Medical Journal. PMID: 29082903

Study seven (paper seven); a retrospective cohort study was conducted over a 12-year period (April 2004-February 2016). ART-naïve, HIV-infected adolescents and adults, assessed for ART eligibility at the (PEPFAR) outpatient clinic at Lagos University Teaching Hospital, Nigeria, were included. Demographic, clinical and laboratory data for each clinic visit were captured in a database and analysed. The findings have been presented as oral presentation at an international conference (EMBO 2016, Cape Town). This manuscript is being written up for submission; we aim to submit to AIDS journal.

All the studies with the exception of study five were commenced and concluded during the timeframe of this PhD. I was involved in recruitment and processing of the samples in all the studies but had to hire nurses and research assistants in the multicentre studies. I collaborated with colleagues (clinical microbiologists, physicians, paediatricians and radiologists) in most of the studies. Dynamiker, a Chinese diagnostic company supported study two by supplying us with free ELISA Aspergillus IgG kits which I perform the assays
on the samples in Nigeria but unfortunately, this was a qualitative kit so the results could not be compared with that of the IMMUNOCAP. Duplicated stored sera was shipped to Manchester and processed in Department of Clinical Immunology, Manchester Royal Infirmary by IMMUNOCAP. Ethical approval was obtained from the local health ethics board where the studies were conducted and also from the University of Manchester Ethics committee. A separate multicentre study assessing the knowledge and awareness of resident doctors in Nigeria which was commenced prior to this PhD program but expanded to include more centres during this period was also completed and has been submitted for publication.

Overall this thesis provides baseline epidemiological data on serious fungal infections in Nigeria. It demonstrates thus far that we have underestimated the burden of the problem in Nigeria. We have also been able to prove conclusively that some cases of smear negative TB could actually be cases of CPA. We have also shown that some serious fungal infections are a sufficiently common problem to be considered a public health issue in Nigeria.
Chapter 1
Introduction and literature review

1.1 Introduction

Fungi are heterotrophic eukaryotes that are conventionally and morphologically categorized into yeast (single-celled) and filamentous (mold) forms. They are saprophytic organisms, which have developed means to survive in mammalian hosts. Fungi are like mammalian cells, that is, they are eukaryotic but their cell membranes differ in that they contain ergosterol, while mammalian cells contain cholesterol. Also, the fungi cell wall is made up of chitin, mannan and both α- and β-glucans. Most fungi are abundant in the ecosystem, and inhaling spores or small yeast cells exposes humans to an increased likelihood of infection if they are immunosuppressed or if the fungal burden is large [1]. Over 5 million species of fungi are estimated to be in existence [2] with only ~300 species accounting for disease in humans [3,4]. Although the epidemiology of fungal diseases has greatly changed over the past few decades, *Aspergillus*, *Candida*, *Cryptococcus* species, *Pneumocystis jirovecii*, endemic dimorphic fungi such as *Histoplasma capsulatum* and *Talaromyces marneffei* remain the main fungal pathogens responsible for the majority cases of serious fungal disease. Cryptococcal meningitis, pneumocystis pneumonia, and disseminated histoplasmosis are always lethal unless diagnosed and treated early and correctly [5].

In many rural communities, especially in tropical areas, fungal disease is linked to poverty (mycetoma, chromo-blastomycosis, phaeohyphomycosis, sporotrichosis, and fungal keratitis) [6]. Furthermore, globally there are areas where fungal infections are endemic and affect healthy people, including histoplasmosis, coccidiodomycosis, blastomycosis, paracoccidioidomycosis, and *Talaromyces marneffei* infection. GAFFI has called for 95% of the world’s population to have access to fungal disease diagnostics and treatment by 2025 (95–95 by 2025); an aspect of this call is provision of reference laboratories in every country in the world, and provision of a critical mass of expertise and a complete portfolio of diagnostic tests [7].

Fungi are associated with a vast range of diseases in humans and animals, varying from acute self-limiting pulmonary manifestations and cutaneous infections in immunocompetent individuals to inflammatory conditions and severe life-threatening
infections in immunocompromised individuals. Serious fungal infections occur as a consequence of underlying health problems such as the HIV/AIDS pandemic, tuberculosis, chronic obstructive pulmonary disease (COPD), asthma and the increasing incidence of cancers; these are the major drivers of fungal infections in both developed and developing countries globally [8]. As the population of immunosuppressed individuals has increased (secondarily to the increased incidence of HIV/AIDS, cancer chemotherapy, major surgery and radiotherapy, organ and haematopoietic stem cell transplantation and immunosuppressant treatment of autoimmune diseases), so has the incidence of fungal diseases also increased [9,10]. Fungi often infect mammalian hosts via the respiratory route. Environmental exposure to spores of pathogenic fungi can result in subclinical and unrecognised syndromes, allergic manifestations, or even overt disease.

Fungi infections are generally classified into superficial, cutaneous, subcutaneous, systemic and opportunistic [3]. Superficial fungal infections account for a significant proportion of fungal infections. Table 1.1 shows the variety of fungi infection.

Table 1.1 Major fungal pathogens, their habitats and associated diseases

<table>
<thead>
<tr>
<th>Organism</th>
<th>Habitat</th>
<th>Manifestation of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Opportunistic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus</em> spp.</td>
<td>Soil; decaying organic materials; indoor air environments</td>
<td>• Aspergilloma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Invasive aspergillosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Acute brochopulmonary aspergillosis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Severe asthma with fungal sensitisation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Chronic pulmonary aspergillosis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cerebral aspergillosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Allergic rhinitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Aspergillus bronchitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Aspergillus rhinosinusitis</td>
</tr>
<tr>
<td><em>Pneumocystis</em> jirovecii</td>
<td>No known environmental habitat;</td>
<td>• Pneumonia, rarely extra-pulmonary infection</td>
</tr>
</tbody>
</table>
| **Cryptococcus spp.** | Environment, in association with decaying materials and trees | • Pneumonia  
• Meningitis  
• Disseminated disease (skin, bone usually) |
| --- | --- | --- |
| **Candida spp.** | Commensal of human gastrointestinal tract, skin and vagina | • Mucocutaneous infections (oropharyngeal, oesophageal, skin and nail infections)  
• Vaginitis  
• Candida bloodstream infection  
• Disseminated infections, notably endocarditis, renal, osteomyelitis, endophthalmitis  
• Intra-abdominal infection (peritonitis) |
| **Pathogenic/Endemic** |  |  |
| **Blastomyces dermatitidis** | Soil, in association with decaying wood | • Acute and chronic pneumonias skin lesions  
• Disseminated disease |
| **Coccidioides immitis/posadasii** | Alkaline soil | • Self-limited influenza-like syndrome  
• Pneumonia  
• Disseminated disease |
| **Histoplasma capsulatum** | Soil contaminated with bird or bat guano | • Self-limited influenza-like syndrome  
• Acute and chronic pneumonias  
• Acute and subacute disseminated disease  
• Chronic cavitary pulmonary  
• Skin, subcutaneous tissue, lymph nodes, bone, (African Histoplasmosis) |
| **Talaromyces** | Bamboo rats (Rhizomys) | • Disseminate disease |
It is estimated that about 25% of the world population suffers from superficial fungal infections of the skin and nails at any given point of their lifetime [11]. These infections refer to conditions commonly known as athletes’ foot, ringworm, onychomycosis, etc. caused by dermatophytes and Candida species. Superficial acute and chronic mucosal infections of the gastrointestinal and genital tracts are also common. It has been suggested that about 50–75% of all women in the reproductive age group suffer from vulvovaginal candidiasis (VVC) and about 492 million women have recurrent VVC (RVVC) in their lifetime [12], however, this has been demonstrated to be inadequately documented [13]. After superficial infections; which is estimated to affect 1 billion affected persons and mucosal candidiasis (most women at some time and oral and oesophageal candidiasis), pulmonary fungal disease is the third most common fungal disease group, and the most serious [7]. Current global estimates have found 3,000,000 cases of chronic pulmonary aspergillosis, ~223,100 cases of cryptococcal meningitis complicating HIV/AIDS, ~700,000 cases of invasive candidiasis, ~500,000 cases of Pneumocystis jirovecii pneumonia, ~250,000 cases of invasive aspergillosis, ~100,000 cases of disseminated histoplasmosis, over 10,000,000 cases of fungal asthma and ~1,000,000 cases of fungal keratitis occur annually [14–16]. Mortality associated with fungal disease is >1.6 million, which is similar to that of tuberculosis and >3-fold more than malaria [17].

Fungi are a significant cause of opportunistic infections in AIDS patients. *Pneumocystis jirovecii* is the most common cause of respiratory infection and *Cryptococcus neoformans* the most common cause of CNS infection in patients with AIDS globally. *Histoplasma capsulatum* (endemic in the Americas) and *Talaromyces* (formerly *Penicillium*) *marneffei* (endemic in south and Southeast Asia) are thermally dimorphic fungi that cause

| *Paracoccidioides marneffei* | faeces, liver, lungs and spleen | • Cutaneous lesions  
• Disseminated (blood, bone marrow, skin, lungs and reticuloendothelial tissues.  
• Subacute pneumonia |
|---------------|-----------------------------|--------------------------------------------------|
| *Paracoccidioides brasiliensis/lutzii* | Soil and digestive tract of some animals | • Asymptomatic  
• Acute and chronic pneumonias |

(Adapted and modified from Romani, 2011) [4].
disseminated infections in this group of patients [18]. *Coccidioides* spp has been documented extensively with AIDS patients in the Americas and *Emmonsia* sp in South Africa [19,20]. Mucocutaneous and oesophageal candidiasis are also common with stage 3 and 4 HIV disease; alongside, fungal skin and nail infections which are major causes of morbidity in HIV-infected individuals [21]. With the increased availability of ART and earlier testing and treatment for HIV, the incidence of serious fungal infections has decreased significantly in people living with HIV in high-income countries; however the contrary is the case in many regions with high HIV prevalence, particularly sub-Saharan Africa, there is little evidence for a substantial decrease in cases and this has been attributed to continued late diagnosis and challenges with retention in HIV care [21,22]. A significant number of patients still present with advanced HIV and with a low CD4 cell or re-present with persistent low CD4 cell counts because of poor adherence, resistance to antiretroviral drugs, or both in sub-Saharan Africa [23].

Fungal infections also compromise cancer outcomes, intensive care, renal dialysis, major gastrointestinal surgery; hyperalimentation, sepsis, and pancreatitis are all linked to hospital-acquired fungal infections, with mortality rates of around 50% [24]. These infections often have no pathognomonic clinical or radiologic features and so laboratory diagnosis is necessary. A major challenge with diagnosing some serious fungi infections such as endemic mycoses, chronic pulmonary aspergillosis, pneumocystis, etc may be frequently confused with tuberculosis or other diseases. Increased awareness, a high index of clinical suspicion and capacity building training of laboratory personnel are imperative for their diagnosis. Capacities for diagnosing mycoses are sadly lacking in most low middle-income countries (LMICs) such as Nigeria. The absence of diagnostic tools and antifungal drugs, plus insufficient training of health-care staff, ensures that the mortality and morbidity of fungal infections remains unacceptably high in resource limited settings [25]. There is also the challenge of documented emerging resistance of some of these causative organisms to some of the currently available antifungal agents [26].

In the last four years, the Leading International Fungal Education (LIFE) portal has facilitated the estimation of the burden of serious fungal infections in several countries and for over 5.7 billion people (>80% of the world’s population) [25]. However, the precise estimate of global prevalence and incidence remains unknown and, this is even
scantier in the developing world. The Global Action Fund for Fungal Infections (GAFFI) recently highlighted a list of serious fungal diseases that are of public health importance, and amenable to improved diagnosis and better treatment outcomes. These diseases include pneumocystis pneumonia, cryptococcal meningitis, chronic pulmonary aspergillosis, disseminated histoplasmosis, and fungal keratitis [7]. Modelling with existing outcome data demonstrates that mortality associated with these serious fungal diseases concomitantly with the UNAIDS 90-90-90 campaign could save the lives of approximately 1.6 million persons living with HIV/AIDS globally over the next five years [8]. Table 1.2 demonstrates the current estimates of fungal infections globally.

Table 1.2: Current estimates of fungal infections globally

<table>
<thead>
<tr>
<th>Fungal diseases</th>
<th>Annual incidence</th>
<th>Global burden</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superficial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin, hair, nail</td>
<td>~2,000,000</td>
<td>~1,000,000,000</td>
<td>HIV only, 90% of those not on ARVs</td>
</tr>
<tr>
<td>Fungal keratitis</td>
<td></td>
<td>~1,000,000</td>
<td></td>
</tr>
<tr>
<td><strong>Mucosal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral candidiasis</td>
<td>~1,300,000</td>
<td></td>
<td>HIV only, 20% on those with CD4 counts &lt;200 and 5% of those on ARVs</td>
</tr>
<tr>
<td>Oesophageal candidiasis</td>
<td></td>
<td>~1,300,000</td>
<td></td>
</tr>
<tr>
<td>Vulvovaginal candidiasis episode</td>
<td></td>
<td></td>
<td>70% affected in their lifetime</td>
</tr>
<tr>
<td>Recurrent vulvovaginal candidiasis</td>
<td>~134,000,000</td>
<td></td>
<td>Annual prevalence. Nearly 500 million lifetime experience</td>
</tr>
<tr>
<td><strong>Allergic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic bronchopulmonary aspergillosis in asthma</td>
<td>~4,800,000</td>
<td></td>
<td>Adults only, rare in children</td>
</tr>
<tr>
<td>Allergic bronchopulmonary aspergillosis in cystic fibrosis</td>
<td>6,675</td>
<td></td>
<td>Adults only, starts from age 4</td>
</tr>
<tr>
<td>Severe asthma with fungal sensitisation</td>
<td>~6, 500,000</td>
<td></td>
<td>Adults only, probably uncommon in children</td>
</tr>
<tr>
<td>Fungal rhinosinusitis</td>
<td></td>
<td>~12,000,000</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic severe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic pulmonary aspergillosis</td>
<td>~3,000,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycetoma</td>
<td>~9,000</td>
<td></td>
<td>1950-2013 case reports</td>
</tr>
<tr>
<td>Chromoblastomycosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coccidioidomycosis</td>
<td>~25,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table of Fungal Infections

<table>
<thead>
<tr>
<th>Infection</th>
<th>Incidence</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracoccidioidomycosis</td>
<td>~4,000</td>
<td></td>
</tr>
<tr>
<td>Blastomycosis</td>
<td>~3,000</td>
<td></td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>~500,000</td>
<td>~25,000 Most of the new infections are asymptomatic based on skin testing</td>
</tr>
<tr>
<td>Sporotrichosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute invasive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive candidiasis</td>
<td>~750,000</td>
<td>Includes 60,000-100,000 cases of intra-abdominal candidiasis</td>
</tr>
<tr>
<td>Invasive aspergillosis</td>
<td>&gt;300,000</td>
<td>From about 10 million at risk annually</td>
</tr>
<tr>
<td><em>Pneumocystis jirovecii</em></td>
<td>~500,000</td>
<td></td>
</tr>
<tr>
<td>Cryptococcosis in AIDS</td>
<td>~223,000</td>
<td>HIV-related</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td>&gt;10,000</td>
<td>Based on French data = 4,200. Based on Indian data = 910,000</td>
</tr>
<tr>
<td>Disseminated histoplasmosis</td>
<td>~100,000</td>
<td>No reliable estimates</td>
</tr>
<tr>
<td><em>Talaromyces marneffei</em> infection</td>
<td>~8,000</td>
<td>SE Asia only;</td>
</tr>
</tbody>
</table>

Adapted from Bongomin et al 2017 [17]

### 1.2 Endemic mycosis - Histoplasmosis

Histoplasmosis has been severally reported in the African continent and in Nigeria particularly. Histoplasmosis was first described by Darling in 1906, regarding a patient presenting with features suggestive of disseminated tuberculosis [27]. It is caused by *Histoplasma capsulatum*, a thermally dimorphic ascomycete that has a global distribution, but extensively reported in the Ohio–Mississippi river valley in the United States [28]. The mould phase (saprophytic form) may contain macroconidia and microconidia. The latter form is smooth walled with a diameter of 2 to 4 μm and are the infectious elements. The yeast phase (parasitic form) in body tissues develops as tiny oval budding cells with a diameter of 2 to 4 μm, predominantly observed within macrophages and histiocytes. In the African strain, *Histoplasma capsulatum var. duboisii* (Hcd), the yeast form is mostly thick walled and larger, 8 to 15 μm in diameter [29].

#### 1.2.1 Epidemiology

While the central USA seems to have the highest incidence of *Histoplasma capsulatum* var. *capsulatum* (Hcc), Brazil, Argentina, India, and South Africa have all documented
small case series, and *Histoplasma capsulatum var. duboisi* (Hcd) is assumed to exist predominantly in West Africa [28]. However, infrequent cases have been documented from Europe, Oceania, Central and South America and northern sub-Saharan Africa [28]. Skin-test surveys using histoplasmin as a reagent, similar to a tuberculin test, showed some exposure throughout Central America and parts of South America as well as Puerto Rico, Dominica, and Mexico in addition to the central USA with almost no skin-test sensitivity positivity in Europe however there have been documented reports of microfoci of histoplasmosis due to Hcc in Italy [30].

Disease manifestations of histoplasmosis vary depending on the competency of an individual’s immune system. Most cases of histoplasmosis in immunocompetent individuals are asymptomatic and spontaneously self-resolve [31]. The picture is however different among immunocompromised patients with acquired or congenital cellular immune deficiency; in these patients histoplasmosis accounts for significant morbidity and mortality [32,33]. Although immunosuppressants used in organ transplant patients and or chronic inflammatory diseases add to this upturn, the major part of the problem is due to the spread of the HIV/AIDS epidemic [33]. Disseminated histoplasmosis was classified as an acquired immunodeficiency syndrome (AIDS)-defining infection in 1987 [34]. In highly endemic regions, histoplasmosis occurs in about 2–25 % in HIV-infected patients resident in these areas [35]. In one endemic region, prior to the highly active antiretroviral therapy (HAART) period, subclinical or symptomatic histoplasmosis was reported in 12/100 person-years at risk in a group of HIV-infected patients [36]. However, with the introduction of effective antiretroviral therapy (ART), there has been significant reduction in the incidence of histoplasmosis in HIV-infected patients in USA [34].

Recently, the WHO broadened their list of core neglected tropical diseases (NTDs) to include deep mycosis, of which histoplasmosis is one [37]. The greatest attributable risk factor for histoplasmosis is the spread of HIV, although immunosuppressive agents used in transplant patients or chronic inflammatory diseases also contribute to its increase [38]. Disseminated histoplasmosis was classified as an acquired immunodeficiency syndrome (AIDS)-defining infection in 1987 [39].

Despite highly active antiretroviral therapy (HAART), morbidity and mortality due to histoplasmosis still remains a public health problem in low and middle-income countries...
(LMICs) [34]. Primary infection is extremely common in highly endemic areas based on the prevalence of skin test reactivity, with 23%-81% and 5%-50% % of the population testing positive in Guatemala and Mexico respectively [40,41]. In these areas progressive disseminated histoplasmosis (PDH) can occur in 5-20% of patients infected with HIV [28,42].

Histoplasmosis is still widely misdiagnosed as multidrug-resistant tuberculosis, leading to numerous preventable deaths, even if they are easily discernible [43]. Figure 1.1 shows the global distribution of histoplasmosis (Adapted from Bahr N et al., 2015) [28].

Figure 1.1. The global distribution of histoplasmosis

Historically, \textit{H. capsulatum} was considered to be divided into three strains on basis of morphologic characteristics: \textit{H. capsulatum var. capsulatum} (Hcc), prevalent in the Americas; \textit{H. capsulatum var. duboisi} (Hcd), majorly reported in central and western Africa; and \textit{H. capsulatum var. farciminosum}, isolated from equines in Africa and the Middle East [29]. Remarkably, clinical differences in histoplasmosis disease appearance have been described among the different strains [44]. African histoplasmosis caused by Hcd is regarded as a distinct entity, causing mainly cutaneous and subcutaneous lesions rather than respiratory disease. Unlike \textit{var. capsulatum, var. duboisi} is still not classified as an AIDS-defining illness [45].
In HIV-infected patients histoplasmosis is disseminated in 95% of the cases, and in 90% of the cases, it concerns patients with CD4 counts below 200/mm³ [46]. A subacute clinical presentation is most common, with symptoms progressing for 1 or 2 months, the clinical picture is misleading, the symptoms being nonspecific. The general symptoms include fever, fatigue and weight loss. Respiratory symptoms such as cough or breathlessness and dyspnea are observed in 50% of the cases, and may be associated with hepatosplenomegaly (25% of cases) and/or superficial lymph node enlargement (25% of cases). Gastrointestinal, neurological or mucocutaneous features are irregularly seen (10–20% of cases) and are polymorphous. Isolated respiratory signs have also been demonstrated in HIV infected patients with CD4 > 200 [46]. Histoplasmosis has been categorized as severe if patients require ICU management; moderately severe if only hospital admission was required and mild if patients are managed on out-patient basis [47,48].

A number of risk factors for histoplasmosis have been documented in HIV-infected patients, they are classified under occupational/environmental factors and host factors. First is environmental exposure to nitrogen rich soil containing bat or bird guano, this has been shown to increase the risk of histoplasmosis in the general populace [36,49]. A past history of cave exploration, presence/participation in excavation sites, woodcutting, exposure to bird roost, farms or poultry have also been documented [50]. In a case controlled study involving HIV-infected patients with histoplasmosis, compared to controls without histoplasmosis, a strong association between histoplasmosis and exposure to chicken coops was reported [50]. A recent study from French Guiana identified host factors with a strong association with histoplasmosis in HIV infected patients to be CD4 nadir <50/mm³, CD4 count less than 200/mm³, a CD8 count in the lowest quartile, herpes co-infection, and recent antiretroviral treatment initiation (less than 6 months). The other variables identified with reduced incidence of histoplasmosis were antiretroviral treatment for more than 6 months, fluconazole treatment, and pneumocystosis co-infection [51]. The researchers also found 13.5% of deaths at 1 month, 17.5% at 3 months, and 22.5% at 6 months following a diagnosis of histoplasmosis; they concluded that the most significant predictors for death within 6 months of histoplasmosis diagnosis were low CD4 counts and antiretroviral treatment [51]. An earlier multicentre study in the USA by Hajjeh and colleagues, demonstrated similar findings [36]. Seasonal variations are also associated with histoplasmosis. Using
climatic records, in one endemic area, it was observed that 70% of incident HIV-related histoplasmosis cases could be forecasted compared to observed cases [52]. Another study demonstrated a clear relationship with a seasonality pattern [53]. Histoplasmosis was more common in the short wet season-long dry season.

1.2.2 Histoplasmosis in Nigeria
Classical histoplasmosis is caused by \textit{Histoplasma capsulatum} var. \textit{capsulatum} and African histoplasmosis by \textit{Histoplasma capsulatum} var. \textit{duboisii}; both strains are endemic in the African continent [54]. African histoplasmosis rarely manifests as pulmonary disease, more commonly as infection of skin, lymph nodes, subcutaneous (abscesses) and bone lesions. It occurs primarily in central and western Africa, though there has also been a documented case from Madagascar [55,56]. In the African continent there have been 250 documented cases with Nigeria accounting for 50% of cases [54]. In a survey performed in Nigeria, over three decades ago, the majority of the participants who had positive skin test to Hcc were also positive to Hcd confirming antigen similarities [57]. In the same study, there were also similarities in biochemical characteristics of the culture filtrate of both strains [58]. In Eastern Nigeria, a natural reservoir of Hcd was discovered in soil of caves mixed with bat guano [54]. There were documented 20 cases of African histoplasmosis in an outbreak amongst cave explorers in Nigeria [59]. There have been several case reports of histoplasmosis spanning five decades in Nigeria. All documented cases appear to be caused by Hcd. The table 1.3 below revealed the distribution of the case reports across Nigeria. There have also been three histoplasmin skin sensitivity screening done, all before the HIV/AIDS epidemic with rates ranging between 4.7-35% [58,60,61].
<table>
<thead>
<tr>
<th>Nigerian City/Town</th>
<th>Type of histoplasmosis</th>
<th>HIV status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immigrant (Europe)</td>
<td>Disseminated histoplasmosis (3 cases); Hcd</td>
<td>Positive</td>
<td>[62]</td>
</tr>
<tr>
<td>Immigrant (Saudi Arabia)</td>
<td>Disseminated histoplasmosis; Hcd</td>
<td>Positive</td>
<td>[63]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Histoplasmosis bone lesion; Hcd</td>
<td>Negative</td>
<td>[64]</td>
</tr>
<tr>
<td>Enugu</td>
<td>Disseminated histoplasmosis in a child; Hcd</td>
<td>Negative</td>
<td>[65]</td>
</tr>
<tr>
<td>Enugu</td>
<td>Cutaneous histoplasmosis; Hcd</td>
<td>Negative</td>
<td>[66]</td>
</tr>
<tr>
<td>Ilorin</td>
<td>Histoplasmosis lymphadenopathy; Hcd</td>
<td>Negative</td>
<td>[67]</td>
</tr>
<tr>
<td>Middle belt</td>
<td>Subcutaneous histoplasmosis; Hcd</td>
<td>Negative</td>
<td>[68]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Cutaneous histoplasmosis (52 cases); Hcd</td>
<td>Negative</td>
<td>[69]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Histoplasmosis, jenunal lesion; Hcd</td>
<td>Negative</td>
<td>[70]</td>
</tr>
<tr>
<td>Enugu</td>
<td>Histoplasmosis, bone lesions (6 cases) in children; Hcd</td>
<td>Negative</td>
<td>[71]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Histoplasmosis, vocal cord lesion</td>
<td>Negative</td>
<td>[72]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Histoplasmosis, oral and bone lesions; Hcd</td>
<td>Negative</td>
<td>[73]</td>
</tr>
<tr>
<td>Jos</td>
<td>Cutaneous histoplasmosis; Hcd</td>
<td>Negative</td>
<td>[74]</td>
</tr>
<tr>
<td>Borno</td>
<td>Histoplasmosis, oral lesions</td>
<td>Negative</td>
<td>[75]</td>
</tr>
<tr>
<td>Ile ife</td>
<td>Histoplasmosis, bone lesions; Hcd</td>
<td>Negative</td>
<td>[76]</td>
</tr>
<tr>
<td>Enugu</td>
<td>Histoplasmosis bone lesions; Hcd</td>
<td>Negative</td>
<td>[77]</td>
</tr>
<tr>
<td>Calabar</td>
<td>Histoplasmosis colonic mass; Hcd</td>
<td>Negative</td>
<td>[78]</td>
</tr>
<tr>
<td>Calabar</td>
<td>Cutaneous histoplasmosis (12 cases); Hcd</td>
<td>Negative</td>
<td>[79]</td>
</tr>
<tr>
<td>Kaduna</td>
<td>Histoplasmosis, bone lesion in child; Hcd</td>
<td>Negative</td>
<td>[80]</td>
</tr>
<tr>
<td>Enugu</td>
<td>Cutaneous and subcutaneous histoplasmosis (2 cases); Hcd</td>
<td>Negative</td>
<td>[81]</td>
</tr>
<tr>
<td>Borno</td>
<td>Cutaneous and osteolytic histoplasmosis (2 adults and 2 children); Hcd</td>
<td>Negative</td>
<td>[82]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Subcutaneous histoplasmosis (3 cases); Hcd</td>
<td>Negative</td>
<td>[83]</td>
</tr>
<tr>
<td>Kaduna</td>
<td>Histoplasmosi, oral lesion in a child; Hcd</td>
<td>Negative</td>
<td>[84]</td>
</tr>
<tr>
<td>Ile ife</td>
<td>Cutaneous and osteolytic histoplasmosis in an child; Hcd</td>
<td>Negative</td>
<td>[85]</td>
</tr>
<tr>
<td>Kano</td>
<td>Cutaneous and osteolytic histoplasmosis</td>
<td>Negative</td>
<td>[86]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Disseminated histoplasmosis in 2 children</td>
<td>Negative</td>
<td>[87]</td>
</tr>
<tr>
<td>Kaduna</td>
<td>Histoplasmosis bone lesion; Hcd</td>
<td>Negative</td>
<td>[88]</td>
</tr>
<tr>
<td>Kaduna</td>
<td>Cutaneous histoplasmosis (10 cases)</td>
<td>Negative</td>
<td>[89]</td>
</tr>
<tr>
<td>Port harcourt</td>
<td>Histoplasmosis, bone lesion; Hcd</td>
<td>Negative</td>
<td>[90]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Pulmonary histoplasmosis (2 cases); Hcd</td>
<td>Negative</td>
<td>[91]</td>
</tr>
<tr>
<td>Enugu</td>
<td>Histoplasmosis orbital cyst; Hcd</td>
<td>Negative</td>
<td>[92]</td>
</tr>
<tr>
<td>Ibadan</td>
<td>Histoplasmosis orbital lesion in a child; Hcd</td>
<td>Negative</td>
<td>[93]</td>
</tr>
<tr>
<td>Ile ife</td>
<td>Histoplasmosis, skull bone lesion; Hcd</td>
<td>Negative</td>
<td>[94]</td>
</tr>
</tbody>
</table>
1.2.3 Diagnosis

Diagnosing histoplasmosis requires a multifaceted approach [95]. Factors to be considered include the clinical picture, radiologic findings, and laboratory test results (such as histology, culture, serology [96]. Results from routine laboratory investigations are non-specific, but increased levels of liver function enzymes (lactate dehydrogenase and alkaline phosphatase), ferritin, pancytopenia with or without hemophagocytosis syndrome, are classically reported [97]. The choice between one diagnostic test and another depends on the type and stage of disease (which informs specimen type), availability, cost, sensitivity and specificity, and the urgency of the result.

The simplest and cheapest test is direct microscopy after staining with Wright or Giemsa stains on an array of samples such as bronchalaveolar lavage (BAL), bone marrow aspirate, tissue biopsy, or peripheral blood [98]. The disadvantage of this technique is its low sensitivity, which is approximately 50% in samples of HIV-infected patients and less than 5% in acute forms with limited symptoms [99,100]. Histology of tissue specimens using special stains such as PAS/methenamine silver will delay results by at least one week because more equipment is required and a pathologist needs to review and report. This also has a sensitivity of less than 50% [101]. Culture, which is the definitive diagnosis, is potentially hazardous for laboratory personnel and requires a level 3 biosafety facility; it remains the reference method for disseminated histoplasmosis particularly in HIV- infected patients though growth requires 1-6 weeks incubation, thus resulting in delay of treatment initiation [98]. Though specificity of the culture method is 100%, sensitivity depends on the fungal load. Bone marrow aspirates yield the highest proportion of positive cultures (70–90%) [46]. Culture is not very useful in diagnosing primary infections in immunocompetent patients with low fungal load [102]. In disseminated cases, blood cultures using the centrifugation–lysis system or automated blood culture systems have been used with significant results [103,104].

Serology for anti-Histoplasma antibody is particularly useful in cases of low fungal load, like in asymptomatic or chronic pulmonary histoplasmosis [98]. Antibody detection by immunodiffusion or complement fixation is less sensitive in immunocompromised HIV-infected patients than in immunocompetent patients [35,105]. Serologic detection of histoplasmosis is only positive in 50–70% of HIV-infected patients [96]. The rise of antibody titres is usually observed 2–6 weeks after primary exposure, and does not
differentiate between active and passive infection [106]. However, antibody testing of CSF in suspected cases is critical for diagnosing neuromeningeal forms [107]. Antibody detection by immunodiffusion or complement fixation methods is less sensitive in HIV-infected patients than in immunocompetent patients [35,96,97]. Cross-reactions with other fungal pathogens, lymphoma, sarcoidosis and tuberculosis have been reported [108].

Diagnosing disseminated histoplasmosis has been significantly facilitated by the development of Histoplasma antigen testing. The detection of Histoplasma capsulatum var. capsulatum circulating antigen has been performed with several EIA methods. The M and H antigens, which are the major H. capsulatum antigens, have homology to catalase and β-glucosidase, respectively however, a recent study demonstrated N-acetylated α-linked acidic dipeptidase of H. capsulatum being recognized as a major antigen detected during Hcc infection [109]. The sensitivity of antigen detection in disseminated histoplasmosis is greater in immunocompromised patients than in immunocompetent patients and in patients with more severe illness. It has been observed that the sensitivity for detection of antigenemia is similar to that for antigenuria in disseminated infection [48,110]. The polyclonal MiraVista Diagnostics Histoplasma antigen EIA allows the quantitative detection of Histoplasma galactomannan polysaccharide circulating antigen with a sensitivity of 95–100% in urine and 92–100 % in serum [110,111]. The antigen levels are higher in the immunocompromised patients with a disseminated form of the disease. Also, the antigen level has been shown to correlate with the severity of the disease [112]. Specificity is 99%; the negative and positive predictive values are 99.5% and 91%, (for a calculated 10 % prevalence) [111], bronchioloalveolar lavage samples antigen testing showed a 93% sensitivity [112]. Histoplasma antigen testing despite the evolution of the test, still has cross–reactions with sporotrichosis, aspergillosis (10%), coccidioidomycosis (60 %), paracoccidioidomycosis (80 %) and blastomycosis (90 %) [112,113]. In spite of this, Histoplasma antigen testing still remains the mainstay of diagnosing histoplasmosis in immunocompromised patients. This test is presently yet to be commercialized.

The Immuno---Mycologics (IMMY) ALPHA Histoplasma antigen EIA has been FDA approved and commercialised since 2006, however its sensitivity is 71 % and specificity is 98 % in urine [114]. The CDC- Mycotic Diseases Branch developed an EIA Histoplasma
antigen detection method meant for low resource settings with a sensitivity of 81 % and a specificity of 95 % in the urine of HIV patients in Guatemala. Cross-reactions were only observed for paracoccidioidomycosis [115]. A recent multicentre study by Zhang et al. (2015) on the different Histoplasma antigen EIAs available had the following findings: “Compared to the MiraVista antigen assay, both the IVD kit and the analyte-specific reagent enzyme immunoassay (ASR-EIA) were significantly less sensitive in detecting Histoplasma antigen in the urine of patients with histoplasmosis. The ASR-EIA and MiraVista assay had comparable specificity. In conclusion, the ASR-EIA has improved performance compared with the IVD kit in the detection of Histoplasma antigen in the urine. However, users should be aware of the potential for false negative results using the currently recommended cut-off value” Zhang [116].

Molecular diagnostic methods have been reported for diagnosis of histoplasmosis, with inconsistent accuracy [117]. Multiple typing methods have been developed to study the epidemiology of Hcc [118,119]. Several genotype-based techniques, such as restriction fragment length polymorphism, hybridization of target genes (probes), random amplified polymorphic DNA (RAPD) analysis, chromosomal DNA typing, (RFLP) analysis, and sequencing, has been described for Hcc [120]. No single approach based on DNA assays has been the dominant method. Molecular methods using nucleic acid amplifications are suitable in detecting Histoplasma in tissue biopsies and in accelerating culture validation of Histoplasma, but are not sensitive in identifying Histoplasma from the noninvasive routine clinical samples such as urine and serum [121,122] thus its use in clinical practice is debatable. A recent study demonstrated the development of a loop-mediated isothermal amplification (LAMP) assay for histoplasmosis, this will provides an affordable method of molecular detection that can be carried out and interpreted without costly equipment in resource limited settings [122].
Table 1.4: Comparison of the diagnostic tests used in cases of histoplasmosis

<table>
<thead>
<tr>
<th></th>
<th>Disseminated cases (n 158) (%)</th>
<th>Pulmonary cases (n 5 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AIDS (n=56)</td>
<td>OIC (n=87)</td>
</tr>
<tr>
<td>Culture</td>
<td>34/48 (71)</td>
<td>57/75 (76)</td>
</tr>
<tr>
<td>Pathology</td>
<td>18/25 (72)</td>
<td>32/43 (74)</td>
</tr>
<tr>
<td>Antigen</td>
<td>53/56 (95) [13.80-7.67]</td>
<td>81/87 (93) [10.48-7.62]</td>
</tr>
<tr>
<td>Antibody</td>
<td>15/19 (79)</td>
<td>37/53 (71)</td>
</tr>
</tbody>
</table>

NOTE: Data are number of patients with positive test results / number of patients tested (%). NIC= nonimmunocompromised; OIC, other causes of immunocompromise. Mean antigen concentration, standard deviation in ng/mL. Among the OIC group, antibody tests were positive in 2 (18.2%) of 11 patients who had undergone organ transplantation, 12 (85.7%) of 14 who were receiving tumor necrosis factor antagonists, and 20 (62.5%) of 32 with other causes for immunocompromise. (Adapted from Hage et al., 2011) [48].

1.2.3 Treatment

While waiting for laboratory confirmation of histoplasmosis in a patient with a strong suspicion of histoplasmosis with or without severe symptoms, physicians have two choices for the treatment induction: intravenous (IV) amphotericin B or oral itraconazole [46]. Although amphotericin B is fungicidal and has shown its efficacy in terms of survival, it is also nephrotoxic [123]. Itraconazole is fungistatic and is associated with drug-drug interactions that confound patient management in the relation to profound immunosuppression. When these factors are added to the diminished bioavailability of itraconazole in HIV patients, there are challenges to achieving the recommended serum concentration level [46].

Published guidelines recommend liposomal amphotericin B as first line treatment of moderately severe to severe histoplasmosis in immunocompromised patients [124].
Liposomal amphotericin (3mg/kg/day) is given as induction therapy for two weeks and oral itraconazole (200 mg three times a day for 3 days, then twice a day) for consolidation/maintenance therapy [124]. An area that requires further resolution is duration of treatment of immunosuppressed patients with disseminated histoplasmosis [46]. This should be dependent on the degree of immunosuppression. In one study, patients on TNF-α inhibitors were successfully managed with a shorter duration of treatment than patients with HIV infection [48,125]. Guidelines advocate that antifungal therapy should not be stopped in AIDS patients with CD4 counts less than 150 cells/µL, but that therapy could be discontinued in patients on ART who have been on treatment for at least 1 year and have immune reconstitution with low urine or serum histoplasmin antigen levels [126].

The American Society of Infectious Diseases recommends prophylaxis with itraconazole for as long as the CD4 count remains below 150/m³ in zones of endemic disease, where the incidence of histoplasmosis is 10 cases per 100 person-years [124]. Unfortunately, dosing of efavirenz and similar ARVs and itraconazole together is not clear, because of a 2-way interaction (efavirenz is a cytochrome activator so it reduces the levels of itraconazole).

1.3 Pneumocystosis

1.3.1 Epidemiology

In 1981, two case reports of PCP in five previously healthy homosexual males who were injection drug users announced the beginning of the HIV/AIDS pandemic [127,128]. PCP is the commonest AIDS defining opportunistic infections in HIV infected persons in the US and Europe [129,130] but was initially assumed to be rare in LMIC such as African countries [131,132]. However, more recent studies have reported contrary findings [133–135]. There are plausible reasons for the low PCP rates previously reported in LMICs. One is the widespread poverty combined with low quality of healthcare that may result in most HIV infected patients to dying from infection before they can develop PCP. Another is the lack of diagnostic facilities and trained personnel to identify Pneumocystis in most of these countries. This lack of standard diagnostic facilities may cause significant numbers of PCP cases to be misdiagnosed.
Studies from Asia reveal varying rates of PCP in the last two decades, ranging from 18.7-25.4% in HIV infected patients in Thailand with attendant high mortality [136–140] and 16.7% in Bangladesh [141], 8.4% in Cambodia [142] and 5% in Vietnam [143]. Earlier data from India demonstrated rates of 5-6.1% of PCP in HIV-infected individuals [144–146]. However, with better detection techniques [PCR and (loop-mediated isothermal amplification (LAMP))], higher PCP rates of 12.2-26.5% are being reported [147–149]. A recent study from India reported an incidence of 14% in 94 immunocompromised children of which 14 were HIV-infected [150].

In South America, the picture is practically the same. Though there is paucity of data, studies on PCP in HIV-infected persons there revealed a 24% incidence rate in Mexico [151]; 48% in Panama [152]; 27% in Guatemala [153]; 32% in an autopsy study of HIV-infected Cubans [154]; and a 35% incidence in Haiti [155]. Data from Venezuela revealed that 36.6% of HIV patients had PCP [156], Only two of 16 (12.5%) patients had confirmed PCP in a study from Peru [157] but 38% in Chile [158] and 27% in Brazil [159].

1.3.1.1 Burden in Africa

On the African continent, PCP was previously assumed to be uncommon among the HIV population [132,134,160,161]. Early studies from Uganda and Zambia reported no cases of PCP among HIV-infected patients [131,160]. A South African study reported similar findings of one (0.6%) positive sample out of 181 patients tested for PCP [162]. However, in the same period an incidence of 3.6-11% was documented among HIV-infected persons in Tanzania, Congo and Ivory Coast [132,163–165]. However, in a setting that had better diagnostic facilities and increased access to ART, a PCP prevalence of 33% from 64 smear negative tuberculosis (TB) patients in Zimbabwe using methenamine silver staining on bronchoalveolar lavage (BAL) samples was reported [166]. A similar study from Kenya, using immunofluorescence (IF) and toluidine blue staining identified Pneumocystis in 37.2% and 27.4% respectively of 51 HIV/AIDS infected patients [167]. In an Ethiopian report, P. jivovecii was detected by PCR in 42.7% of 131 BAL samples from HIV-infected patients with atypical radiological reports who were acid fast bacilli (AFB) smear negative [168] and 29.7% by IF [169]. In Nigeria, 12.6% was reported positive using Pneumocystis PCR [170].
With respect to paediatric HIV-infected patients, the situation is very similar. A recent study from Mozambique demonstrated a 6.8% prevalence of PCP with 14.3% in HIV-infected children and 3.3% in non HIV-infected children [171]. At the start of the HIV/AIDS pandemic, the incidence of PCP was 1.3 cases per 100 child-years from early childhood to adolescence and went up to 9.5 cases per 100 child-years in infancy [172,173]. Postmortem studies of lung tissues from children with AIDS revealed an incidence of 67% in Zimbabwe [133]; 31% in children less than 15 months old in Ivory Coast [174] and 48% in HIV infected children under 12 months in Botswana [175]. PCP appears to occur early amongst HIV-infected infants (median age: approximately 13 months), suggesting that exposure to Pneumocystis is relatively extensive. One of the challenges with diagnosis in infancy is that age 3-6 months has been shown to be a period of high incidence of PCP [176]. However, the child’s HIV status is usually undetermined at that period in most resource poor settings because these patients are not routinely presented for care [177]. Anti-Pneumocystis antibodies were demonstrated in HIV-negative children in early years of life (aged 1.9–19 months; mean, 7.1 months; median, 5 months; SD, 4.9) [178] and as early as 2-6 months in African children, often with it being the first presentation of HIV related disease [179]. Following improvement of prenatal HIV testing and introduction of ART to prevent vertical spread, there has been a significant decrease in paediatric HIV infections. The incidence of PCP also reduced substantially in children from 1992 to 1997, with a sharp decline from 1995 and this was attributed to improving ART administration in labour [180]. Despite this, a study from Mozambique amongst children less than 5 years of age reported a prevalence of 6.8% in newly presenting children with severe pneumonia, of whom 25.7% had HIV infection and 59% of the PCP cases were in those with HIV infection [171]. Table 1.5 shows the distribution across resource limited countries.

1.3.2 Outbreaks

Outbreaks of PCP suggestive of human transmission were first documented amongst hospitalized oncology and transplant patients in United States and Europe [181–184]. These were followed by reports of outbreaks amongst hospitalised AIDS patients and immunosuppressed rheumatoid arthritis patients [185–188]. The possibility of transmission of P. jirovecii to and by healthcare workers (HCWs) has also been investigated with some studies reporting substantial differences in antibody titer levels in HCWs exposed to PCP [189]. Another study demonstrated a significant increase in those
that been exposed to PCP, keeping in mind that this is an aerosol transmitted disease [189,190]. A study measuring levels of antibodies to the major surface glycoprotein (Msg) of *Pneumocystis* demonstrated higher levels in healthcare workers exposed to PCP than in non-HCWs that were not exposed to the infection [191] implying that HCWs can serve as a reservoir for *P. jirovecii*. More recently, a group of researchers designed a short tandem repeat (STR) based molecular typing method for *P. jirovecii* genome [192]. They selected six genomic STR markers located on different contigs of the genome and used these to identify a specific genotype (Gt21) which may have been transmitted *Pneumocystis* between 10 patients including six renal transplant recipients. These reports pose a challenge in the management of PCP patients considering that current international guidelines do not advocate respiratory isolation for these patients. Single room isolation for PCP patients to minimize transmission is desirable for the first week of therapy but not realistic in most LMICs.

1.3.3 Clinical presentation
The clinical presentation differs between HIV infected patients and non HIV immunosuppressed patients. In the HIV infected population, the diagnosis of PCP presents a clinical dilemma because there are no specific signs and symptoms of the disease. There is also the challenge of co-existing morbidities with other pathogens (examples are *Salmonella, Pneumococci, Mycobacteria, Cytomegalovirus, Toxoplasmosis*, and others) and the use of prophylactic drugs in managing these patients [193,194]. HIV infected patients will present with sub-acute onset of gradual dyspnea, nonproductive or minimally productive cough, low-grade fever and malaise or they might even be asymptomatic. Acute dyspnea with associated pleuritic chest pain is likely indicative of pneumothorax. In contrast, non-HIV immunosuppressed patients tend to present more acutely, with significant dyspnea, high fever, chills and in some cases with respiratory failure which could result in 40% mortality [195]. In children, there might also be cyanosis, nasal flaring, and intercostal retractions. Physical examination tends to reveal tachycardia, tachypnea and a ‘clear chest’ on auscultation but sometimes inspiratory crackles are heard [196]. Rarely, extrapulmonary manifestations are seen especially in patients who are on prophylaxis and in those with advanced AIDS disease. Some of these manifestations include; pneumocystic lesions of bone, brain, kidney, liver, spleen, eye, thyroid and the gastrointestinal tract [197–203].
HIV infected PCP patients generally have more *Pneumocystis* organisms with fewer neutrophils in their bronchoalveolar lavage (BAL) specimen than non-HIV immunosuppressed patients [196]. This greater burden of infecting organism correlates with a significantly higher diagnostic yield [204]. However, the smaller number of inflammatory cells does not seem to result in worsening oxygenation or impact on survival. In fact, the opposite tends to occur, HIV infected PCP patients appear to have increased arterial oxygen tension and a reduced alveolar-arterial oxygen gradient than non-HIV immunosuppressed PCP patients [205]. This probably explains why non-HIV immunosuppressed PCP patients are more likely to develop respiratory failure than HIV infected PCP patients [205].

Radiological features of PCP are basically the same in both populations of patients. These include diffuse bilateral interstitial infiltrates extending from the perihilar region that become increasingly homogeneous and diffuse as the disease progresses. Other features less common but documented include solitary or multiple nodules, upper-lobe infiltrates in patients receiving aerosolized pentamidine, pneumatoceles, pneumothorax and patchy asymmetric infiltrates [177,206]. A recent case of documented PCP case report showed symmetric biapical cystic spaces in chest radiographs [207]. High-resolution computed tomography, which is more sensitive than chest radiography is used when chest radiographic features appear normal in cases with high index of suspicion and this, may reveal extensive ‘ground-glass’ appearance or cystic lesions.

PCP is graded based on clinical features into mild, moderate and severe to aid in the management of patients (see table 1.5). Patients with mild PCP are often treated as outpatients with oral cotrimoxazole and close follow-up, while patients with moderate/severe PCP with significant hypoxemia are admitted and treated with recommended intravenous therapy. Intensive care admission is necessary for patients with respiratory failure.
Table 1.5: Grading of severity of PCP in HIV and non-HIV patients

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Mild PCP</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dypsnoea</td>
<td>Dypsnoea on mild exercise +/- cough and sweats</td>
<td>Dypsnoea on mild exercise, fever (+/- sweats)</td>
<td>Breathlessness at rest, persistent fever and cough.</td>
</tr>
<tr>
<td>Arterial blood gases and oxygen saturation at rest, on air</td>
<td>PaO₂ &gt;11 kPa; SaO₂ &gt;96%.</td>
<td>PaO₂ 8-11 kPa; SaO₂ 91-96%.</td>
<td>PaO₂ &lt;8 kPa; SaO₂ &lt;91%.</td>
</tr>
<tr>
<td>Radiological findings</td>
<td>Normal or minor perihilar infiltrates</td>
<td>Diffuse interstitial shadowing.</td>
<td>Extensive interstitial shadowing, with or without alveolar shadowing</td>
</tr>
</tbody>
</table>

The pathogenesis of *Pneumocystis* infection is not yet fully understood. It is generally assumed that *Pneumocystis* is transmitted as aerosols from host to host. Historically, it was assumed that the infection is acquired during early childhood most likely manifesting as a self-limiting upper respiratory tract infection [208-210]. This is buttressed by findings that demonstrated detectable levels of anti-*Pneumocystis* antibodies in the majority of children by 2 to 4 years of age [211–213].

Recent findings have demonstrated anti-*Pneumocystis* IgG antibodies and *Pneumocystis* DNA in an apparently healthy simian population [214] thus suggesting that healthy humans could be a reservoir for the infection. This implies that PCP occurs through recrudescence of dormant infection when there is immunosuppression or re-infection and that protection from infection is not conferred by prior colonisation. Also, *Pneumocystis* has been identified as colonizers in adults with chronic obstructive pulmonary disease (COPD) and with consequent immunosuppression and reactivation of the infection [215]. Several researchers have demonstrated new *Pneumocystis* exposure either from environmental or colonized sources resulting in transmission of the infection [185,187,188,216–219].

A key point is that individuals with unknown HIV status and poorly compliant patients are particularly at risk of this infection. The most substantial risk factor for PCP in HIV infected patients is a CD4+ cell count below 200 cells/µl; the lower the CD4+ count falls below this level the more likely the development of PCP [220–223]. CD4 cells are necessary for the clearing of *Pneumocystis*. They play a significant part in regulating *Pneumocystis* infection and this is apparent in the glaring association between decreasing CD4+ cell count in HIV-infected patients and intensified risk of acquiring PCP [220].
Fortunately, the advent of ART, which boosts successful reconstitution of CD4+ cell count levels have led to significant reduction in rates of PCP in HIV infected patients [224]. This is the picture in industrialized countries where the majority of HIV positive patients have access to ART, however the contrary is the case in resource poor countries such as Nigeria.

1.3.4 Diagnosis
There is an increase in survival with earlier diagnosis [225]. Laboratory diagnosis of PCP is a two-step procedure that involves sample collection and pathogen identification. Since *P. jirovecii* cannot be cultured in routine clinical laboratories, diagnosis relies on microscopy, serology and molecular detection techniques of the organism in respiratory samples. A variety of respiratory specimens have been used for diagnosis, including lung biopsy, bronchoalveolar lavage (BAL), induced and expectorated sputum, nasopharyngeal aspirates and, more recently, oral washings. The high morbidity associated with biopsy specimens has limited their clinical utility so BAL has largely been the sample of choice [226, 227]. Bronchoscopy is required for lung biopsy and BAL. It is unpleasant, expensive, invasiveness and the expertise it requires renders it impractical in resource poor settings and so the use of induced sputum has been preferred [228–232].

The induction of sputum using nebulised saline has generally been preferred over spontaneously expectorated sputum because it is believed to yield higher quality clinical material. However, sputum induction may be unsafe in some patients, particularly infants and weaker AIDS patients, both because of the risk of haemoptysis in patients with tuberculosis or chronic pulmonary aspergillosis and because the healthcare worker is potentially exposed to *M. tuberculosis* (including MDR and XDR TB). It also cannot be done in children less than about 4 years old, because of their limited ability to comply with or comprehend instructions, and usually swallow anything they cough up. Therefore the correct detection of *Pneumocystis* presents a myriad of challenges. Though the procedures to obtain oral washes, nasopharyngeal aspirates and sputa are less invasive than that for bronchoalveolar lavage, they are also less efficient at obtaining adequate amount of *Pneumocystis* for detection with standard stains. Thus, methods that do not necessitate the use of expensive specialised equipment or technical expertise would be useful in resource poor settings. Several studies have shown that *P. jirovecii* can be detected by PCR in the nasopharynx usually using nasopharyngeal aspiration (NPA) [233].
In direct comparisons of diagnostic performance NPA (with or without induced sputum) and immunofluorescence microscopy in 105 HIV-infected children with severe pneumonia in Johannesburg, NPA was 33% sensitive or 75% if combined with induced sputum [234]. In children in Capetown, PCR detected more infections compared to immunofluorescence microscopy in NPAs [87/183 (48%) vs. 4/183 (2%)] [235]. Compared to bronchoscopy specimens analysed by silver staining in adults, NPA results by PCR were positive in all 15 BAL positive patients (100%) and an additional 4 patients, giving a PPV of 96.1% and an NPV of 78.9% [236]. In recent work on expectorated sputum from HIV infected and smear negative TB patients in Namibia, of 475 samples analysed, 5.3% samples were positive for \textit{P. jirovecii}, (13.6% using both qPCR and GMS staining and 1.7% using qPCR only) [237].

1.3.4.1 Microscopy

There are two major life forms of the organism. The ascus (cyst) stains positively with Gomori–Methenamine–Silver (GMS) and is the most frequently considered feature for pathologic diagnosis in respiratory specimens (induced sputum or bronchoalveolar lavage). The trophic form which is generally stained with Wright–Giemsa stain but due to its relatively small size and non-specific staining pattern makes it unsuitable for diagnosis [238]. There are several methods that can be engaged for \textit{Pneumocystis} detection on all sample types, they include immunofluorescence microscopy utilizing monoclonal antibodies; special stains using light microscopes. The sensitivity and specificity of these methods depends on a number of issues such as the type and quality of the collected sample, the burden of \textit{Pneumocystis} in the sample, and the expertise of the laboratory technician with the particular method used. These different methods have their advantages and challenges. A comparison of three stains (IFA, Diff-Quik and Toludine blue O) demonstrated a sensitivity of 92% for IFA, 76% for Diff-Quik and 80% for toluidine blue O with no false positives for IFA [239].

Calcofluor white staining is quick, convenient, and can detect simultaneously the presence of other fungi in samples but expertise is required for identification of \textit{Pneumocystis}. Immunofluorescence microscopy using monoclonal antibodies is most sensitive and can detect both cyst and trophic forms of \textit{Pneumocystis}; however, it is more time consuming and expensive. All of these microscopy methods can have false-
negative results, especially in samples from non-HIV immunosuppressed patients and is probably as a result of fewer organisms in collected specimen [205].

1.3.4.2 PCR

Molecular detection assays are presently widely accessible; they have higher specificity and sensitivity compared to other methods, and is particularly useful in the PCP patient population. PCR has been shown to be the most sensitive detection technique [240]; however, it is not technically and financially viable for resource poor settings where the burden of this disease is high. A cost effectiveness analysis by the CDC resolved that for the detection of *Pneumocystis*, use of PCR assays, combined with less-invasive patient specimens such as expectorated or induced sputum, NPA, oral washings, represent more cost-effective alternatives than any other diagnostic technique using BAL, or radiological findings alone. [193]. False negatives are however seen when fewer copies are present than the lower limit of detection for a given assay [215]. So despite increasing specificity and sensitivity of *Pneumocystis* detection, PCR interpretation still has challenges in differentiating between active infection and colonization.

The main genes targeted are the major surface gene (MSG) [241]; the heat shock protein gene (HSP70) [227], the dihydrofolate reductase gene (DHFR) [242,243], the dihydropteroate synthase gene (DHPS) [242,244], the cell division cycle 2 gene (CDC2) [245] and more recently B-cell epitope, Meu 10, that encodes a glycosylphosphatidylinositol- anchored polypeptide [246]. The sensitivity of the test can be improved by choosing a multicopy gene target (MSG or mtLSU gene) or by nPCR that amplifies detection by using two rounds of PCR [247–249]. The most common target for sequencing is the mtLSU gene [227,247]. In BAL fluid studies, it had reduced specificity but marked sensitivity, sensitivity was increased by using a mtLSU gene which differentiates colonization from active infection [250].

Real-time PCR is now what is widely used in most clinical laboratories due to reduced inter-run contamination thus improving the specificity of the technique. Quantitative touchdown PCR (QTD PCR) using the MSG locus gene of *Pneumocystis* reported a sensitivity of 88% and a specificity of 85% in oral washes from HIV-infected patients, and this increased to 100% specificity with the application of a post hoc cut-off of 50 copies/tube. [251].
1.3.4.3 β-1-3-D-glucan (BG)

Beta-1-3-D-glucan (BG) is a polysaccharide that is present in the cell wall of most fungi including *Pneumocystis* cyst wall [252,253]. It has been demonstrated to trigger an intrinsic immune reaction that can be detected in patients’ BAL and serum specimens infected with *Pneumocystis* [253,254]. Though BG specificity for *Pneumocystis* is not so distinct, it is however highly sensitive for PCP [255–258]. The high sensitivity and ability to exclude a diagnosis of PCP when negative, has resulted in the inclusion of BDG testing in algorithms for management of PCP [259].

In a retrospective study of specimens from 295 HIV infected patients suspected of having PCP, when BG was compared with microscopy with a BG cut-off level of 31.1 pg/ml, the sensitivity and specificity of the assay were 92.3% and 86.1%, respectively with which the positive and negative predictive values of 0.610 and 0.980, respectively. In this same study, comparison was made with the three other serum markers (CRP, LDH and KL-6) and the receiver operating characteristic curves demonstrated BD as the most reliable indicator for PCP (figure 1.2). Another comparison of the four serological biomarkers (BG, Krebs von den Lungen-6 antigen-KL-6, lactate dehydrogenase (LDH) and S-adenosyl methionine (SAM/Adomet)) confirmed BG as the most reliable serologic biomarker for PCP diagnosis, followed by KL-6, LDH and then SAM; and that the BG/KL-6 combination test was the most precise serologic assay for PCP diagnosis, with 94.3% sensitivity and 89.6% specificity respectively [260]. A positive BG assay result might be a good indication to begin anti-PCP treatment [261–263].
A meta-analysis of 12 studies in adults with possible PCP revealed a pooled sensitivity of 96%, a specificity of 84%, a diagnostic odds ratio of 102.3 receiver operating characteristic curve of 0.96, considerably superior to its performance for other invasive fungal infections such as invasive aspergillosis and candidiasis [264]. However, BG assay is not suitable for the vast majority of laboratories, especially those in resource poor settings due to cost constraints, challenges with environmental contamination and the test requires a kinetic reading ELISA machine. All these make it unsuitable for both a clinical laboratory and laboratories in LMIC countries.

1.3.4.4 S-Adenosyl-L-methionine (AdoMet)

S-Adenosyl-L-methionine (AdoMet) plays a vital role in the physiology of all cells, both as a methyl donor in countless numbers of metabolic processes and as a precursor of polyamines. About 95% of AdoMet is used for transmethylation reactions in which the \( N \)-methyl group of the methionine portion is transferred to large molecules such as proteins, complex lipids, and DNA or to smaller molecules to form lecithin and reproduce methionine [265,266]. It was assumed that the organism lacks SAM synthetase so must source this middle compound from its mammalian host, however, another study has shown that *Pneumocystis* possesses a working SAM synthetase [267]. *Pneumocystis* does not synthesize AdoMet, so it scavenges it from the infected host thus suggesting that low
plasma AdoMet levels might be a useful marker for PCP [268][266]. Presently the test is not currently recommended for clinical use.

1.3.4.5 Lactate dehydrogenase (LDH)

Increased serum lactate dehydrogenase (LDH) levels have been documented in patients with PCP in some studies but it is most probably due to the underlying lung inflammatory responses and damage rather than a precise biomarker for PCP [269][258].

1.3.4.6 Culture

A recent study from Germany described a ‘groundbreaking’ method to culture *Pneumocystis jirovecii* using differentiated pseudostratified CuFi-8 cells that were inoculated with BAL fluid (confirmed positive by PCR for *P. jirovecii*) [270]. Although the efficacy of such a culture system for propagating the organism and/or directed therapy selection has yet to be determined, it is nevertheless a breakthrough discovery that will potentially impact on the diagnosis and management of PCP.

Table 1.6: Comparison of different diagnostic methods for *Pneumocystis* detection

<table>
<thead>
<tr>
<th>Method</th>
<th>Real time PCR</th>
<th>IFA</th>
<th>GMS</th>
<th>Toludine Blue O</th>
<th>Gram Weiger</th>
<th>Giemsa</th>
<th>Diff Quik</th>
<th>BG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Cyst forms</td>
<td>Cyst forms</td>
<td>Cyst forms</td>
<td>Cyst forms</td>
<td>Trophic forms</td>
<td>Trophic forms</td>
<td>Cyst form</td>
<td></td>
</tr>
<tr>
<td>Target</td>
<td>Trophic forms</td>
<td>Cyst forms</td>
<td>Cyst forms</td>
<td>Cyst forms</td>
<td>Trophic forms</td>
<td>Trophic forms</td>
<td>Cyst form</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Specificity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Total time (duration) of procedure</td>
<td>50-150³</td>
<td>200</td>
<td>100</td>
<td>50</td>
<td>60</td>
<td>80</td>
<td>33</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Affordable</td>
<td>Expensive</td>
<td>Expensive</td>
<td>Affordable</td>
<td>Affordable</td>
<td>Affordable</td>
<td>Affordable</td>
<td>Affordable</td>
<td>Expensive</td>
</tr>
<tr>
<td>Skills/Expertise required</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

IF = immunofluorescence; GMS = Gomori Methenamine Silver; *cyst form = asci; trophic forms similar a cell wall deficient yeast cell; new nomenclature for cysts, trophozoites and sporozoites. + = 0-25%; ++ = 26-50%; +++ = 51-75%; ++++ = 76-85%; ++++++ 86-95%. Adapted and modified from Procop, G.W. et al, 2004 [271].
1.3.5 Treatment

The drug of first choice in treating PCP is cotrimoxazole, which is widely available and relatively cheap. However, in case of treatment failure, hypersensitivity, drug intolerance or toxicity the alternative therapeutic agents are pentamidine, atovaquone, trimethoprim plus dapsone and clindamycin plus primaquine which are more expensive and not readily available in resource poor setting, thus creating a dilemma when clinicians are confronted with these cases. Researchers have shown a direct relationship between contact with sulfa containing agents and transmutations of the dihydropteroate synthase gene of *P. jirovecii*, however the association between these alterations and therapeutic failure is yet to be proven [134,272,273]. This information is of utmost importance in the development of guidelines for clinicians managing PCP patients. Table 1.7 shows the recommended drugs for prophylaxis and treatment while table 1.8 summarizes possible side effects of the drugs.

Table 1.7: Recommendations for prophylaxis and treatment

<table>
<thead>
<tr>
<th>Prophylaxis</th>
<th>Treatment of PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preferred Therapy</strong></td>
<td><strong>Preferred Therapy</strong></td>
</tr>
<tr>
<td>• Cotrimoxazole, 1 DS PO daily (AI) or</td>
<td><strong>Moderate to Severe PCP—Total</strong></td>
</tr>
<tr>
<td>• Cotrimoxazole , 1 SS PO daily (AI).</td>
<td>Duration = 21 Days (AI):</td>
</tr>
<tr>
<td></td>
<td>• Cotrimoxazole: (TMP 15–20 mg and</td>
</tr>
<tr>
<td></td>
<td>SMX 75–100 mg)/kg/day IV given q6h or</td>
</tr>
<tr>
<td></td>
<td>q8h (AI), may switch to PO after clinical</td>
</tr>
<tr>
<td></td>
<td>improvement (AI).</td>
</tr>
<tr>
<td></td>
<td><strong>For Mild to Moderate PCP—Total</strong></td>
</tr>
<tr>
<td></td>
<td>Duration = 21 days (AI):</td>
</tr>
<tr>
<td></td>
<td>• Cotrimoxazole: (TMP 15–20 mg/kg/day and SMX 75–100</td>
</tr>
<tr>
<td></td>
<td>mg/kg/day), given PO in 3 divided doses</td>
</tr>
<tr>
<td></td>
<td>(AI) or</td>
</tr>
<tr>
<td></td>
<td>• Cotrimoxazole DS - 2 tablets TID (AI).</td>
</tr>
</tbody>
</table>

**Indications for prophylaxis:**
CD4 count <200 cells/mm³ (AI) or
• Oropharyngeal candidiasis (AII) or
• CD4% <14% (BII) or
• History of AIDS-defining illness (BII) or
- CD4 count >200 but <250 cells/mm³ and if CD4 cell count monitoring (e.g., every 3 months) is not possible (BII).
  Note—Patients who are receiving pyrimethamine/sulfadiazine for treatment or suppression of toxoplasmosis do not require additional prophylaxis for PCP (All).

<table>
<thead>
<tr>
<th>Alternative Therapy</th>
<th>Alternative Therapy</th>
</tr>
</thead>
</table>
| • Cotrimoxazole 1 DS PO TIW (BI) or | Moderate to Severe PCP (AIi):
| • Dapsone 100 mg PO daily or 50 mg PO BID (BI) or | • Primaquine 30 mg (base) PO once daily + clindamycin [IV 600 q6h or 900 mg q8h] or [PO 300 mg q6h or 450 mg q8h] (AI). Or
| • Dapsone 50 mg PO daily + pyrimethamine 50 mg + leucovorin 25 mg PO weekly (BI) or | Pentamidine 4 mg/kg IV once daily infused over at least 60 minutes (AI); may reduce the dose to 3 mg/kg IV once daily because of toxicities (BI)
| • Dapsone 200 mg + pyrimethamine 75 mg + leucovorin 25 mg) PO weekly (BI) or | For Mild to Moderate PCP:
| • Aerosolized pentamidine 300 mg via Respigard II™ nebulizer every month (BI) or | • Dapsone 100 mg PO daily + TMP 15 mg/kg/day PO (3 divided doses) (BI) or
| • Atovaquone 1500 mg PO daily with food (BI) or | • Primaquine 30 mg (base) PO daily + clindamycin PO (300 mg q6h or 450 mg q8h) (BI) or
| • Atovaquone 1500 mg + pyrimethamine 25 mg + leucovorin 10 mg PO daily with food (CIII). | • Atovaquone 750 mg PO BID with food (BI)

Key to Abbreviations: BID = twice daily; DS = double strength; IV = intravenously,;; PO = orally; q “n” h = every “n” hour; SS = single strength; TID = three times daily; TIW = thrice weekly. A: Strong recommendation for the statement. B: Moderate recommendation for the statement. C: Optional recommendation for the statement. I: One or more randomized trials with clinical outcomes and/or validated laboratory endpoints. II: One or more well-designed, non-randomized trials or observational cohort studies with long-term clinical outcomes. III: Expert opinion. *IDSA guidelines [177].
### Table 1.8: Anti PCP drugs and their toxic effects

<table>
<thead>
<tr>
<th>Drug</th>
<th>Toxic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotrimoxazole</td>
<td>Neutropenia and anaemia (40%), fever (25%), skin rash (19%), nausea, headache, bone marrow suppression, thrombocytopenia (5%), interstitial nephritis, liver function abnormalities (10%), aseptic meningitis, distributive shock syndrome and Stevens-Johnson syndrome or toxic epidermal necrolysis (TEN).</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>Renal dysfunction (60%), leucopenia (50%), hypotension (50%), nausea and vomiting (25%) dysglycemia, pancreatitis, hypoglycaemia (20%), hyperglycaemia, electrolyte dysfunction, bone marrow suppression, cardiac dysrhythmia such as torsades de pointes.</td>
</tr>
<tr>
<td>Dapsone</td>
<td>Rash, fever, methemoglobinemia and hemolytic anaemia</td>
</tr>
<tr>
<td>Primaquine</td>
<td>Rash, fever, diarrhoea, methemoglobinemia and hemolytic anaemia</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>Fever, rash, anaemia and diarrhoea. Long term use predisposes to Clostridium difficile infection</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>Nausea, diarrhoea, headache, rash, and liver function abnormalities</td>
</tr>
</tbody>
</table>

#### 1.4 Aspergillosis

The term "aspergillosis" refers to a disease spectrum due to allergy, airway or lung invasion, cutaneous infection, or extra pulmonary dissemination caused by species of *Aspergillus*. Both inherited, acquired immunodeficiency and chronic pulmonary disease predispose to the development of these varieties of pulmonary syndromes in response to *Aspergillus spp* [274]. The clinical features, course and prognosis of Aspergillus infections largely depend on the degree of immune suppression of the host, although there is increasing recognition of the importance of genetics [275]. Tissue invasion is uncommon and occurs most frequently in the setting of immunosuppression associated with therapy for hematologic malignancies, hematopoietic cell transplantation, or solid organ transplantation. They cause serious mycotic infections in man and account for a hospitalisation rate of 36 per million per year in the USA [276].

*Aspergillus* species are ubiquitous in nature, and inhalation of infectious conidia is a frequent event. There are around 300 known species of *Aspergillus*, of which only a few cause disease in humans [277]. The genus *Aspergillus* is subdivided into eight subgenera, with each subgenus divided into subdivisions that include several related species [278]. The section *Fumigati*, includes more than 30 species, with at least 10 isolated from clinical specimens [278]. *Aspergillus fumigatus* is the most common pathogen, but other
species such as *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans* have also been connected with human disease [279]. Known characteristics of these opportunistic pathogens are the ability to survive and grow in a wide range of environmental conditions, the small size of their spores (2-3 µm), which permits deposition deep within the lungs following inhalation and swift adaptability to the host environment [280,281]. Extensive research has been conducted on *Aspergillus* spp. to determine its biology, pathogenicity, virulence factors and molecular biology [282-285].

“Pulmonary aspergillosis refers to a range of diseases that include invasive aspergillosis from angioinvasive disease, simple aspergilloma from passive colonization of pulmonary cavities, and chronic cavitary pulmonary aspergillosis from fungal germination and immune activation Allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS), driven by allergic responses, has an important place along this spectrum as well”[274].

1.4.1 Hypersensitivity/allergic disease

‘Fungal asthma’ includes the syndromes SAFS and ABPA [286]. Modelling estimates that >6.5 million people have severe asthma with fungal sensitizations (SAFS), up to 50% of adult asthmatics presenting at secondary care have fungal sensitization, and 4.8 million adults have allergic bronchopulmonary aspergillosis (ABPA) [287]. ABPA is characterized by asthma, recurrent pulmonary infiltrates and bronchiectasis [288]. ABPA occurs almost exclusively in patients with asthma or CF, whereas fungal sensitization elicits a strong hypersensitivity response characterized by elevated total serum immunoglobulin (Ig) E and *Aspergillus*-specific IgE and IgG antibodies [289]. In severe asthma with fungal sensitization (SAFS), exposures to fungi can account for asthma exacerbations; the diagnosis often requires escalation to maximal asthma therapy [290]. *Aspergillus* sensitization without ABPA can be clearly separated by negative GM and slgG markers [291]. Allergy to *Aspergillus* can also be a cause of fungal rhinosinusitis with a profound Th2 lymphocyte response with eosinophilic mucin in the sinus as the hallmark of allergic fungal rhinosinusitis [290,292]. A total IgE of more than 1000 IU/L subjectively differentiates allergic bronchopulmonary aspergillosis from severe asthma with fungal sensitisation [293]. Complications of allergic bronchopulmonary aspergillosis include bronchiectasis and pulmonary fibrosis (including chronic pulmonary aspergillosis).
Fungal colonisation is the terminology used when; (1) one (or preferably two or more) respiratory sample(s) are positive for a fungus by culture or PCR method, (2) no new major respiratory symptoms, (3) no evidence of ABPA or other forms of aspergillosis, (4) negative fungal specific IgG in serum [294].

Management of fungal asthma includes using inhaled or oral corticosteroids, or both, immunisation against Streptococci pneumoniae and Haemophilus influenzae, management of excess mucus with therapeutic bronchoscopy (for severe plugging), and hypertonic saline nebulisers and azithromycin as an airway anti-inflammatory agent. Oral or inhaled antifungal therapy, if tolerated, is beneficial [290].

1.4.2 Invasive aspergillosis (IA)

More than 90% of cases of IA are invasive pulmonary aspergillosis [295,296]. A report from a multicenter European registry gave 5% prevalence in patients with acute leukemia [297] and mortality rates of 50 to 100% (Corzo-Leon et al 2015). It is typically a disease of highly immunocompromised persons and is a significant cause of infection-related mortality in hospitalized patients with acute leukemia and recipients of allogeneic hematopoietic stem-cell transplants (HSCT) [298]. However, IA has also been diagnosed in immunocompetent hosts after massive exposure to fungal spores [275]. There is a paucity of data on IA from Africa. In a study of invasive aspergillosis among 105 hematology patients in Tunisia, 16(15.2%) were diagnosed with probable aspergillosis [299]. A more recent study from same Tunisia demonstrated 7.5% (11) cases of proven IA (using EORTC criteria) and 12 cases of putative IA [300]. The researchers also documented A. niger accounting for 35% of Aspergillus spp. isolated from the sputum culture of affected patients contrary to studies from developed countries that gave A. fumigatus as dominant species [300]. There have also been a number of case reports from other African countries [301–307].

The major risk factor in IA is prolonged neutropenia, commonly seen in hematological malignancies and HSCT patients [308,309]. Other risk factors in this group of patients include allogeneic stem cell transplantation (SCT), iron overload, graft-versus-host disease (GVHD), prolonged corticosteroid use, monoclonal antibody use, infection by other microbes (CMV and/or respiratory viruses) [274]. Exposure to Aspergillus is unavoidable, but increased exposure to high levels of the organism during building construction or housing in contaminated environments increases risk of IA and also
recently, genetic predisposition has been recognized as a risk factor for IA [274]. Other risk factors for IA include prior steroid use, ICU admission, diabetes and AIDS [308–311]. More recent studies suggest additional populations at risk; including patients with COPD, end-stage liver disease, or alcoholic hepatitis [274]. IA is the most common infection-related missed diagnosis at autopsy in ICU patients [312]. There have however been no documented reports of IA from Nigeria despite having a significant burden of the at risk population.

1.4.2.1 Diagnosing IA

Diagnosis of invasive aspergillosis remains a challenge because the clinical manifestations are non-specific; radiological findings are non-conclusive, and cultures of respiratory specimens lack sensitivity. Proven invasive aspergillosis is histologic demonstration of invasive hyphae or a positive culture from a normally sterile environment (e.g., pleural fluid) [298]. Probable invasive aspergillosis entails a combination of host factors (e.g., prolonged neutropenia and organ transplantation), compatible radiologic findings, and mycologic criteria [313]. These diagnostic criteria were designed for research purposes but can be applied to clinical practice [298].

Computed tomography (CT) scan of the lungs is a more sensitive radiological tool than radiography for the detection of early pulmonary aspergillosis and is recommended in patients with 10 to 14 days of neutropenia (neutrophil count, <500/mm$^3$) and persistent or recurrent fever of undetermined origin, which does not responds to empirical broad spectrum antibiotics [314]. In a recent report by Kabbani and colleagues, CT scans were successfully used to aid early detection of an outbreak of IA amongst heart transplant recipients [315]. The first radiologic indication of IA is a nodule; then a macronodule - “halo sign”; which is described as a ‘macronodule’ circumscribed by an edge of ground-glass opacity equivalent to alveolar hemorrhage, is indicative of IA in patients with compatible host factors [316]. However, other fungi (e.g., Mucorales) and bacterial agents such as Pseudomonas aeruginosa are also capable of angio-invasion and the sign [316], or reverse halo sign. Other radiographic features consistent with that are IA are consolidation, wedge-shaped infarcts, and cavitation [298].
1.4.2.2 Laboratory diagnosis of IA

A positive culture of *Aspergillus* spp. from a clinical sample was the conventional laboratory method of diagnosing IA prior to the availability of galactomannan (GM) and PCR [317]. One of the advantages of culture over other diagnostic method is the ability to identify the organism and demonstrate its’ susceptibility profile. Culture is also cheap and takes approximately 48 hours to grow; however, a study has shown 17% increase in yield if incubation duration is extended from 2 to 5 days [318]. In patients where a positive culture is not representative of the patient’s clinical picture, such results are classically interpreted as colonisation [319,320].

1.4.2.2.1 Biomarkers

Galactomannan (GM) is routinely used and if positive supports a probable diagnosis of IA. It is a carbohydrate made up of a core of mannose residues with side chains of β-1–5 linked galactofuranosyl residues [321]. GM is a constituent of the *Aspergillus* cell wall along with chitin, β-1–3, and 1–4 glucans [322]. The release of GM glycoproteins is assumed to only happen during cell wall lysis that may occur *in vivo* where nutrients are few and growth is restricted by the host immune response and in dying oxygen deprived tissue [323]. It has been demonstrated that GM is freed during hyphal growth rather than from conidia thus hypothetically permitting the distinction between colonisation and active infection [321]. The proportion of neutropenic patients diagnosed with IA by GM positive BAL who are culture positive varies from 10% to 58% [321]. Five years ago, a monoclonal antibody that binds GM-like antigens was discovered and used to create a lateral flow assay to reassess the possibility of a non-invasive method of diagnosing IA by detecting GM in urine [324]. The major challenge with GM is that it is also found in variable amounts in other fungi such as *Penicillium, Fusarium, Alternaria,* and *Histoplasma* [325–327]. There is also documented cross-reaction with antigens from *Cryptococcus neoformans* thus giving positive results in patients with cryptococcosis and also the same with yeast infections caused by *Geotrichum capitatum,* with low levels of positivity with *Candida* spp [325,328,329].

Another significant challenge with GM is false positive samples in patients receiving certain antibiotic agents. False positives resulting from the use of piperacillin tazobactam have been reported since 2003 in patient sera and confirmed on testing antibiotic preparations [330,331]. Some drug manufacturers have mitigated this occurrence
Finally, most recently, GM has been found in formulations of electrolyte solutions such as PlasmaLyte; gluconate in some of these formulations is prepared from A. niger, and GM from A. niger is present to the level that it may trigger false positive reactions” [321].

A meta-analysis of 34 studies of the use of serum GM to diagnose IA from 1991 to 2008 revealed that the collective sensitivity was as low as 66% and specificity was 90% [333]. Further studies of the usefulness of serum GM to diagnose IA published more recently between 2009 and 2012 suggest that authors findings on the proportion of haematological patients with IA that are positive for GM continues to vary ranging from 13% to 87.5% [334]. Mould active prophylaxis in neutropenic patients also reduces the GM sensitivity in serum [335]. In most studies so far the specificity of the galactomannan assay was greater than 85% [323].

BD forms a large part of glycan, which constitute a major part of the Aspergillus cell wall [336]. However, many fungi including Fusarium, Pneumocystis and Candida produce BDG and it is present in minimal amount in Cryptococcus (not usually detected by assays) not by Mucorales [257]. Studies have shown that BD is secreted into culture liquid of A. fumigatus [337]. Manufacturers of the different available assays have their own recommended cutoffs for reporting positive results. Detecting BD is however, time consuming, labor intensive, prone to contamination, requires considerable technical skill and is expensive, so is not practical for use in LMICs like Nigeria.

1.4.2.2.2 Other “biomakers”

‘Aspergillus antibody measurement plays a peripheral role in the diagnosis of invasive aspergillosis and data on the comparative efficacy of the different techniques are limited’ [338]. A new test that has been recently developed for the detection of Aspergillus and diagnosis of IA is an antigen test detecting an extracellular glycoprotein antigen only produced during active growth of the fungus using a lateral flow device [339,340]. This assay has been reported to be specific for Aspergillus spp. and was comparably sensitive to GM and BDG assays in sera from patients diagnosed with IA [339,340]. In a guinea pig model of IA, the assay was positive earlier than either GM or BDG [341]. Other targeted A. fumigatus antigens that have been proposed for a new assay include Cf2 found on the
surface of the growing *Aspergillus* [342]. The diagnostic accuracy of the main biomarkers of *Aspergillus* is shown in the table below:

Table 1.9: Diagnostic accuracy of the main biomarkers for IA.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Sample</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>DOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mengoli et al. 2009</td>
<td>PCR</td>
<td>Blood</td>
<td>0.88 (0.75–0.94)</td>
<td>0.75(0.63–0.84)</td>
<td>22.11 (7.77–62.92)</td>
</tr>
<tr>
<td>Mengoli et al. 2009</td>
<td>PCR</td>
<td>Blood</td>
<td>0.75 (0.54–0.88)</td>
<td>0.87(0.79–0.93)</td>
<td>21.33 (6.86–46.63)</td>
</tr>
<tr>
<td>Leeang et al. 2008</td>
<td>GM</td>
<td>Blood</td>
<td>0.87(0.71–0.83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun et al 2010</td>
<td>GM</td>
<td>Blood</td>
<td>0.66 (0.61–0.70)</td>
<td>0.90(0.89–0.90)</td>
<td>19.1 (12.67–28.79)</td>
</tr>
<tr>
<td>Guo et al 2013</td>
<td>GM</td>
<td>BAL</td>
<td>0.79 (0.61–0.93)</td>
<td>0.89(0.85–0.92)</td>
<td>51.0c</td>
</tr>
<tr>
<td>Onishi et al 2012</td>
<td>BDG</td>
<td>Blood</td>
<td>0.83(0.82–0.84)</td>
<td></td>
<td>23.2 (9.9–54.4)</td>
</tr>
</tbody>
</table>

a: Sensitivity and specificity as proportions with confidence intervals (CI)
b: DOR- diagnostic odd ratios
c: Calculated from sensitivity and specificity data in reference

Other diagnostics for invasive aspergillosis include, 2-Pentylfuran, a metabolic by-product of *A. fumigatus*, which can be detected in the breath of patients with *A. fumigatus* infection or colonisation with at least comparable sensitivity to culture, [343]; and more recently a study by Koo and colleagues demonstrated that a breath fungal secondary metabolite signature can be used in the diagnosis of invasive aspergillosis [344]. Detection of other *Aspergillus* antigens such as thioredoxin reductase GltT has also been explored for future *Aspergillus* diagnostics [345].

1.4.2.2.3 PCR

Multiple PCR assays have been commercialized for the detection of *Aspergillus* spp. and other pathogenic fungi [321]. The major benefit of the molecular detection method is that unlike almost all other detection methods except for culture, there is the element of amplification of the *Aspergillus* signal and therefore the method is potentially highly sensitive [321]. Additionally, due to extensive use in clinical laboratories in industrialised nations especially for diagnosing viral infections, the technology and expertise required is available in most clinical laboratories, and consumable costs for PCR assays, once expensive, have gradually become more competitive.
1.4.3 Chronic pulmonary aspergillosis (CPA)
CPA is a spectrum of diseases comprising of aspergilloma, aspergillus nodules, chronic cavitary pulmonary aspergillosis and chronic fibrosing pulmonary aspergillosis, [289]. Duration of disease more than three months differentiates CPA from IPA [346,347]. CPA has been identified in patients with the presence of the following: 1) at least one pulmonary cavity on chest imaging; 2) positive anti-Aspergillus immunoglobulin G antibodies in blood, cultures or biopsy identifying Aspergillus and 3) symptoms (usually weight loss, fatigue, cough, haemoptysis and breathlessness) for more than 3 months, with slowly progressive destruction of the lung with or without one or more fungal ball in a cavity (chronic cavitary pulmonary aspergillosis (CCPA), or chronic fibrosing pulmonary aspergillosis (CFPA), or a fungal ball in a cavity without evidence of progression, few symptoms or patients might be asymptomatic (i.e simple aspergilloma) [348]. Prior pulmonary disorders are often obvious in CPA patients [346,349]. Subacute invasive pulmonary aspergillosis or chronic necrotizing aspergillosis develops in four to twelve weeks affects mainly moderately immunocompromised patients and is managed as IA [350].

The majority of CPA patients are men aged 50–75 years (60%) with long-standing constitutional symptoms of pulmonary symptoms of productive cough, haemoptysis of variable degree, chest discomfort, and shortness of breath, profound fatigue and weight loss. Chest imaging reveals at least one cavity, which can be small or large, thick or thin-walled and often, the cavity appears with or without an aspergilloma or with solid or cavitating nodules [290]. Pleural thickening is also a characteristic feature of chronic pulmonary aspergillosis. Patients often have recurrent infections with Streptococcus pneumoniae or Haemophilus influenzae [290].

1.4.3.1 Epidemiology
The global burden is estimated at about 3 million people, of whom about 1·2 million have had pulmonary tuberculosis [25]. In low-income countries, the burden of chronic pulmonary aspergillosis closely tracks that of tuberculosis [6]. Other underlying conditions associated with CPA are emphysema or COPD, non-tuberculous mycobacterial infections, pneumothorax or bullous lung disease, ABPA and asthma, pulmonary fibrotic sarcoidosis, lung irradiation and ankylosing spondylitis [290]. About 2-10% of cases have demonstrated no discernable underlying disorder [290].
Some patients have multiple underlying pulmonary conditions. CPA should be considered when upper lobe cavitary or fibrotic disease and systemic symptoms are present in those with lung disease [346]. In CPA, *Aspergillus* progressively destroys lung tissue, leading to the formation and expansion of cavities. This cavity could contain a fungal ball (aspergilloma) [277,351]. It is often asymptomatic but can lead to life threatening haemoptysis which necessitates surgical resection of the affected area of lung [352]. If left untreated, it will eventually encompass and destroy an entire lobe or lung; even with treatment, the morbidity and mortality of CPA remains elevated [345,351].

Studies of aspergilloma in Taiwan, South Korea, China and India identified tuberculosis as the aetiological factor in up to 93% of cases of CPA [353–356]. The importance of tuberculosis in relation to CPA is demonstrated by the results from other studies of tuberculosis patients. The link between TB and *Aspergillus* has, however been established in publications from around the globe. In Japan 20% of treated TB patients had antibodies to *Aspergillus* [357]. In India *Aspergillus* antibodies are present in 27% of patients with TB at one Centre [358] and 23% of patients with “chronic lung diseases”, of whom 96% had prior TB, at another Centre [359]. In the same India, 26% of all HIV positive children had raised anti-*Aspergillus* IgG levels in another study [360]. In Brazil, 21% of in-patients with TB at a tertiary chest clinic with *Aspergillus* antibodies had an aspergilloma [361]. In Hong Kong the rate of antibodies against *Aspergillus* in patients without an aspergilloma, but suffering from haemoptysis following TB treatment is 36%. This compares to a rate of 7% in those treated for TB without haemoptysis and 2.5% in those with haemoptysis associated with other underlying conditions [362]. A large survey in UK found that out of 544 patients who were left with a residual cavity of 2.5 cm 1 year post TB therapy, 36% had positive *Aspergillus* antibodies and 22% had radiological aspergillomas after 3 years [363,364]. This survey has not been repeated since.

Tuberculosis is much more common in Africa than in Europe or America [365] and the relationship between tuberculosis and HIV/AIDS have been extensively reported. This raises the possibility that CPA is much more common in Africa than in Europe or America however there is no data to back this. The total global prevalence of CPA secondary to TB has been estimated at between 0.8 and 1.37 million cases with 43 cases per 100,000 population in Congo and Nigeria [366]. There has never been a survey of CPA secondary to TB in Africa to confirm this prediction. However, the first documented case of CPA in
Africa was that of an aspergilloma in a South African farmer in 1965 [367]. Subsequent case series have documented presentations of CPA in Ivory Coast [368] Senegal [303], Ethiopia [369], and Nigeria [301]. More recent reports has come from Uganda [370], Tanzania [371], a South African report revealed a 9.9% rate in post TB treatment patients [372] and another from Senegal involved 35 patients diagnosed as CPA using *Aspergillus* IgG and histology [373].

The likely interface between pulmonary aspergillosis, TB and HIV in Africans fosters two crucial assumptions; (1) the possibility that some HIV positive patients with ‘smear-negative TB’ maybe cases of CPA. (2), the estimated rate by Denning and colleagues of pulmonary aspergillosis secondary to treated TB is even higher than that predicted, as a result of additional immunosuppression due to HIV [366]. Both these assumptions raise the possibility of a major unacknowledged public health burden in Africa, especially sub-Saharan Africa with high level of HIV/AIDS. Confirming this is especially important as surgical treatment for aspergillosis has been successfully documented in Africa [303].

The key diagnostic test for chronic pulmonary aspergillosis is the detection of IgG to *Aspergillus* (or the less sensitive precipitins), which is elevated in more than 90% of patients with cavitary disease [374] but only in about 60% of patients with *Aspergillus* nodules [290]. PCR or positive culture of aspergillus or the detection of galactomannan in sputum or BAL samples also aids diagnosis [375]. Elevated total IgG or *Aspergillus* specific IgE can be seen in some patients.

Presently, there are only three classes of antifungal agents available to treat aspergillosis and orally administered azole antifungals are the first-line drugs for the treatment of these diseases. Azole resistance is fast becoming an issue and this has been attributed to mutations of the target protein (CYP51A) with a leucine to histidine amino acid substitution at position 98 the most frequent, predominantly conferring resistance to itraconazole [376]. A recent Japanese study found that long-term itraconazole treatment induced G54 substitution in CYP51A, causing itraconazole-resistance [377].

Long-term oral antifungal therapy is recommended for most patients [378]. The objectives of therapy are to minimise symptoms particularly fatigue, weight loss, cough, sputum production, and risk of haemoptysis. Follow-up without antifungal therapy is advocated for patients with resected *Aspergillus* nodules and for patients with minimal
symptoms and no radiological progression in several months [289]. Haemoptysis can be controlled with tranexamic acid and bronchial artery embolization [290]. Haemoptysis can be a sign of therapeutic failure or antifungal resistance, or both. Acquired azole resistance can develop, as is more likely, following prolonged therapy, poor compliance, and a high burden of infection [290]. Intermittent or long-term intravenous therapy is recommended for these cases.

1.4.4 Tracheobronchial aspergillosis
Aspergillus bronchitis accounts for a small population of immunocompetent patients who have evidence of Aspergillus (either microbiological or serological) but without pulmonary parenchymal disease especially in patients with bronchiectasis and cystic fibrosis [275]. These patients usually have a history of recurrent chest infections unsuccessfully managed with antibiotics and repeated isolation of Aspergillus from sputum or positive PCR. A good response to antifungals may be observed, but may be followed by relapse. In a few patients, bronchoscopy shows localized invasion of hyphae [275].

1.5 Cryptococcosis
Cryptococcus neoformans is the most common etiological agent of meningitis in adults in Sub-Saharan Africa, accounting for 20 to 25% of AIDS–related deaths in Africa [379]. With approximately 35.3 million individuals living with HIV and an estimated peak of 2.3 million HIV-associated deaths in 2012 [380], the increase in the incidence of previously rare opportunistic infections has been noteworthy, leading to tremendous pressure on healthcare resources and loss of lives and income especially in LMICs[381]. Cryptococcal meningitis (CM) is an AIDS-defining disease.

1.5.1 Epidemiology
Globally, an estimated 1 million new cases of CM occur per year, with more than 600,000 deaths [382]. It is also estimated that 88% of all global cases and more than 90% of deaths from CM occur in sub-Saharan Africa and Southeast Asia [383,384]. In South Africa, CM is the most frequent cause of adult meningitis, responsible for 63% of all laboratory-confirmed cases [385]. A study in Benin City, Nigeria amongst ART naïve patients gave a seroprevalence of 12.7% [386] and this was used to make an estimate of 57,866 cases of cryptococcosis in Nigeria [387]. A review by Veltman and colleagues found higher rates of CM (19-68%) than TB meningitis (1-36%) [388].
**Cryptococcus neoformans** was first identified in 1894 [389]. Subsequently, it has been isolated from numerous sites round the globe, where its main natural niche is soil, particularly in connection with pigeon excreta [390–392]. Cryptococci grow as unicellular, thickly encapsulated cells in the asexual state or as basidiomycetous filaments in the sexual state [393,394]. The lungs are the portal of entry for this organism, with frequent dissemination to the central nervous system as well as a variety of other organs including bone and skin [395-396].

“Cryptococcosis is one of the few infections in which the manifestation of disseminated infection can be proven even while the patients remain asymptomatic” [381]. Asymptomatic cryptococcosis (positive CrAg test with/without minimal symptoms) is a sub-clinical infectious condition known to herald clinically obvious disease by weeks to months and is significantly associated with risk of incident meningitis [397-399]. Cryptococcal antigenemia (positive CrAg test) is common in persons with AIDS and is inversely proportional to CD4 count [386,400–403]. Reports from sub-Saharan Africa demonstrated that patients with CD4 ≤100 cells/µL have a CRAG seroprevalence between 2.2 and 21.0 % or up to 11.5 % in studies including only asymptomatic, ART-naive HIV-infected patients [386,398,399,401,402,404–408].

Prior to the global HIV pandemic, cryptococcosis was a disease primarily of patients with immunosuppression, especially in solid organ transplant patients, in patients receiving long term immunosuppressants, haematological or other malignancies, innate immune deficiency, advanced liver or renal disease, sarcoidosis, rheumatologic disease, and diabetes mellitus, to name but a few [409–413]. Although the incidence of CM has declined significantly in industrialised nations with the introduction of ART, sub-Saharan Africa still continues to struggle with a high prevalence of HIV and opportunistic infections, with cryptococcal meningitis as the foremost cause of meningitis among HIV-infected adults [381,403]. As access to ART increases, an increasing proportion of cryptococcal meningitis cases develop in patients who have recently started ART. In Cape Town, South Africa, 1 in 5 HIV-infected patients was documented to develop CM while receiving ART (after a median duration of 41 days) with 29% mortality [382]. Over the last three decades of the HIV epidemic, extensive research has gone into improving CM management that has resulted in the revision of international guidelines focusing on diagnosis and management of the disease however, actual implementation of these
guidelines has been varied in different nations to suit their epidemiological picture [381]. A recent review of over 200 million hospital admissions in the US from 1997 to 2009 equally found a 5.8 % annual reduction of HIV- related CM and proportional increase in non-HIV CM (patients with solid-organ transplant and long-term use of immunosuppressant) over this period from 16 to 29 % of all cases [413].

Despite this trend in industrialised nations, treatment of cryptococcosis is still below standard in most African settings, given limited access to ART, the poor availability of first-line antifungal drugs used in treatment of CM and muted uptake of recommendations for management of increased intracranial pressure. Therefore, cryptococcal-associated mortality in Africa remains disappointingly high, ranging from 20% to 50% even in settings with available first-line drugs [414]. When comparing the estimate of deaths in sub-Saharan Africa with other diseases excluding HIV infection, deaths associated with CM are higher than those from tuberculosis (350,000), with the former causing 20-25% of AIDS-related mortality in Africa [384]. The CM case fatality rate is 35-65% in HIV-infected African patients compared to 14-26% amongst HIV-infected patients living in industrialised countries [415–418]. One of the main reasons for this discrepancy in case fatality is the limited diagnostic resources available in many hospitals across sub-Saharan Africa. There are also no clinical criteria that consistently predict the diagnosis of CM, and laboratories in these settings more often than not, lack the ability to perform fungal cultures or CrAg testing [419].

In 2011, the World Health Organization pronounced early ART initiation followed by preemptive fluconazole therapy in areas with high incidence of CrAg as the most significant and cost-effective preventive approach to lowering the incidence of CM-associated mortality [420]. They further declared (as a provisional recommendation, given the low grade of evidence) that routine clinical screening of and therapy for cryptococcal antigenemia should be considered before ART initiation for ART-naïve adults patients with a CD4+ count <100 cells/μL in areas with a high prevalence (3% or more) of cryptococcal antigenemia [403]. ‘With a prevalence of detectable sub-clinical cryptococcal infection averaging 7.2 % (95 % CI 6.8–7.6 %) among 36 cohorts with CD4 <100 cells/μL in Africa, together with data showing that preemptive fluconazole prevents cryptococcal disease in this group of patients, implementing a screen and treatment strategy as part of HIV care practice among patients with CD4 <100 cells/μL could
prevent the incidence of often fatal cryptococcal meningitis in the setting of the HIV pandemic’ [381].

1.5.2 Laboratory diagnosis

Early diagnosis of cryptococcal infection is the key to improving outcomes. Historically, cryptococcal infection has been diagnosed by India ink microscopy, latex agglutination for cryptococcal antigen, or culture. Culturing of the organism is considered the gold standard diagnostic method, however, it has poor sensitivity, requires a large quantity of specimen, requires laboratory infrastructure and delays in obtaining a result make culture clinically unhelpful for initial management decisions. Other diagnostics include microscopy or detection of CrAg in body fluids. Detection of CrAg in serum and CSF has extensively been utilized with very high sensitivity and specificity. CrAg is measurable in serum a median of 3 weeks before the onset of symptoms of CM, thus making screening for serum CrAg and subsequently the treatment of those with a positive test result a conceivable means of lowering CM-associated mortality [397]. However, these methods require refrigeration, a cold chain for specimen transport, and technical expertise; therefore, they are often performed only in reference/diagnostic labs far removed from patients, potentially limiting their clinical utility. In addition, they are expensive. Thus cryptococcosis often goes undiagnosed in resource-limited countries.

CrAg assays include enzyme immunoassay (EIA), latex agglutination assay (LA), or a newly developed rapid point-of-care lateral flow assay (LFA) (IMMY, Inc., Norman, Oklahoma), which has been approved by the US Food and Drug Administration for use with serum or CSF since July 2011. The test uses an immunochromatographic test strip that contains gold-conjugated monoclonal antibodies which binds to glucuronoxylomannan (GXM) cryptococcal antigen and is capable of detecting all cryptococcal serotypes. The assay has a number of qualities that make it perfect for use in resource-limited settings; these include, it is cheap (approximately. $4), highly sensitivity/specificity, point-of-care testing, rapid turnaround time, does not requires electricity (major challenge in sub-Saharan Africa), stability of diluent and test strips at room temperature with a long shelf life (up to 2 years), easy to perform/does not require much training, and no need for processing of samples (e.g., pre-treatment, heat inactivation) or specialized laboratory equipment. Additionally, titres can be performed using this assay for CrAg quantification.
The LFA has excellent sensitivity and specificity (both in plasma and serum) that is comparable with results from other antigen-based tests [407,421–423]. Serum LFA had a sensitivity of 99.6 to 100 % and a specificity of 92 % [379,421,424,425]. The plasma LFA test seems to perform equally with the serum test [424]. A study from Tanzania demonstrated a 100 % agreement between serum LFA and LA in evaluating CrAg prevalence in asymptomatic, ART-naïve patients; thus supporting LFA as a good substitute to LA assay for use in CrAg screening [407]. A recent finding from Africa demonstrated that there is 100 % agreement between whole blood, serum, and plasma CRAG LFA testing, signifying that finger prick is a feasible alternative for point of care testing of CrAg, especially in the absence of a phlebotomist [426].

Published data however reveals poor performance of LFA with urine (has good sensitivity but poor specificity) and saliva samples (excellent specificity but poor sensitivity), resulting in a reduced positive predictive value for cryptococcosis [408,421,427]. However, systematic review of LFA studies, revealed median CSF sensitivity of 100 %, and median specificity of 97.7 %; for sera; median sensitivity was 100 %, and median specificity was 99.5 % [428].

1.5.3 Treatment
Management of CM and its complications has several components. These include: identifying the infection promptly; the role of appropriate antimicrobial therapy; handling of the increased intracranial pressure; and managing the associated neurological challenges, systemic complications (e.g. disseminated infection and syndrome of inappropriate antidiuretic hormone secretion). Delaying or mismanaging any of these components can lead to substantial increase in mortality and morbidity. In 2011, with the advent of LFA, WHO advocated a change to the diagnostic work-up of CM to include early lumbar puncture (LP) [It is also critical to measure opening pressure when performing LPs. If elevated (>20 cm H2O)] with measurement of CSF CrAg or serum CrAg [379,429]. In zones of high HIV prevalence, including CrAg screening in the initial work-up for meningitis has been shown to be highly cost-effective [430]. The screen and treat strategy in ARV-naïve patients with CD4 counts <100 cells/μL have been conducted in various settings, including South Africa, Uganda, Vietnam and Cambodia [431–434]. All studies have demonstrated the cost-effectiveness of this strategy, even in settings with a lower prevalence estimate of 2 % [381].
Flucytosine combined with amphotericin B (AmB), which is the recommended first line of drug for induction therapy, leads to quicker and increased sterilization of CSF when compared to using AmB alone. However, flucytosine is most often not available in developing countries; this is especially the case in sub-Saharan African countries (e.g., in Nigeria, flucytosine is not licensed despite the high burden of HIV infection) [435]. Government commitment and health policies are urgently needed for procuring this drug for HIV treatment programs. AmB also require rigorous laboratory monitoring, including a minimum of biweekly potassium and creatinine measurements, prehydration with 1 L normal saline prior to amphotericin dosing and management of electrolyte abnormalities to maintain potassium levels. If amphotericin B is not available, high-dose fluconazole plus flucytosine, or high-dose (800mg) fluconazole alone, is recommended [436].

Previous trials to provide primary prophylaxis with fluconazole or itraconazole did not result in better mortality outcomes [437]. Also daily therapeutic LPs should be done until the opening pressure measurement is normal on more than two consecutive days.

Deciding when ART should be introduced after diagnosing cryptococcal meningitis entails balancing the survival benefit bestowed by ART against the risk of the immune reconstitution inflammatory syndrome (IRIS), a paradoxical reaction that happens during immunologic recovery with ART notwithstanding effective therapy for the opportunistic infections[438]. Early Initiation of ART early after diagnosing cryptococcal meningitis (<2weeks) has been revealed to increase overall mortality due to immune reconstitution syndrome (IRS); therefore, it is therefore advisable to wait either 2 - 4 weeks if AmB is used for induction or 4-6 weeks if fluconazole is used before introduction of ART [379]. A recent report revealed ‘that deferring ART for 5 weeks after the diagnosis of cryptococcal meningitis was associated with significantly improved survival, as compared with initiating ART at 1 to 2 weeks, especially among patients with a paucity of white cells in cerebrospinal fluid’[379].
Table 1.10: Distribution of mortality rates from opportunistic infections in first year of ART in Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Deaths in first year of ART (%)</th>
<th>Total</th>
<th>Cryptococcal meningitis</th>
<th>PCP</th>
<th>TB</th>
<th>Undiagnosed infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morocco[440]</td>
<td>57/1243 (4.6)</td>
<td>7/57 (12)</td>
<td>?</td>
<td>20/57 (35)</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Zimbabwe + Uganda [441]</td>
<td>179/3316 (5.4)</td>
<td>20/179 (11)</td>
<td>1/179 (1)</td>
<td>14/179 (8)</td>
<td>33/179 (18)</td>
<td></td>
</tr>
<tr>
<td>Malawi [442]</td>
<td>190/1584 (12)</td>
<td>7/190 (4)</td>
<td>2/190 (1)</td>
<td>10/190 (5)</td>
<td>35/190 (18)</td>
<td></td>
</tr>
</tbody>
</table>

ART = antiretroviral therapy; PCP = Pneumocystis pneumonia; TB = tuberculosis.

In sub-Saharan Africa and in Nigeria in particular, diagnosis is often limited, and wider availability of accurate and low-cost laboratory diagnostics is urgently needed for prompt diagnosis and initiation of appropriate treatment. Wider acceptance and adoption of available preventative modalities can decrease the incidence of potentially fatal central nervous system infections in African patients living with HIV.

Figure 1.3: Therapeutic lumbar puncture and acute mortality in HIV-infected individuals with cryptococcal meningitis.

Repeated therapeutic lumbar puncture is associated with improved survival regardless of initial CSF opening pressure. Data obtained from Rolfes et al [443]. CSF.-Cerebrospinal fluid. OP. Opening pressure.
In resource-limited settings such as Nigeria, the tools for repeated lumber punctures and manometry are not available, or they exist in such limited supply thus impeding the provision of optimal care for majority of persons with HIV-associated cryptococcal meningitis. In addition to these economic barriers, there are also cultural barriers to care; for example, permission for additional lumbar punctures for management of increased intracranial pressure is often denied by patients or their families due to misunderstanding and fear [444].

The controversial role of corticosteroid in the management of cryptococcal meningitis has recently been laid to rest. A double-blind, randomized, placebo-controlled trial, that recruited 451 adult patients with HIV-associated cryptococcal meningitis in Vietnam, Thailand, Indonesia, Laos, Uganda, and Malawi did not show any mortality benefit in using adjunctive dexamethasone but rather adverse events and disability in patients that received it [445].

1.5.3 Treatment outcomes
Mortality

Cryptococcal meningitis accounts for up to 15% of HIV-related deaths [13]. It is one leading cause of early mortality among HIV-infected adults in sub-Saharan Africa [446]. Without treatment, 2 week mortality associated with acute cryptococcal meningitis is almost always 100% [447]. The early mortality associated with acute cryptococcal meningoencephalitis in the developed countries with access to ART is as few as 10-20% when managed with combination antifungal therapy. In patients treated late, with fluconazole monotherapy, the outcome is much worse, with over 50% mortality at 10 weeks [448,449,417]. Tenforde and colleagues comprehensively reviewed published mortality rates attributed to cryptococcal meningitis in resource-limited settings [22]. In their review, ten-week mortality with amphotericin-based treatment regimens ranged between 22 and 36% and twelve-month mortality between 41 and 56% [22]. A more recent report of a cohort study from Botswana enrolling 236 individuals with HIV-associated cryptococcal meningitis showed an overall mortality of 62%. The two-week, 10-week and 1-year mortality were 26%, 50%, and 65% respectively [450].
Relapses and persistent cryptococcal disease

Without consolidation and maintenance antifungal therapy, recurrence is very common occurring in up to 40-50% of the patients after a successful induction antifungal therapy [451]. The diagnosis of persistence and relapse is based on CSF culture and not on biochemical markers, microscopy or antigen tests. Management involves re-initiation of the induction agents at a higher dose and longer duration (≥4 weeks). Determination of antifungal susceptibility profile of the relapse/persistent isolates is crucial as this might indicate antifungal drug resistance. Adjunctive interferon gamma supplementation may be considered in selected patient groups [452]. Both relapsed and persistent cryptococcal disease should be clearly differentiated from C-IRIS as the later may require steroid therapy without alteration/re-initiation of induction antifungal agents [420]. The best predictors of recurrence-free survival are fluconazole treatment, a lower serum cryptococcal-antigen titre, and more prolonged primary therapy with flucytosine [451]. Maintenance therapy with fluconazole is highly effective in preventing recurrent cryptococcal infection and it remains the treatment of choice for maintenance therapy for AIDS-associated cryptococcal disease. Flucytosine may contribute to the prevention of relapse if used during the first two weeks of primary therapy [451,453]. Immune restoration and low serum cryptococcal antigen titres are associated with lower cryptococcosis relapse rates [454].

1.5.4 Cryptococcal immune reconstitution inflammatory syndrome (C-IRIS)

C-IRIS is an emerging problem in resource-limited settings, it can present any time after initiation of ART and as late as 2 years after, and the explanation for this is the persistence of CrAg in the body circulation [455,456]. The exact burden of C-IRIS is unclear in this setting probably due to both difficulties in diagnosis and the absence of diagnostic criteria [456]. C-IRIS usually presents with central nervous system manifestations, but can also display pulmonary disease and/or lymphadenitis. The meningitis with C-IRIS is characterized by a higher cerebrospinal fluid (CSF) white blood cell count and a lower cryptococcal antigen titre compared with the typical clinical picture for cryptococcal meningitis. C-IRIS can be potentially life threatening and has been associated with increased mortality [438]. The management of IRIS with corticosteroids may increase the risk of relapse which further increases the risk of recurrent IRIS and resulting complications including death.
1.6. Rationale of research described in this thesis

Presently, there is dearth of data on serious fungal infections in sub-Saharan Africa and in Nigeria especially, which is the most populous African country. A search of the literature revealed some data from the 1970s to the early 1980s and then a serious lack of data (apart from some data on superficial fungal infections). Interestingly, this period coincides with the period of ‘brain-drain’ in Nigeria, where skilled health care professionals and academia, pressed by economic hardship migrated in droves to industrialised nations and the gulf (Middle East Asia) nations [457]. The existing literature reveals numerous case reports of African histoplasmosis, three previous histoplasmin skin screening surveys (the last was over twenty years ago), a couple of case reports on opportunistic fungal infections, three decades old report on aspergillomas and a couple of reports of deep mycosis. Immunosuppression and underlying pulmonary diseases are the hallmark of most serious fungal diseases, and Nigeria with a HIV prevalence of 3.7% and an estimated population of 170 million people has the highest number of people living with HIV in the African continent. Also existing data reveals increasing incidence of other malignancies and other immunosuppressive condition. A study by Oladele and colleagues gave a hospital-based frequency of candidaemia at 5.2% in critically ill patients [458].

Pulmonary fungal infections have no pathognomonic clinical or radiologic pattern and laboratory facilities for diagnosing mycoses are sadly lacking in developing countries such as Nigeria. Thus, these diseases may be frequently confused with tuberculosis or other diseases. Greater awareness, a high index of clinical suspicion and training of laboratory personnel are important for their diagnosis. A recent report by Oladele and Denning (2014) [387] estimated that 11.8% of Nigerians have a serious fungal infection annually; an estimated 57,866 cases of cryptococcal meningitis. 75,000 patients with AIDS cases are expected to develop Pneumocystis pneumonia (40% rate in children). There were 78,032 cases of pulmonary TB in 2010, and we anticipate 19,000 new cases of chronic pulmonary aspergillosis with a five year period prevalence of 60,377 cases [387]. Epidemiological studies are required to either substantiate or debunk these estimates.

1.7. Aims of research

The general aim of this study was to investigate the epidemiology of serious fungal infections in Nigerians.
1.7.1 - Specific aims

- To evaluate chronic pulmonary aspergillosis as a possible cause of smear negative TB and or treatment failure in patients being managed for pulmonary tuberculosis in Nigeria.

- To determine the prevalence of cryptococcal antigenemia in antiretroviral (ART)-naïve and ART-experienced HIV/AIDS patients with varying CD4 counts and to ascertain possible factors associated with asymptomatic/cryptococcal antigenemia in patients attending a PEPFAR clinic in Lagos.

- To analyse the burden of histoplasmosis in Africa and determine if it is an emerging or neglected disease.

- To ascertain areas of endemicity of histoplasmosis in different localities in Nigeria using a histoplasmin skin sensitivity screening survey.

- To assess the current state of serious fungal infections and identify the missing gaps in diagnosing these infections in Nigeria.

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Chapter 2

Methodology

2.1 - Study 1 - Seroprevalence of cryptococcal antigenemia in HIV infected patients in Lagos, Nigeria

Study population

This was a prospective cross sectional study at the PEPFAR (US President’s Emergency Plan for AIDS Relief) clinic, Lagos University Teaching Hospital. The clinic has over 5,000 registered HIV-infected patients. All consecutive consenting HIV-infected adults with CD4+ count of 250 cells/mm³ or less were recruited into the study irrespective of their ART status. The exclusion criteria were non-consenting patients, CD+ counts >250 cells/mm³, patients diagnosed with cryptococcosis and/or meningitis and less than 18 years of age. For very sick patients, informed consent was obtained from the next of kin/surrogate. Ethical approval was obtained from the institutional ethics review board (NHREC:19/12/2008a; ADM/DCST/HREC/522). Recruitment of patients took place in the PEPFAR clinic and medical wards in LUTH, Lagos from November 2014 to August 2015.

Sample size determination: Assuming the prevalence of cryptococcal antigenemia is 12.7%, based on a previous study [1], in a new study sample of 200 patients the 95% CI width would be 4.6% in both directions. Approximately 25 patients would be expected to have Cryptococcus from the sample of 200 patients

Recruitment targets were;

- 200 HIV infected patients with CD4+ counts of 250 cells/mm³ or less.
  - 100 ART naïve patients
  - 100 ART experienced patients
  - 50 HIV infected patients with CD4+ count above 350 cells/mm³ and ART naïve as controls.

Data collection

A structured questionnaire was used to collect data on sociodemographic, medical history and laboratory results. Other pertinent data such as clinical examination reports,
final clinical diagnosis, drug history (including ART and antifungals) and viral load results were obtained from patients’ case files. Participants’ personal details were coded and stored in a locked file.

Sample collection and processing

Venous blood (5mL) was collected from each patient into an ethylenediaminetetraacetic acid (EDTA) vacutinised tube. The CD4+ cell count was done first (following laboratory standard operating procedures) using the Partec cyflow counter. The CrAg lateral flow assay (LFA) testing was performed on the residual sample according to manufacturer’s instruction (Immuno-Mycologics). The LFA uses immunochromographic test strips that have been impregnated with monoclonal antibody against capsular polysaccharide antigens common to pathogenic Cryptococcus spp [2]. Samples were stored at 2-8°C for up to 72 hours if there was delay in testing.

We ensured that when handling patient specimens, adequate universal control measures were to prevent exposure to etiologic agents potentially present in the specimen. Also we wore gloves when handling reagents in the kits since some reagents are preserved with 0.095% (w/w) sodium azide.

The test results were communicated to the managing clinicians to assist in management of the participants. Loss to follow-up was defined as no follow-up visits or not having visited the clinic within the last 6 months of the study period.

Statistical analysis

All analyses were performed using SPSS 20.0 (Texas, USA) program. Medians and frequencies (%) were used to describe patients’ characteristics. Fisher’s exact test was used to compare categorical variables where appropriate. Student’s t-test was performed to assess the differences between means. Binary logistic regression was used to determine factors associated with positive serum CrAg. For strength of association, adjusted odds ratios and a p value of ≤ 0.05 was considered significant.

Patients were approached to participate in the study if they were HIV positive and had a recent CD4 count (<6 months).

Data recording and storage
All data collated was entered into a password protected excel spreadsheet. The hard copy (questionnaires) data collected during the study has been stored within a locked filling cabinet; it will be kept for 5 years. Soft copy of data is only accessible to members of our research team who are working on the study. The results of the study will be stored on electronic media and accessed by password only. All patient details will be anonymized; identifying codes will be used throughout the study. The PC computer used was encrypted to University of Manchester standards. Data will be stored on University of Manchester computers for up to 10 years after the time of the study and may be used by staff at the University of Manchester, College of Medicine, University of Lagos to inform and guide future research.
2.2- Study 2 - Evaluation of chronic pulmonary aspergillosis (CPA) as a cause of smear-negative TB and or anti-TB treatment failure in HIV-infected Nigerians.

Study Population

This was a prospective cross-sectional study conducted in three centers in Nigeria, namely National Institute for Medical Research (NIMR) ART (Antiretroviral therapy) and TB (tuberculosis) clinic; DOTS (Directly Observed Therapy, Short-course) clinic at Lagos University Teaching Hospital (LUTH) and DOTS clinic at University of Ilorin Teaching Hospital (UITH). NIMR and LUTH are located in Lagos State, which is in Southwestern Nigeria with an estimated population size of 18 million people; UITH is located Kwara state which is in northwestern Nigeria with an estimated population of 3 million people. HIV positive and negative consenting adults who are at the end or are in their last month of TB treatment (smear + GeneXpert® positive) or currently being treated for ‘smear-negative TB’ were recruited into the study. In addition, two control groups were included: 50 blood donors and 50 HIV positive patients on ART with good HIV control and without a history of TB. Controls underwent venepuncture only.

Sample size: A sample size of 200 was required for this study. With the constraint of finances and time, we anticipate ~40-60 patients will be recruited with chronic pulmonary aspergillosis. This is the first study to be done, so any power calculation is speculative. However, To calculate the proportion of patients with CPA in the TB patient population with 95% confidence interval is not greater than 10% (±5% in either direction from the estimated proportion of CPA), based on an expected CPA rate of 15%, at least 196 patients are required.

Recruitment targets were thus;

- 100 HIV positive patients with previously treated TB
- 100 HIV negative patients with previously treated TB
- 100 healthy controls who had never had TB

We aimed to recruit 200 patients and 100 controls over a 12 months period. We finally recruited 208 patients with 100 controls.
Ethical approval was obtained from the University of ManchesterManchester ethics committee (Ref: ethics/16456) and the institutional Ethics Committee of the study site hospitals; LUTH (NHREC:19/12/2008a: ADM/DCST/HREC/497) and UITH (ERC PIN/2014/07/10/1342). Informed verbal and written consent was obtained from each patient and control after adequate explanation of the study and its objectives. In the centers of study, it is part of the protocol to screen all TB patients for HIV infection.

Study Design

The study was carried out in two phases;

First phase: This involved patient recruitment, administering questionnaires, sample (sputum and blood) collection and chest X-ray (CXR).

A simple clinical examination including chest auscultation and blood oxygen level testing was performed and documented. Where patients have a productive cough, sputum was collected. CXR and 5mls venous blood were obtained in all patients.

During clinical assessment, demographic data were recorded including patient name, age, gender, religion, tribe, occupation and telephone number. Details of TB treatment were taken including date of commencement of treatment; smear status at diagnosis, culture results, GeneXpert PCR results and whether the patient’s symptoms resolved with treatment. The TB reference number was noted as was the HIV status was recorded together with the date of diagnosis. The most current (<6 months) CD4 count and HIV viral load was recorded.

The presence and duration of symptoms including a cough, hemoptysis, fatigue, breathlessness, fevers, night sweats, and chest pain were recorded. The score on the MRC Dyspnea scale was also recorded. A research assistant was available to administer the questionnaire and clarify questions. The ability of the patient to work normally or not was recorded. Lung percussion and auscultation were performed and the results recorded together with oxygen saturations.
Table 2.1. Medical Research Council Dyspnoea Scale (MRC Scale)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Degree of breathlessness related to activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not troubled by breathlessness except on strenuous exercise</td>
</tr>
<tr>
<td>2</td>
<td>Short of breath when hurrying on the level or walking up a slight hill</td>
</tr>
<tr>
<td>3</td>
<td>Walks slower than most people on the level/stops after a mile or so/ or stops after 15 minutes walking at own pace</td>
</tr>
<tr>
<td>4</td>
<td>Stops for breath after walking about 100 yards or after a few minutes on level ground</td>
</tr>
<tr>
<td>5</td>
<td>Too breathless to leave the house, or breathless when undressing</td>
</tr>
</tbody>
</table>

Potential CPA risk factors were recorded, including living in a traditional ‘grass-thatch’ house in comparison to a modern brick dwelling with metal or slate roof. Other factors recorded were the presence of visible dampness in the patient’s house, regular exposure to wood smoke through cooking, farming or handling farm products and cigarette smoking.

Venepuncture and blood handling

Venepuncture was performed by three trained phlebotomists working in the PEPFAR and hospital throughout the recruitment period. Samples were transported immediately to the laboratory and stored. In the laboratory, serum was separated and stored at -20°C till when assays were run; duplicates were stored in labeled cryotubes at -80°C. The -80°C freezers are put on dedicated power lines with uninterrupted power supply.

Control samples

Blood donors gave verbal consent to having some of their blood used in the study. Phlebotomists working for the blood transfusion service in Lagos University Teaching Hospital (LUTH) collected the blood and screened for HIV status. Only HIV negative samples were transported immediately to the laboratory, where serum was separated and stored as described above. 50 blood donors were recruited. A further 50 consenting HIV positive patients who were clinically stable and on ART (with no history or symptoms suggestive of TB) and have achieved goals of treatment were bled and samples stored as described above. Their names and contact details were not recorded.
Data Collection

A structured questionnaire was used to collect their basic sociodemographic data, clinical parameters and a structured medical history including possible risk factors for developing CPA. Other pertinent data such as clinical examination reports, final clinical diagnosis, drug history (including ART and antifungal medications) latest (within last six months) CD4 count and HIV viral load results were obtained from patients’ case files. Participants’ personal details were coded and stored in a locked file.

Second phase: Frozen sera were transported to the Mycology Reference Centre, Manchester, UK for Aspergillus–specific IgG serology.

Laboratory processing

Sputum culture

Sputum was collected from participants with a productive cough in a sterile container and transported to the laboratory. Each sputum sample was mixed in an equal volume of pancreatin 0.5% and centrifuged for 10 min at 3,000 rpm. The supernatant was discarded and the sediment was vortexed for 30 seconds. The sediment was then divided into two samples; one for fungal culture and the other for direct microscopic examination. The sample for fungal culture was inoculated unto Sabouraud dextrose agar (Oxoid) supplemented with chloramphenicol (0.5 mg/mL) (SC) and incubated at 27–30 °C for up to 4 weeks with twice-weekly examination (macroscopic and microscopic) and results documented. Pictures of plates both front and reverse view were taken and sent from Ilorin, which is 400KM away from Lagos by the clinical Microbiologist there. Identification of isolates was done by myself, a clinical microbiologist who did a four weeks preceptorship course at the Mycology Research Centre in UHSM and in Mycology laboratory in Royal Free Hospital in London. I also have a Masters in medical microbiology and have attended a fungal identification course in Bristol, UK. All isolates were stored at =80°C. The remaining part of the sediment was mounted with 20% potassium hydroxide (KOH). Any Aspergillus species grown were identified by subculture onto Czapek Dox agar medium and described according to macroscopic and microscopic characteristics of each colony.
Antibody testing by ELISA

The commercially produced *Aspergillus* IgG test kits by Bio-Enoche (now Dynamiker) were used locally in Nigeria to run the samples. The manufacturer’s instructions were strictly adhered to and stored samples were processed in batches of twenty to minimize error. The assay is qualitative.

All duplicate samples were stored at ≈80°C pending their transfer in dry ice to Manchester, UK. Sera were measured for *Aspergillus*-specific IgG antibodies using the ThermoFisher Scientific ImmunoCAP® system following the manufacturer’s instructions. The results are expressed in mg/L. A serum-specific IgG more than 40 mg/L was considered as elevated.

Results were communicated to managing clinicians.

Radiology

All x-rays were performed in the radiology department of the various Centres by qualified radiographers; the CDs and films were reviewed and reported by Dr. Nicholas Irurhe, a consultant radiologist.

The following pre-determined abnormalities were specifically noted as they have been associated with chronic pulmonary aspergillosis [3];

1. – Cavitation (single or multiple – size of cavities was noted)
2. – Aspergilloma (deemed probable or possible after further review by Prof Denning)
3. – Pleural thickening
4. – Pericavitary opacification

Definitions

CPA was diagnosed based on the criteria proposed by Denning et al in 2003[3] and later modified by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and European Respiratory Society clinical guidelines statement [4]. It required the presence of all of the following; 1 - underlying pulmonary disease, 2 - symptoms, 3 – characteristic radiological findings and 4 - microbiological evidence of aspergillosis, in this case, IgG antibody positivity with the ImmunoCAP.
Pulmonary tuberculosis was diagnosed (documented TB) if a patient had a positive sputum smear test for acid and alcohol fast bacilli (AAFB) or a positive GeneXpert 2110 polymerase chain reaction (PCR) test for MTB; culture results were not used due to the long incubation period of MTB.

Anti-tuberculous (anti-TB) treatment failure TB was diagnosed in a patient who was sputum smear positive at 5 months after the initiation of anti-TB treatment or in persistently symptomatic patients in the last one month of anti-TB therapy.

A patient is described as symptomatic if they have at least one of: haemoptysis, cough, productive cough, and severe fatigue. A patient is classed as having radiological features indicative of CPA if they have at least one of: cavitation, fungal ball, pleural thickening, or fibrosis. An unspecified fungal ball is diagnosed in patients with an apparent aspergilloma on CXR, but with normal *Aspergillus*-specific IgG levels.

Data recording and storage

All data were entered into a structured questionnaire and entered into a pass worded excel spreadsheet file. The hard copy (questionnaires) data collected during the study were stored in a locked filling cabinet and will be kept for 5 years. Soft copy of data is only accessible to members of our research team who are working on the study. The results of the study will be stored on electronic media and accessed by password only. All patient details will be anonymised; identifying codes will be used throughout the study. The PC computer used was encrypted to University of Manchester standards. Data will be stored on University of Manchester computers for up to 10 years after the time of the study and may be used by staff at Manchester University, College of Medicine of the University of Lagos to inform and guide future research.

Data analysis

Analyses were performed using SPSS 22 and a 5% significance level was used throughout the paper unless otherwise specified. Summary statistics were presented using frequencies and percentages for binary and categorical variables, means and standard deviations for normally distributed, continuous variables, and medians and interquartile ranges (IQR) for non-normally distributed, continuous variables. Natural logarithm
transformations have been used to perform analyses on the non-normally distributed variables.

Chi-squared tests and Fisher’s exact tests were used to compare proportions between groups and independent samples, Student t-tests were used to compare means. Linear regression was used to assess the relationship between continuous variables. To produce a model to predict IgG positive patients, logistic regression models were used. Eight variables were considered for selection, entered individually in logistic regression models and those that were significant at the 10% level (p<0.1) were included in the initial multivariable logistic regression model. This model was run and the least significant variable removed before running the model again. The process was repeated until only variables significant at the 10% significance level remained. A forward stepwise method was used to assess the robustness of the final model. The regression coefficients were used to produce a scoring model, with higher scores indicating a higher probability of being IgG positive.

The scoring model was evaluated for its discriminative ability using the AUROC (area under the receiver operating characteristic curve) and its performance was assessed by choosing a cut-off score and looking at its sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value).
2.3 Study 3 - Histoplasmosis in Africa

A review of published literature on histoplasmosis in African was conducted using the following search strategy and selection criteria:

Literature searches for publications on histoplasmosis in Africans preceding 30 March 2017, were performed using PubMed, Web of Science, Google Scholar, Cochrane Library, African Journals Online (AJOL), Africa-Wide: NiPAD, CINAHL (accessed via EBSCO Host) databases and grey literature to identify all published papers regarding the topic. Articles published in other languages (e.g., French, German and Portuguese) were considered if they were cited in any of the databases searched. The main search comprised individual searches using detailed medical subject heading (MeSH) terms for histoplasmosis, Africa (also the names of the 54 African countries), and HIV/AIDS combined with terms relevant to histoplasmosis including broad terms diagnosis and management. The Boolean operator ‘AND’ and ‘OR’ were used to combine and narrow the searches. Only reports with patients’ country of origin identified were included. The references in all relevant papers were reviewed for additional publications that may not have been cited elsewhere ("snow balling"), as well as our own paper files. We did not systematically search all meeting abstracts and other ‘grey literature’, primarily because only a very limited number of scientific conferences related to mycological topics have been held in Africa.

The case definitions employed were based on an international consensus statement by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC/IFICG) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG).[5] Species were distinguished based on morphological, biochemical and immunological properties. Cases were classified according to their clinical presentations; the demonstration of the fungus from blood, bone marrow, or from one site plus systemic complaints or two non-contiguous sites was considered indicative of disseminated disease; and localised if a single site was involved in the absence of any signs and symptoms indicative of systemic involvement (e.g, fever, weight loss, hepatomegaly, splenomegaly, pancytopenia).
2.4 Study 4 - Histoplasmin skin sensitivity survey

Study population

Both HIV infected patients attending a PEPFAR clinic with CD4 count ≥ 350 cells/mm regardless of their ART status and healthy persons from the community (whose HIV status are unknown) were recruited consecutively from six centres across five geopolitical zones of Nigeria (South Eastern Nigeria- Calabar; Northern Nigeria- Yola; North central Nigeria – Ilorin; South southern Nigeria – Benin City and South Western Nigeria- Ibadan; Lagos). This was done following a detailed literature search for reports on histoplasmosis in Nigeria. We chose areas where there have been documented reports of cases of histoplasmosis and also areas with no documented cases. Ethical approval was obtained from University of Manchester (Ref: ethics/16457) and the institutional Ethics Committee of the study sites Health management boards; Ministry of Health, Calabar (RP/REC/2016/444), Institute for Advanced Medical Research and Training, Ibadan, NHREC/05/01/2008a (UI/EC/16/0432), University of Benin Teaching Hospital Ethics and Research Committee (ADM/E22/A/VOL.VII/1468), Ministry of Health, Yola (FMCY/SUB/96N/T/X), Lagos University Teaching Hospital, Health Research and Ethics Committee. NHREC 19/12/2008a (ADM/DCST/HREC/662) and Ministry of Health, Ilorin (MOH/KS/EC/777/115). Informed verbal and written consent was obtained from each patient and control after adequate explanation of the study and its objectives.

Participants with a previous medical history of histoplasmosis and those who did not return for skin test reading were excluded.

Sample size determination

A minimum sample size of 150 will be required [6]. It was calculated using the Fisher statistical formula and then adjusted for 10% attrition due to incomplete documentation.

Data collection

A structured questionnaire relating to socio-economic and demographic data; known risk factors associated with Histoplasma infection, such as recent (up to a year before) or past (more than a year ago) activities involving soil (gardening, civil construction or agriculture) or visits to farms or caves, or the presence of birds or bats in the home or neighborhood; and travel history was administered to consenting participants. Clinical
and laboratory data, such as time of HIV diagnosis, CD4 cell count, ART and antifungal therapy was obtained from the known HIV-infected patients’ records.

Antigen preparation

The *Histoplasma* antigen was prepared by Dr. Conchita Toriello at the Universidad Nacional Autónoma de México (UNAM), Mexico City. In brief, *Histoplasma capsulatum* EH53 was obtained from the fungal collection of the Department of Microbiology and Parasitology, School of Medicine, UNAM. Strains were maintained in Sabouraud dextrose agar (Bioxon, Mexico) at 4°C and routinely inoculated in mice to check and regain virulence. Three-week-old mycelia from solid Sabouraud dextrose cultures at 28°C were inoculated into 1L Erlenmeyer flasks with 250 ml of Smith's asparagine medium [7]. They were maintained in static cultures at 28°C for 2 months. Cultures were checked for their characteristic morphology. Two-month-old cultures of the organism were killed with the addition of 0.05% thimerosal (final concentration) at 28°C for one week. The killing was routinely checked by the culture of treated mycelia in Sabouraud dextrose agar. Killed cultures were filtrated through a 0.45μm Millipore membrane; the filtrate was dialyzed and concentrated 10-fold using an Amicon ultrafiltration system with a PM-10 membrane (Amicon Corp., Lexington, MA). Each filtrate containing crude antigens, including histoplasmin was subjected to further treatment by phenol extraction, ethyl alcohol precipitation, and deproteinization by pronase and Sevag [7]. Purified antigen (PPC-histo) was the products of this last procedure. The purified antigens were adjusted to 2.5mg carbohydrates/mL and 0.5-1.0mg protein/mL [8]

Skin testing

Skin tests were performed by intradermally injecting 0.1 mL of histoplasmin antigen in a 0.1 ug protein/0.1 mL ST into the inside of the left forearm of each participant. Retractable tuberculin-type syringes were used for each consenting participant. The same investigator using the same measuring instrument performed the intradermal tests and readings. Tests were read at 48/72 hours (because some participants were recruited on a Friday reappeared on the following Monday for reading) after injection and those that produced induration ≥ 5 mm in transverse diameter after 48 or 72 hours were considered to be histoplasmin positive.

Definition
High-risk occupations were classified as agriculture/farming, builders, labourers, factory workers, and those who work with wood i.e. carpenters, furniture makers, wood cutters, wood sellers, and artisans.

Data analysis

Analyses were performed using SPSS Statistics v22.0 and a 5% significance level was used throughout the study unless otherwise specified. Summary statistics were presented using frequencies and percentages or means, standard deviations and ranges, as appropriate. Chi-squared tests and Fisher’s exact tests were used to compare proportions between groups and independent samples t-tests were used to compare means. A 0.5% significance level was used to account for multiple testing in the single variable analysis. An exploratory logistic regression analysis, with skin sensitivity as the outcome, was used to assess whether any variables that were statistically significant in the single variable analysis were independently statistically significantly associated with skin sensitivity.

Data management

This will be as documented in the other studies comprising this thesis.
2.5 Study 5 - Intensive Care Unit acquired infections in the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria.

This study was prospectively conducted in the ICU of the Lagos University Teaching Hospital, Nigeria (LUTH), with patient’s recruitment from September 2011 to July 2012. The hospital is located in the cosmopolitan city of Lagos. It is one of the largest teaching hospitals in Nigeria with 761 patients’ bed, rendering services in specialized areas in medicine such as paediatric surgery, haemodialysis, neuro-surgery, cancer treatment, eye surgery and maternal health. Its six bedded ICU admits critical patients from Medical, Surgical, Accident and Emergency and Obstetrics and Gynaecological patients. One out of the six beds is always reserved for patients that need to be isolated, for example, burns patients, so only five beds are actively in use. The ICU admits approximately 220 patients annually. Approval to carry out this research was obtained from the Ethics and Research Committee of the Lagos University Teaching Hospital. Informed consent was also obtained from the patients or their relatives.

Study design

The study was an observational, prospective study. The patients included in the study were followed up prospectively until they were either discharged from the ICU or dead.

Data collection

A structured proforma was used to collect patients’ information. The proforma was divided into sections: socio-demographic, medical and drug history, possible risk factors, and outcome. The severity of underlying diseases was assessed within 24 hours of admission using Acute Physiological and Chronic Health Evaluation index (APACHE II score). A score of twenty and above was taken as a severe premorbid state where the patient has about 40.0% probability of dying.

Inclusion criteria

All patients that were fifteen years of age and above whose relations gave informed consent over the study period were recruited into the study.

Exclusion criteria
Those unwilling to participate or whose relations did not grant consent to be recruited into the study and also patients who are anticipated to stay less than 48 hours in the ICU were excluded.

Case definition

ICU-acquired infection was defined as infection developing within 48 hours of admission into ICU and/or infections manifesting 48 hours after discharge from ICU. This means that the infection was not present, nor incubating on admission to the ICU. The definition of individual ICU-acquired infection adopted was that proposed by the Centres for Diseases Control and Prevention with minimal modification [9].

Candidaemia was defined as the isolation of Candida spp from at least one positive blood culture sample in patients with clinical signs of blood stream infection.

Case outcome

The outcome of each participant was based on whether the patient died or survived and was discharged from the ICU. The crude mortality rate for fatal cases was also ascertained.

Sample collection

Samples were collected from patients on the first day of admission into the ICU. Repeat samples were taken after 48 hours of admission. Further samples were collected whenever there was clinical suspicion of infection e.g. fever; otherwise, it was collected on every other seven days. The samples that were collected included urine, blood, and endotracheal aspirate. Others were cerebrospinal fluid, wound aspirate and stool. Most specimens were collected based on clinical diagnosis and suspicion. Each specimen was collected aseptically.

Sample processing

All samples were examined microscopically (Gram stain and wet preparation) before being cultured. The samples were cultured on appropriate media (Bactec culture system, blood agar/chocolate, and MacConkey agar) and incubated aerobically at 37°C for 18 to 24 hours. Isolates obtained after incubation were identified to the species level.
Gram-positive cocci were subjected to catalase test, catalase positive Gram-positive bacteria were further subjected to coagulase test. Coagulase positive cocci were considered as *Staphylococcus aureus*, while coagulase negative ones were referred to as Coagulase-negative staphylococci. The Gram-positive, catalase negative cocci that grew on McConkey, displaying pin-point colonies and/or magenta coloration were regarded as Enterococcus species. The Gram-negative bacteria were identified with the use of Microbact® (Oxoid, UK)

Procedure

Eight to 10 ml of blood was aseptically collected and then inoculated into the Bactec culture vial (BD Bactec Plus Aerobic/F medium). The inoculated Bactec vial was then placed in the Bactec fluorescent series instrument as soon as possible for incubation and monitoring. Positive vials were subcultured and gram-stained.

Quality control of BACTEC automated blood culture

The media in the vial has a quality control certificate indicating the organisms tested and the acceptability of those tests. Each shipment of media (vials) was tested for acceptable media performance through the use of a positive and negative vial test. The positive vial was inoculated with 1.0 mL of a 0.5 McFarland Standard of either *Escherichia coli* (ATCC 25922) or *Staphylococcus aureus* (ATCC 25923) prepared from a fresh 18-24 culture. This vial and an uninoculated vial were logged into the instrument and tested. The inoculated vial should be detected as positive by the instrument within 72 hours. The negative control vials should remain negative throughout the entire testing protocol. This verifies that the media were not subject to adverse storage or shipping conditions prior to receipt in the laboratory. If either of these vials did not give the expected results, the media (vials) should not be used.

Oxoid-Microbact

Principle

Each kit contains 12 (12A, 12B, & 12E) or 24 (24E) miniature biochemical tests. Organism identification is based on pH change and substrate utilization. Clinical use only: Microbact Gram-negative 12A (strip-format) and 12E (microplate format) may be used alone for the identification of oxidase-negative, nitrate-positive, glucose fermenters
(comprising 15 genera) and is useful for screening pathogenic *Enterobacteriaceae* from enteric and urine specimens.

Microbact Gram-negative 12B can be used in conjunction with 12A for the identification of oxidase-positive, nitrate-negative, glucose non-fermenters (Miscellaneous Gram-negative bacteria) and Enterobacteriaceae. Microbact Gram-negative 24E is a combination of the tests in 12A (or 12E) and 12B in microplate format.

**Procedure**

An 18-24 hour pure culture of the organism to be identified was obtained and Oxidase test was performed on it to determine which kit(s) to use. One to 3 isolated colonies were selected and emulsified in saline and four drops of the bacterial suspension obtained were dropped into each well. Two drops of mineral oil were also added to ‘black wells’. The entire test strip or micro-plate in holding tray was then covered with a transparent seal tape and incubated at 35º C ± 2ºC for 18-24 hours. The tray was removed from the incubator and appropriate reagents added, results were then recorded and interpreted using the Microbact Identification soft-ware package.

**Quality control**

Control strains tested for accuracy of identification were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

For the gram-positive bacteria, a combination of colonial morphology and biochemical tests were used to identify them. Catalase, coagulase, Novobiocin biochemical tests were used to differentiate the three commonest species of staphylococcus affecting man, i.e., *S. aureus, S. saprophyticus* and *S. epidermidis*. *Enterococcus spp.* (the only other gram positive isolated) was identified using its ability to grow on MacConkey, forming pin-point magenta colonies and aesculin hydrolysis.

Positive samples were examined microscopically using direct gram staining; those showing yeast were cultured unto Sabourauds dextrose agar and CHROMagar (France); germ tube test was used for presumptive diagnosis of *C. albicans*, all isolates were identified to specie level using API20AUX and or API32C.

**Antimicrobial susceptibility testing**
All Gram positive isolates were tested for their susceptibility to Ampicillin/Sulbactam 20/10μg, Amoxicillin/Clavulanate 20/10μg, Piperacillin/Tazobactam 100/10μg, Clindamycin 2μg, Levofloxacin 5μg, Gentamicin 10μg, Cefuroxime 30μg Ceftriaxone 30μg, Ciprofloxacin 5μg, Meropenem 30μg and Vancomycin (E-test was carried out for Vancomycin).

For the gram negative isolates, the following antibiotics were tested: Ampicillin/Sulbactam 20/10μg, Amoxicillin/Clavulanate 20/10μg, Piperacillin/Tazobactam 100/10μg, Levofoxacin 5μg, Gentamicin 10μg, Ceftriaxone 30μg, Cefepime 30 μg, Ciprofloxacin 5μg, Meropenem. Antimicrobial susceptibility testing was determined by Kirby-Bauer disc diffusion method, using Mueller Hinton agar plates. The bacterial suspension was prepared with peptone water to give concentration equivalent of 0.5 Mc Farland standards. The suspension was then inoculated onto Mueller Hinton agar plate by swabbing to give a growth lawn, and antibiotic discs were placed on them and incubated at 37ºC for 24 hours. The zone of inhibition diameter was measured using a calibrated ruler and interpreted as sensitive (S), intermediate (I), or resistant (R) in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Quality control organisms used were ATCC strains of E. coli (25922), S. aureus (25923), P. aeruginosa (27853).

Antifungal susceptibility testing was performed using Clinical Laboratory Standards Institute (CLSI) method [10]. Susceptibility testing to fluconazole was performed using a broth microdilution method (M27-A2). The microtitre plates were incubated at 35°C for 24-48 h. The amount of growth in a well containing the antifungal agent was compared with the amount of growth in an antifungal free growth control well. The minimum inhibitory concentration (MIC) was read as the lowest concentration of antifungal that inhibited 50 per cent growth of the organism detected visually. Quality control was ensured by testing the CLSI recommended quality control strains Candida parapsilopsis ATCC 22019 (MIC range 2-8 mg/ ml) and Candida krusei ATCC 6258 (MIC range 16-64\ mg/ml). Isolates with MIC < 8 mg/ml were considered to be susceptible to fluconazole, whereas isolates with MIC > 64 mg/ml were considered to be resistant. Isolates with MICs between 16-32 mg/ml were fluconazole-susceptible-dose dependent (S-DD).

Antibiotic resistance testing
All Staphylococci isolated were screened, according to Clinical Laboratory Standards Institute guideline [10]. The inoculum used was a direct colony suspension. After inoculating the test isolate on an MHA, the cefoxitin disc (30µg) was placed on the plate, incubated at 35 ± 2ºC for 16-18 hours. The isolate was described as MRSA or MRCoNS if the zone of inhibition was ≤21mm. The quality control strains used was ATCC S. aureus (ATCC 25923) for positive control.

Extended Spectrum Beta-lactamases (ESBLs)

The presence of ESBLs was suspected if an isolate of *Klebsiella pneumoniae* or *E. coli* demonstrated resistance to one or more of the indicator beta-lactam antibiotics – third or fourth generation cephalosporin antibiotics [10]. The screening and confirmation of ESBLs production in the gram *E. coli* and *Klebsiella spp.* was carried out using CLSI criteria. For the confirmatory test (phenotypic), a double-disc diffusion synergy test was performed. A suspension of each of the test organisms, equivalent to 0.5 McFarland standards was inoculated on the surface of MHA plates using sterile swab stick. This was allowed for 20 minutes to prediffuse into the agar. A disc of cefotaxime (30µg) and ceftazidime (30µg) were placed 20 mm apart, center to center on both sides of the disc containing clavulanic acid (10µg). The plates were inoculated for 18-24 hours at 37ºC. An extended zone of inhibition toward the disc containing clavulanic acid (dumbbell shape) was interpreted as synergy, indicating the presence of an ESBL. The quality control strain used was *E. coli* (ATCC 25922).

Macrolide-Lincosamide-Streptogramin B Resistance Testing

All *Staphylococcus* spp. isolated were subjected to this test. It was carried out using CLSI guidelines; 15µg Erythromycin and 2µg Clindamycin discs were placed 15- 26 mm apart, center to center on MHA plate. After incubation for 16-18 hours at 35 ±2ºC ambient air, a flattening of the zone of inhibition of the Clindamycin disc adjacent to the Erythromycin disc (referred to as D-zone), or presence of hazy growth within the zone of inhibition around Clindamycin even without the classical D-zone, was to be reported as “Clindamycin resistant”.

Vancomycin Resistance Testing
All Enterococcus spp. isolated was tested for Vancomycin resistance using E-test method (AB, Biodisk, Solna, Sweden) on MHA according to the manufacturer’s instructions. The plate was incubated at 37°C for 24 hours. Minimum Inhibitory Concentration (MIC) result was interpreted according to CLSI guideline. Isolate with MIC breakpoint of >16µg/ml was to be reported as resistant and the isolate would be termed Vancomycin resistant Enterococcus.

Data analysis

Data were entered into Epi Info software (Epi In, version 3.4, CDC, Atlanta GA, USA). The analysis was done with SPSS software (version 17.0, SPSS Inc, Chicago, IL., USA). Continuous variables were presented as the mean ± standard deviation. Categorical variables were presented as actual numbers and percentages or as bar charts. Categorical variables were compared using Pearson’s Chi-square test or Fisher’s exact test. P-values < 0.05 were considered significant for all tests. Multivariate logistic regression analysis was employed to determine the independent contribution of clinical variables to the prediction of acquisition of ICU infections in the hospital as dependent variables. The same statistical test was used to determine independent predictors of ICU mortality except that APACHE II-adjusted model was used. The model was employed to remove any possible confounding effect of APACHE II score on ICU mortality. Variables that had a value of P≤ 0.2 on univariate analysis were entered into a forward stepwise logistic regression model. Goodness-of-fit was evaluated by Hosmer-Lemeshow test. Two --tailed p values were reported. The association between the independent determinants of ICU-acquired infections and hospital mortality were estimated using odds ratios and 95% confidence intervals. Variables were assessed among patients based on presence or absence of infection. For outcome analysis, patients were distributed into two subgroups according to survival status (died or discharged).

Prevalence and incidence rates calculations

Prevalence rate = Number of infected patients at the time of study/Number of patients observed at the same time X 100.

In this rate, a patient with 2 infections was counted once.
Incidence rate = Number of new ICU infections acquired in a period/Total of patient-days for the same period X 1000.

Patient-days = total number of days that patients were in the ICU during the period of study.
2.6 Study 6 – Invasive Candida infections in a Nigerian neonatal intensive care unit (NICU)

A microbiology laboratory audit of all results for NICU patients in LUTH was conducted.

This study was a retrospective descriptive study of all cases of culture-proven invasive neonatal candida infection admitted to the NICU of LUTH between January 2012 and December 2015 (4 year period). Ethical approval was obtained from the Health Research and Ethics committee of the institution (NHREC: 19/12/2008a ADM/DCST/HREC/APP/101). The study participants were identified from microbiology records of all neonates admitted to the NICU with a positive candida culture. Medical records of identified neonates were reviewed, and demographic and medical information was obtained.

Definitions

Cases of invasive candida infection were defined as those that fulfilled the following criteria:

(1) A clinical picture compatible with fungal sepsis, defined by patients meeting at least 5 of the following parameters [11]: admission to the NICU, history of broad-spectrum antibiotic coverage >5 days, use of a third-generation cephalosporin, negative bacterial blood culture results despite persistent features suggestive of sepsis, need for intubation and mechanical ventilation, severe cardiovascular instability, indwelling central venous catheters, delayed feeding, preterm delivery.

(2) A *Candida* pathogen isolated from a sterile site such as blood culture; urine culture; or cerebrospinal fluid (CSF) culture.

Sample collection and processing

Blood, urine and cerebrospinal fluid samples collected aseptically from babies with a clinical diagnosis of suspected sepsis were transported to the clinical microbiology laboratory for immediate processing. Urine samples were collected by supra-pubic tap or sterile catheterization. Blood was cultured in the BACTEC culture system 9050 (Becton Dickinson, New Jersey, US) while urine and cerebrospinal fluid samples were processed according to established standardized protocol. They were cultured on Sabouraud dextrose agar (Oxoid UK) and Mueller Hinton agar (Oxoid UK) to which 5–7% blood had been added. Incubation was in room air for 24 hours at 35–37°C (Oxoid, UK). Gram stain was used to characterize isolates as yeast cells and they were further identified using germ tube test and were categorised as *Candida albicans* and non-*C. albicans* spp.
Medical records of identified Candida positive neonates were retrieved and reviewed. Demographic and medical information were obtained and entered in a pre-designed proforma. Data obtained included: gestational age, age at the culture which is also taken as age at diagnosis, sex, procedures done on the baby, antibiotics use, the day of commencing enteral feeds, other co-morbidities identified and outcome (survival or death).

Data analysis

Data were entered into Epi Info software version 3.4 (CDC, Atlanta GA, USA). The analysis was done with Statistical Package for Social Sciences (SPSS) software version 22.0 (SPSS Inc., Chicago, IL., USA). Data were presented using descriptive statistics. Discrete data were compared by Chi-square or Fisher exact test. Categorical variables were compared using Pearson’s Chi-square test or Fisher’s exact test. P-values < 0.05 were considered significant for all tests.
2.7 – Study 7 - A 12-year retrospective study of opportunistic fungal infections in HIV infected patients attending a PEPFAR clinic in Nigeria

A retrospective cohort study was conducted over a 12-year period (April 2004-February 2016). ART-naïve, HIV-infected adolescents, and adults, assessed for ART eligibility at the PEPFAR outpatient clinic at Lagos University Teaching Hospital, Nigeria, were included. Demographic, clinical and laboratory data for each clinic visit were captured in a database. A panel of laboratory tests was observed to be performed at baseline and follow-up visits, including CD4+ T-lymphocyte cell (CD4) counts and HIV-1 viral loads (VL). Opportunistic infections, including fungal infections, were diagnosed clinically. Laboratory records were accessed from the PEPFAR clinic database. Data pertaining to all microbial isolates, to bacterial, parasitic and viral infection, and to CD4%, the absolute CD4 count, and CD4/CD8 ratio were collected. Absolute CD4 count was reported, as CD4/CD8 ratio may reflect the adequacy of antiretroviral treatment. Data were tabulated into Microsoft Excel and analyzed separately for the predominant microbiologic isolates using SPSS version 21.0. Ethical approval was obtained from the institutional ethics board (NHREC: 19/12/2008a ADM/DCST/HREC/APP/129)

Definitions

We focused on opportunistic diseases that are included in Category C of the AIDS surveillance criteria of the Centre for Disease Control and Prevention [12]. The following diagnoses and criteria were used in our analyses:

1. *Pneumocystis carinii* pneumonia: a) microbiological identification using induced sputum or bronchoalveolar lavage fluid or b) symptomatic presentation with compatible chest radiologic study and clinical response to an appropriate therapeutic regimen.

2. Cytomegalovirus infection: retinitis diagnosed by clinically compatible examination by an ophthalmologist; colitis and esophagitis diagnosed by histopathologic confirmation of cytomegalovirus inclusions.

4. *Mycobacterium tuberculosis* disease: isolation in culture from a pulmonary specimen, blood, or other tissue.

5. *Toxoplasma gondii* encephalitis: symptomatic clinical presentation with a compatible computed tomographic scan or magnetic resonance image of the brain and response to
appropriate therapy; nonresponse to therapy shown by histopathologic evidence of the organism; neurologist-confirmed diagnosis.

6. *Candida* esophagitis: symptomatic presentation of dysphagia or difficulty in swallowing with endoscopic evidence of invasive fungi or with clinical response to appropriate therapy.

7. Cryptococcal meningitis: evidence or culture of the organism in the cerebrospinal fluid.

Data analysis

All analyses were performed on the diagnoses data set which contained a total of 7034 patients. Descriptive statistics were calculated for the categorical variables using frequencies (given as the number of patients and the percentage). For continuous variables, assessment of normality was carried out. If the variable was found to be normally distributed, the mean, standard deviation and range was given. Non-normally distributed variables were described using their median, range and inter-quartile range. All descriptive statistics were calculated using SPSS 22.0.

Analysis

All analyses were performed on the diagnoses dataset which contained a total of 7034 patients. Descriptive statistics were calculated for the categorical variables using frequencies (given as the number of patients and the percentage). For continuous variables, assessment of normality was carried out. If the variable was found to be normally distributed, the mean, standard deviation and range were given. Non-normally distributed variables were described using their median, range and inter-quartile range. All descriptive statistics were calculated using SPSS 22.

Data are presented as mean (±SD) values. Comparisons between the studied groups were done using Student’s *t*-test, with *p* < 0.05 considered statistically significant. The study was approved by the research and ethics committee of the hospital. This was a purely record-based study with no ethical issues.

References


Chapter 3


Abstract

Introduction and objectives:
Cryptococcal meningitis has a high mortality in HIV-infected persons in Africa. This is preventable with early screening and pre-emptive therapy. We evaluated the prevalence of cryptococcal disease by antigen testing, possible associated factors, and outcomes in HIV-infected patients being managed in a tertiary hospital in Lagos, Nigeria.

Methods:
Sera were collected from 214 consenting HIV infected participants with CD4+ counts <250 cells/mm³, irrespective of their ART status, between November 2014 and May 2015. A cryptococcal antigen lateral flow assay (CrAg®LFA) was used for testing. Pertinent clinical data was obtained from patients and their case notes.

Results:
Of the 214 participants, females 124 (57.9%) outnumbered males. Mean age was 41.3+/=9.4 years. The majority (>95%) were ART-experienced. The median CD4+ cell count was 160 cells/mm³. The overall seroprevalence of cryptococcal antigenemia was 8.9% (19/214); 6/61 (9.8%) in those with CD4+ cell counts <100 cells/mm³, 4/80 (5.0%) in the 100-200 group and 9/73 (12.3%) in 200-250 cells/mm³ group. Amongst ART-naïve patients, one (14%) was CrAg positive. 27 (12.1%) had associated oral thrush. Potential meningitic symptoms were common in the study group but were not statistically significant. Two CrAg positive patients died and 10 (52.6%) were lost to follow up. Empirical fluconazole was routinely given to those with low CD4 counts >100cells/mm³ (p=0.018), unrelated to CrAg positivity.

Conclusion:
We report a prevalence of 8.9% cryptococcal antigenemia in a setting where first line antifungals are not readily available. We recommend CrAg screening for HIV-infected patients, even if on ART.
Introduction
With approximately 35.3 million individuals living with HIV and an estimated peak of 2.3 million HIV-associated deaths in 2012 [1], sub-Saharan Africa continues to struggle with a high prevalence of HIV and consequent OIs. Cryptococcal meningitis (CM) is one of the most common of these and the most common cause of meningitis among HIV-infected adults [2,3]. A recent review by Veltman and colleagues (2014) found higher rates of CM (19-68%) than TB meningitis (1-36%) [4]. Nigeria has an estimated population of 3.2 million HIV-infected persons, second only to South Africa in terms of country burden.

Asymptomatic cryptococcosis proceeds CM by weeks to months [5-7]. Rates of asymptomatic cryptococcosis are inversely proportional to CD4 count [3,8-11]. Reports from sub-Saharan Africa demonstrated that patients with CD4 ≤100 cells/μL have a cryptococcal antigen (CrAg) seroprevalence between 2.2% and 21.0% or up to 11.5% in studies including only asymptomatic, ART-naïve HIV-infected patients [6,7,9-16]

Treatment of cryptococcosis is still below standard in most African settings [17], given the limited access to ART, poor diagnostics including lumbar puncture, the poor availability of first-line antifungal drugs used in the treatment of CM and muted uptake of recommendations for management of the increased intracranial pressure. Therefore, cryptococcal-associated mortality in Africa remains disappointingly high, ranging from 20% to 50% over 10 weeks from presentation even in settings with available first-line drugs [18]. Limited diagnostic resource in many hospitals across sub-Saharan Africa including an inability to perform fungal cultures or CrAg testing [19] is all too common.

Nigeria has an estimated 3.2 million HIV-infected patients, the second largest country burden in the world, after South Africa. In Benin City, a seroprevalence of the cryptococcal antigen of 12.7% amongst ART naïve patients [9] was used to make an estimate of 57,866 cases of cryptococcosis in Nigeria [20]. Here we evaluated the CrAg prevalence in HIV-infected patients in Lagos, with CD4+ count <250cells/mm³, irrespective of their ART status.

Methods
Study population
This was a prospective cross-sectional study at the PEPFAR (US President’s Emergency Plan for AIDS Relief) clinic, Lagos University Teaching Hospital. The clinic has over 5,000 registered HIV-infected patients. Lagos is a cosmopolitan city with an estimated population of 18 million people. All consecutive consenting HIV-infected adults with CD4\(^+\) count of 250 cells/mm\(^3\) or less were recruited into the study irrespective of their ART status. The exclusion criteria were non-consenting patients, CD4\(^+\) counts >250 cells/mm\(^3\), patients diagnosed with cryptococcosis and/or meningitis and less than 18 years of age. For very sick patients, informed consent was obtained from the next of kin/surrogate if patients were too weak to give consent. Ethical approval was obtained from the institutional ethics review board. The study period was between November 2014 and May 2015.

Data collection
A structured questionnaire was used to collect data on sociodemographic, medical history and laboratory results. Other pertinent data such as clinical examination reports, final clinical diagnosis, drug history (including ART and antifungals) and viral load results were obtained from patients’ case files. Participants’ personal details were coded and stored in a locked file.

Sample collection and processing
Venous blood (5mL) was collected from each patient into an ethylenediaminetetraacetic acid (EDTA) vacutinised tube. The CD4\(^+\) cell count was done first (following laboratory standard operating procedures) using the Partec cyflow counter (Partec, Germany). The CrAg lateral flow assay (LFA) testing was performed on the residual sample according to manufacturer’s instruction (Immuno-Mycologics, Oklahoma, US). The LFA uses immunochromographic test strips that have been impregnated with a monoclonal antibody against capsular polysaccharide antigens common to pathogenic Cryptococcus spp [21]. Samples were stored at 2-8\(^\circ\)C for up to 72 hours if there was delay in testing. The test results were communicated to the managing clinicians to assist in the management of the study participants. Loss to follow-up was defined as no refills collected or not having visited the clinic within the last 2 months of the study period [22].

Statistical analysis
All analyses will be performed using SPSS 20.0 (Texas, USA) program. Medians and frequencies (%) were used to describe patients’ characteristics. Fisher’s exact test was
used to compare categorical variables where appropriate. Student’s t-test was performed to assess the differences between means. Binary logistic regression was used to determine factors associated with positive serum CrAg. For strength of association, adjusted odds ratios and a p-value of ≤ 0.05 was considered significant.

Results

A total of 214 HIV-infected outpatients attending the PEPFAR clinic were recruited. None were suspected of having clinical meningitis at sample collection and none had been managed for cryptococcosis previously. Of these, over 95% (204) were ART-experienced (Table 1). ART-naïve accounted for 4.7% (10) and 2.3% (5) were ART defaulters. Females (124 (57.9%)) outnumbered males (90 (42.1%)) with a 1.4:1 ratio. The median age of the studied population was 40 years (IQR 35-48) with a range of 19-74 years. The median CD4+ cell count was 160 cells/mm³ (IQR 90-210). The median viral load in the 164 patients in whom it was done was 6,132 (IQR 200 – 116,179) with a range of 20 – 3.7 million copies/mL (Table 1). The duration of ART use range from <1 to 118 month (Table 2).

Table 3.1: Overview of study population demographics and clinical characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>CD4 ranges (cells/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100 (n=61)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male n (%)</td>
<td>27 (30.0%)</td>
</tr>
<tr>
<td>Female n (%)</td>
<td>31 (27.4%)</td>
</tr>
<tr>
<td>Age Median (IQR)</td>
<td>40 (37-45.7)</td>
</tr>
<tr>
<td>Viral load (copies/mL) Median (IQR)</td>
<td>46512 (4660 158000)</td>
</tr>
<tr>
<td>CD4+ cell count Median (IQR)</td>
<td>53 (29-83)</td>
</tr>
<tr>
<td>ART experienced N (%)</td>
<td>60 (98.4%)</td>
</tr>
<tr>
<td>Fluconazole use N (%)</td>
<td>33 (54%)</td>
</tr>
<tr>
<td>Neck stiffness/pain N (%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Oral thrush N (%)</td>
<td>9 (42.9%)</td>
</tr>
</tbody>
</table>
Table 3.2: CrAg positive patients to date clinical characteristics and outcomes in relation to ART

<table>
<thead>
<tr>
<th>Serial no</th>
<th>Date last seen</th>
<th>Outcome</th>
<th>Current CD4 count (cells/mm$^3$)</th>
<th>Last viral load (copies/mL)</th>
<th>Duration of ART (months)</th>
<th>Current ART regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>04/12/15</td>
<td>Current in treatment</td>
<td>796</td>
<td>Undetectable</td>
<td>37</td>
<td>TDF/3TC/EFC</td>
</tr>
<tr>
<td>2</td>
<td>20/04/15</td>
<td>Lost to follow up</td>
<td>119</td>
<td>1,279,204</td>
<td>23</td>
<td>TDF/3TC/EFC</td>
</tr>
<tr>
<td>3</td>
<td>05/05/15</td>
<td>Died</td>
<td>33</td>
<td>171,481</td>
<td>40</td>
<td>TDF/3TC/ATV/r</td>
</tr>
<tr>
<td>4</td>
<td>23/12/15</td>
<td>Current in treatment</td>
<td>214</td>
<td>Undetectable</td>
<td>8</td>
<td>TDF/3TC/EFC</td>
</tr>
<tr>
<td>5</td>
<td>11/10/15</td>
<td>Lost to follow up</td>
<td>211</td>
<td>25</td>
<td>60</td>
<td>TDF/3TC/ATV/r</td>
</tr>
<tr>
<td>6</td>
<td>08/11/14</td>
<td>Lost to follow up</td>
<td>107</td>
<td>233,867</td>
<td>20</td>
<td>TDF/3TC/EFC</td>
</tr>
<tr>
<td>7</td>
<td>10/12/15</td>
<td>Current in treatment</td>
<td>404</td>
<td>Undetectable</td>
<td>53</td>
<td>TDF/3TC/EFC</td>
</tr>
<tr>
<td>8</td>
<td>12/01/16</td>
<td>Current in treatment</td>
<td>493</td>
<td>Undetectable</td>
<td>97</td>
<td>AZT/3TC/NVP</td>
</tr>
<tr>
<td>9</td>
<td>20/09/15</td>
<td>Lost to follow up</td>
<td>291</td>
<td>33</td>
<td>14</td>
<td>3TC/ABC/EFV</td>
</tr>
<tr>
<td>10</td>
<td>21/12/15</td>
<td>Lost to follow up</td>
<td>352</td>
<td>25</td>
<td>117</td>
<td>TDF/3TC/EFC</td>
</tr>
<tr>
<td>11</td>
<td>06/01/16</td>
<td>Lost to follow up</td>
<td>135</td>
<td>30,468</td>
<td>60</td>
<td>TDF/3TC/ATV/r</td>
</tr>
<tr>
<td>12</td>
<td>26/09/15</td>
<td>Lost to follow up</td>
<td>115</td>
<td>85,290</td>
<td>19</td>
<td>TDF/3TC/EFC</td>
</tr>
<tr>
<td>13</td>
<td>13/01/16</td>
<td>Current in treatment</td>
<td>297</td>
<td>25</td>
<td>98</td>
<td>TDF/3TC/ATV/r</td>
</tr>
<tr>
<td>14</td>
<td>07/01/16</td>
<td>Current in treatment</td>
<td>294</td>
<td>2,077</td>
<td>118</td>
<td>TDF/3TC/ATV/r/AZT</td>
</tr>
<tr>
<td>15</td>
<td>26/09/15</td>
<td>Lost to follow up</td>
<td>221</td>
<td>144,000</td>
<td>33</td>
<td>AZT/3TC/NVP</td>
</tr>
<tr>
<td>16</td>
<td>07/09/15</td>
<td>Lost to follow up</td>
<td>228</td>
<td>33,101</td>
<td>97</td>
<td>AZT/3TC/NVP</td>
</tr>
<tr>
<td>17</td>
<td>15/01/15</td>
<td>Lost to follow up</td>
<td>85</td>
<td>301,266</td>
<td>22</td>
<td>TDF/3TC/EFC</td>
</tr>
<tr>
<td>18</td>
<td>31/08/15</td>
<td>Lost to follow up</td>
<td>31</td>
<td>206,382</td>
<td>63</td>
<td>TDF/3TC/ATV/r</td>
</tr>
<tr>
<td>19</td>
<td>20/05/15</td>
<td>Died</td>
<td>4</td>
<td>474,966</td>
<td>&lt;1</td>
<td>TDF/3TC/EFC</td>
</tr>
</tbody>
</table>

AZT – Zidovudine; 3TC – Lamivudine; EFV – Efavirenz; ABC – Abacavir; NVP – Nevirapine; ATV/r – Atazinavir/ritonavir; TDF – Tenofovir
The overall prevalence of cryptococcal antigenemia was 8.9% (19/214) (Fig 1). The distribution of CrAg positive across the categorized CD4+ cell groups was, 6 (9.8%) in those with CD4+ cell counts <100cells/mm³, 4 (5.0%) in the 100-200 group and 9 (12.3%) in 200-250 cells/mm³ group (Figure 1). The majority of the CrAg positive patients were ART-experienced, while of the ten ART-naïve patients, one (10%) was CrAg positive with a CD4 cell count of 190. There was no statistical significance between CrAg positive and CrAg negative patients in HIV viral load (Mann-Whitney U-test).

Fig 3.1: CrAg positivity, fluconazole use and ART relationship

Twenty-one (12.1%) patients had oral thrush with 12 (57.1%) on fluconazole therapy. The standard preventative dose used is 200mg daily. Unfortunately, fungal culture and sensitivity testing of oral candidiasis was not done so we cannot ascertain if there is fluconazole resistance present. The occurrence of oral thrush was not statistically related to CrAg positivity. Other comorbidities such as diabetes mellitus (n-1) and tuberculosis (n-2) were rare. Amongst the CrAg positive patients, three (14.3%) had headache, (unrelated to lower CD4+ cell count (p = 0.055)) and 3 patients had neck stiffness/pain (unrelated to CrAg positivity).
Thirty three patients (43.4%) with CD4+ cell count less than 100 cells/mm³ were on prophylactic fluconazole compared to 15 (19.7%) of those with >200 cells/mm³; while the 100-200 CD4+ cell count group accounted for 28 (36.9%) (Table 1) (p <0.001).

Of the 19 patients with positive CrAg results, 2 (10.5%) died of cryptococcal meningitis, 10 (52.6%) were lost to follow up and the remaining patients are currently in treatment (table 2). Lumbar puncture was performed at least in one of the patients with CM who was managed with high dose (800mg daily) fluconazole only because of non-availability of amphotericin B and flucytosine; patient had it for only three days prior to demise.

Discussion

Our study revealed an overall prevalence of cryptococcal antigenemia of 8.9% amongst HIV-infected outpatients with CD4+ counts <250 cells/mm³, irrespective of their ART status, This is particularly disturbing because WHO recommends screening for asymptomatic CrAg should be limited to ART-naïve patients with CD4 counts <100 cells/mm³ [2,9,10,11,14,15,16,17]. Amongst the study participants in the <100 CD4+ cell count group, cryptococcal antigenemia was found in 9.8%. This incidence is lower than the 12.7% found in Benin, Nigeria [9]; however, that study was amongst ART-naïve patients. Amongst our 10 ART-naïve patients, only one was CrAg positive and that patient was in the 100-200 CD4 group; further studies will be required in this patient group.

Our CrAg positivity rate of 8.9% is higher than that from some other Sub-Saharan African studies such as Ghana (2.2%), Uganda (5.8%) and Tanzania (3.7%) [4]; but lower than those from Kenya (11.5%), Ethiopia (11%) and Uganda (19%) [4]. A number of reasons have been proffered for these disparities such as seasonal variations [9,15,23,24]. *Cryptococcus* spp. are acquired through inhalation and variable intensity of local environmental presence is also likely as a cause [23,25]. While there are few pigeons in Lagos, lack of town planning allows for poultry farms in residential areas and chickens freely roam the streets.

CD4+ cell count values in this study are probably valid as the test was conducted in the APIN (Aids Prevention Initiative of Nigeria) laboratory which is an offshoot of the Harvard School of Public Health PEPFAR project and the scientists there were trained by Centre for Disease Control personnel. The CD4+ cell count test utilizes control beads as an internal control. The laboratory does quality control with CAP, UK NeQas, and other international agencies.
In the 100-200 CD4⁺ group, we demonstrated a surprisingly high prevalence, comparable to that from a similar study from Ethiopia [11], which demonstrated a 14.6% prevalence of cryptococcal antigenemia. We concur with their suggestion that a likely reason for the high prevalence of cryptococcal antigenemia among persons with a CD4 >100 is our inclusion of patients already on ART and that cryptococcal infection occurred at a lower CD4 count prior to ART initiation but they remained antigenemic as their CD4 count improved. Guidance on the management of CrAg+ patients on ART with a rising CD4 count not taking fluconazole is missing from current recommendations.

In our study site, prophylactic fluconazole has been adopted for patients with low CD4+ cell counts in view of the absence of any diagnostic test for cryptococcosis, the high mortality associated with cryptococcal meningitis, and the non-availability of the recommended first-line drugs (amphotericin B and flucytosine) in Nigeria. Fluconazole use was more common in low CD4 count patients (p=0.001) and yet 4 of 6 (67%) patients with a CD4 <100 with a positive CrAg were on fluconazole compared with those a CD4 >100 of whom only 3 of 13 (23%) were on fluconazole (p=0.13). A randomised clinical trial in Uganda revealed that fluconazole was highly effective and safe in the prevention of invasive cryptococcal disease with a protective effect that occurred both before the start and in the first few months of antiretroviral therapy [26]. Remarkably no deaths occurred in the fluconazole group. Others have found benefit with this strategy too, albeit at higher doses of fluconazole than we use in Lagos [27,28]. Adoption of this preventive strategy in Lagos is one option, but not fully effective and fluconazole resistance is a concern yet to be documented in cryptococcal isolates in Nigeria but seen in Candida spp [29].

An alternative strategy is routine clinical screening for CrAg, giving fluconazole to those who are positive, as advocated by WHO [17]. Presently the point of care test, which we used, is affordable, easy to do, require minimal infrastructure and has excellent sensitive and specificity. Screening for serum CrAg has been demonstrated to be highly sensitive, specific and cost-effective in preventing death in HIV-infected patients with CD4 counts of <100 cells/ mm3 [30]. Currently no center in Nigeria routinely screens for CrAg regardless of the CD4⁺ count.

Two of the patients that died developed cryptococcal meningitis in less than three weeks after CrAg positivity, consistent with findings by French and colleagues that noted that CrAg positivity preceded symptoms of CM by a median duration of 22 days [5]. The COAT
study show that early administrations of ART to patients with CM (which may be asymptomatic when early) do worse than if ART is delayed [30]. This is a major argument for proactive screening prior to starting ART. However, our study further buttresses the beneficial effect of ART in preventing clinical cryptococcosis infection despite cryptococcal antigenemia.

A significant limitation of this study was the limited clinical follow-up of patients, although it was designed as a cross-sectional study, not a longitudinal one. Some of the patients who were lost to follow-up might, in fact, have died without the knowledge of the PEPFAR program. Not all patients had viral load assays done thus limiting our full understanding of the impact of ART on CrAg positivity. The remarkably low number of ART-naïve patients recruited surprised us and this reflects several issues related to patient access to HIV testing and adherence. There is also the challenge of the fluidity of patient clinic attendance due to the phobia of stigmatization.

Despite Nigeria’s population of 160 million of whom 3.2 million are living with HIV infection, none have access to first-line drugs for management of cryptococcal meningitis. It is thus imperative that the public health importance of screening for cryptococcal antigenemia in the ‘at risk’ group be given urgent attention and included in the national guidelines in the management of HIV infected Nigerians.

Funding

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References


Chapter 4


Abstract

Objective: Chronic pulmonary aspergillosis (CPA) complicates pulmonary tuberculosis. We evaluated CPA as an alternative diagnosis of smear-negative TB and treatment failure in TB patients in Nigeria.

Methods: We conducted a cross-sectional multicenter survey in HIV positive and negative adult patients at the end of their TB treatment in clinics in Lagos and Ilorin states. Patients had documented TB or ‘smear-negative TB’ (both smear and GeneXpert® negative). All were assessed by clinical examination, chest X-ray and Aspergillus IgG serology and some for sputum fungal culture. CPA was defined as a positive Aspergillus fumigatus IgG titre with compatible chest X-ray or a positive sputum culture of Aspergillus with a visible fungal ball on the chest X-ray with symptoms.

Findings: 208 patients were recruited between December 2013 and September 2014. 153 (73.6%) were HIV positive. Mean age was 39.8 years, 124 (59.6%) were female and 39 (18.8%) were unable to work. Median CD4 count (range) was 169.5 cells/ml (4-593) in the HIV infected patients with positive Aspergillus IgG. 109 (52.4%) had documented TB and 140 (67.3%) had a productive cough and 50 had hemoptysis. The prevalence of CPA was 8.7%; 10 (6.5%) with HIV infection and 8 (14.5%) HIV negative patients (Fisher’s exact p=0.092).

Conclusion: CPA is a neglected disease in Nigeria, with an annual incidence of ~1,430 HIV positive CPA cases and 43,500 HIV negative CPA cases, most matching the WHO diagnostic criteria for ‘smear-negative’ tuberculosis. CPA is being misdiagnosed as smear=negative TB.
Introduction

Chronic pulmonary aspergillosis (CPA) is a problematic progressive pulmonary disease, complicating many other respiratory disorders such as tuberculosis, and affects an estimated three million people globally [1]. Prior pulmonary disorders are almost universal in CPA patients [2, 3]. The global prevalence of CPA secondary to TB is estimated to be between 0.8 and 1.37 million cases with 43 cases per 100,000 in Congo and Nigeria [4]. CPA is associated with significant morbidity and mortality. Long-term oral antifungal therapy is given because of the high frequency of relapse [2]. Resistance development or intolerable side effects occur in up to 50% of patients [5].

Studies of aspergillomas in Taiwan, South Korea, China and India identified tuberculosis as the aetiological factor in up to 93% of cases of CPA [6-9]. In Brazil, 21% of TB in-patients with Aspergillus antibodies had aspergilloma [10]. A UK survey found that out of 544 patients, who were left with a residual cavity of 2.5 cm one-year post TB therapy, 36% had positive Aspergillus antibodies and 22% had an aspergilloma after 3 years [11, 12]. No similar surveys have been reported since.

Tuberculosis is more common in Africa than in Europe or America [13], and the relationship between tuberculosis and HIV/AIDS is clear. This raises the possibility that CPA is much more common in Africa. The first case of CPA in Africa was an aspergilloma in a South African farmer in 1965 [14]. Subsequent cases have documented CPA in Ivory Coast [15], Senegal [16], Ethiopia [17], and Nigeria [18]. More recently reports have come from Uganda [19] and Tanzania [20]. A South African report revealed a 9.9% rate of aspergillomas in patients with life-threatening haemoptysis in an area of high tuberculosis incidence [21], and another from Senegal reported 35 patients with CPA using Aspergillus IgG and histology [22].

Currently, many CPA cases are either diagnosed late or misdiagnosed. Here, we evaluated CPA as a cause of smear-negative TB and or anti-TB treatment failure in HIV-positive and -negative Nigerians.

Methods

Study Population
Patients enrolled in a cross-sectional study conducted in three centers in Nigeria, namely National Institute for Medical Research (NIMR) ART (Antiretroviral therapy) and TB (tuberculosis) clinic; DOTS (Directly Observed Therapy, Short-course) clinic at Lagos University Teaching Hospital (LUTH) and DOTS clinic at University of Ilorin Teaching Hospital (UITH). HIV -positive and -negative adults who had just completed or were in their last month of TB treatment (smear + GeneXpert® positive) or being treated for ‘smear-negative TB’ were recruited. In addition, two control groups were included: 50 blood donors and 50 HIV positive patients on ART with good HIV control (CD4 count >350 cells/ml) and without a history of TB. Controls underwent venepuncture only.

Ethical approval was obtained from the institutional Ethics Committee of the study site hospitals. Informed verbal and written consent was obtained from each patient after adequate explanation of the study and its objectives. All TB patients were screened for HIV infection.

Study Design

We recruited 208 patients and 100 controls over a 12 months period, from June 2014 to May 2015. This included 153 HIV-positive patients and 55 HIV-negative patients. All were assessed clinically, chest x-ray and by Aspergillus IgG serology. Clinical examination including chest auscultation and blood oxygen level testing was performed. Where patients had a productive cough, sputum was collected. Chest X-ray and 5mls venous blood were obtained in all patients. Sputum was processed for microscopy and fungal culture (Supplementary methods).

Chest X-rays were assessed by two independent consultant radiologists (NI and JY).

Data Collection

A questionnaire was used to collect basic sociodemographic data, clinical parameters and medical history including risk factors for developing CPA. Other pertinent data such as clinical examination reports, final clinical diagnosis, drug history (including ART and antifungal medications) latest (within last six months) CD4 count and HIV serology-status were obtained.
Laboratory processing

Serum transferred in dry ice to Manchester. *Aspergillus fumigatus*-specific IgG antibodies were measured (*Aspergillus* IgG) using ThermoFisher Scientific ImmunoCAP® system. An *Aspergillus* IgG more than the threshold of 40 mg/L was considered as elevated.

Definitions

CPA was diagnosed based on the criteria proposed by Denning and colleagues in 2003 [2] and later modified in 2016 [23]. It required the presence of all of the following: 1 - underlying pulmonary disease, 2 - symptoms, 3 – radiological findings and 4 - microbiological evidence of aspergillosis, *A. fumigatus* IgG antibody positive or culture from sputum of a non-*fumigatus* species of *Aspergillus* in patients with a fungal ball visible on the chest radiograph.

Pulmonary tuberculosis was diagnosed (documented TB) if a patient had a positive sputum smear test for acid and alcohol fast bacilli (AAFB) or a positive GeneXpert 2110 polymerase chain reaction (PCR) test for MTB; culture results were not used due to the long incubation period of *M. tuberculosis*.

Anti-tuberculous (anti-TB) treatment failure TB was diagnosed in a patient who was sputum smear positive at 5 months after the initiation of anti-TB treatment or in persistently symptomatic patients in the last one month of anti-TB therapy.

A patient is described as symptomatic if they have at least one of haemoptysis, cough, productive cough, and severe fatigue. A patient is classed as having radiological features indicative of CPA if they have at least one of cavitation, fungal ball, pleural thickening, or fibrosis. An unspecified fungal ball is diagnosed in patients with an apparent aspergilloma on CXR, but with normal *Aspergillus* IgG levels.

Data analysis

Analyses were performed using SPSS 22 and a 5% significance level was used unless otherwise specified. Summary statistics were presented using frequencies and percentages for binary and categorical variables, means and standard deviations for normally distributed, continuous variables, and medians and interquartile ranges (IQR) for non-normally distributed, continuous variables. Natural logarithm transformations have been used to perform analyses on the non-normally distributed variables.
Chi-squared tests and Fisher’s exact tests were used to compare proportions between groups and Student’s t-tests were used to compare means. Linear regression was used to assess the relationship between continuous variables.

Results

Of the 208 patients recruited into the study, 153 (73.6%) were HIV positive and 55 (26.4%) were HIV negative. Ninety-five (95/141, 67.4%) were receiving anti-TB therapy for the first time, while 46 (32.6%) were being retreated having been diagnosed with anti-TB treatment failure. Thirty-nine (18.8%) were unable to work due to the severity of their illness. There were 124 (59.6%) females, of whom 52 (41.9%) were 30-39 years old. The mean age of the participants was 39.8 years (SD 12.3) (range 16-82). Ninety-nine (64.7%) of those HIV-infected were 30-49 years old and 25 (16.3%) 10-29 years old. The median CD4 count was 212.5 (n=136, IQR 88.5-337.5) in the HIV-infected patients and 169.5 (n=8, range 4-593) in the HIV-infected patients with positive *Aspergillus* IgG. One hundred and nine (52.4%) had TB and 140 (67.3%) had a productive cough. The median duration of a cough amongst the studied population was 3 months (n=120, IQR 1-8), with a range of 1-96 months. Positive sputum cultures for *Aspergillus* spp. were obtained in 28 (20%); *A. fumigatus* in 15, *A. flavus* 10 and *A. niger* 3. Fifty patients had haemoptysis, nine with moderately severe frank blood haemoptysis. Only one patient had a co-existing chronic obstructive airway disease (COPD).

Serology results, CPA and risk factors

Seventeen (8.2%) patients had a positive *Aspergillus* IgG above the 40mg/L cut off. The median titre was 6.2mg/L (IQR 3.7-14.3) with a range of <2 to 194mg/L. Both CD4+ and *Aspergillus* IgG levels were positively skewed so log transformations were performed, allowing a linear regression to be used to determine their statistical relationship; it was not significant. For 150 patients a smear/GeneXpert result was provided, the remaining 58 patients were diagnosed using radiology. There was no significant association between HIV status and smear status – 27.0% (27/100) of HIV-positive patients were smear positive and 36.0% (18/50) of HIV-negative patients were smear positive (p=0.26). In the HIV positive subgroup, there was no significant difference in the proportion of IgG positive patients between patients with confirmed (smear and/or GeneXpert) TB and smear-negative patients (7.4% (2/27) vs 9.6% (7/73); Fisher’s exact p>0.99). Similarly, in
the HIV negative subgroup, there was no significant difference in the proportion of IgG positive patients with confirmed TB and smear-negative patients (5.6% (1/18) vs 18.8% (6/32); Fisher’s exact p=0.40) (table 1). However, there was a significant difference in the geometric mean of *Aspergillus* IgG concentration between the HIV infected and non-HIV infected patients (6.0 vs 10.6 mg/L; p=0.002). None of the controls had *Aspergillus* IgG levels above the threshold.

Table 4.1: Relationship between smear status and clinical features in HIV positive and negative patients separately

<table>
<thead>
<tr>
<th>Smear/GenExpert</th>
<th>HIV positive (153)</th>
<th>p-value</th>
<th>HIV negative (55)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With data: 100</td>
<td></td>
<td>With data: 50</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td></td>
<td>Positive</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>73</td>
<td>&gt;0.99</td>
<td>Negative</td>
<td>32</td>
</tr>
<tr>
<td><em>Aspergillus</em> IgG positive</td>
<td>2 (7%)</td>
<td>1 (6%)</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus</em> IgG titre</td>
<td>6.0 (1.1-135.0)</td>
<td>10.6 (1.0-159.0)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>geometric mean (range)</td>
<td>6.8 (1.0-194.0)</td>
<td>11.9 (1.0-186.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age mean (SD)</td>
<td>40.6 (11.6)</td>
<td>0.70</td>
<td>42.7 (17.6)</td>
<td>0.98</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marked haemoptysis</td>
<td>0 (0%)</td>
<td>2 (11%)</td>
<td>5/23 (22%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Fatigue</td>
<td>11 (41%)</td>
<td>12 (67%)</td>
<td>11/20 (55%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Productive cough</td>
<td>12 (44%)</td>
<td>16 (89%)</td>
<td>19/25 (76%)</td>
<td>0.43</td>
</tr>
<tr>
<td>Chest pain</td>
<td>8/26 (31%)</td>
<td>7 (39%)</td>
<td>15/20 (75%)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Using the definition of CPA, 18/208 (8.7%) had CPA, including three patients with *Aspergillus* IgG <40mg/L had fungal balls on chest X-rays and grew *A. niger* (n=2) and *A. flavus* (n=1) in their sputum. A greater proportion of HIV-negative patients had CPA (8/55, 14.5%) compared to HIV-positive patients (10/153, 6.5%), though the difference
was not significant (Fisher’s exact p=0.092). In the HIV-positive subgroup, there was no significant difference (p=0.74) in the CD4 count between those with CPA (n=9, geometric mean=162.5) and those without CPA (n=127, geometric mean=182.4). A greater proportion of patients with CPA had chest pain than those without CPA for whom accurate data was available, though the difference was not significant (58.8% (10/17) vs 36.2% (63/174); p=0.067).

Radiological findings

The proportions of different radiological features are, for the most part, similar for the whole group and the HIV-positive subgroup (Figures 4.1-4.3). In the whole group, there were 79 (46.5%) patients without CPA with a normal CXR. 84.8% (67/79) of these patients were HIV positive and 70.1% had CD4 counts below 350 cells/mm$^3$ (52.2% had CD4 count <250 cells/mm$^3$); none had Aspergillus IgG above 40mg/L. All nine HIV positive patients with Aspergillus IgG >40mg/L had radiological features suggestive of CPA and in six out of eight of those with a documented CD4 count result, it was less than 250 cells/mm$^3$.

Figure 4.1: A graph showing the percentage with radiological features in IgG positive and negative patients.
Figure 4.2: A graph showing the percentage with radiological features in those with and without CPA, including only HIV positive patients.

Figure 4.3: A graph showing the percentage with radiological features in those with and without CPA, including only HIV negative patients.
Ten of 18 (55.6%) patients with CPA had cavitation compared to 17.1% (29/170) of the patients without CPA. For fungal ball, 77.8% (14/18) of those with CPA had it compared to 4.7% (8/170) of those without CPA. Of those with fungal ball and *Aspergillus* IgG <40mg/L, three had positive culture growth of *Aspergillus* spp. For pleural thickening, 61.1% (11/18) of those with CPA had it compared to 17.1% (29/170) of those without CPA. Fibrosis was seen in 12 of 18 (66.7%) of those with CPA compared to 28.2% (48/170) of those without CPA. In the HIV positive subgroup, 50.0% (5/10) of the patients with CPA had cavitation compared to 11.4% (14/123) of the patients without CPA. Of the patients with CPA, 90.0% (9/10) had a fungal ball compared to 5.7% (7/123) of those without CPA; 60.0% (6/10) of the CPA patients had pleural thickening compared to 14.6% (18/123) of those without CPA; and 80.0% (8/10) of the CPA patients had fibrosis compared to 26.0% (32/123) of those without CPA. No formal statistical testing was done for these four radiological features since they were part of the definition of CPA. There were significant differences between the CPA and non-CPA patients in the proportion with: consolidation (66.7%, 12/18 vs 38.8%, 66/170; p=0.023), opacity (61.1%, 11/18 vs 30.6%, 52/170; p=0.009), collapse (38.9%, 7/18 vs 8.8%, 15/170; p=0.002), air crescent/Monod sign [24] (22.2%, 4/18 vs 5.3%, 9/170; p=0.025), pleural effusion (22.2%, 4/18 vs 6.5%, 11/170; p=0.041), calcification (22.2%, 4/18 vs 4.7%, 8/170; p=0.018) and hilar lymphadenopathy (27.8%, 5/18 vs. 8.2%, 14/170; p=0.023). All the radiological features that were statistically significant in the whole dataset were also significant in the HIV positive subgroup.

Normal CXRs were not found in any of the IgG positive patients but were in 79 (46.2%) of the *Aspergillus* IgG-negative patients. Eight cases of an ‘unspecified’ fungal ball were diagnosed with apparent aspergilloma on CXR, six of these patients had normal *Aspergillus* IgG levels and negative sputum cultures but some symptoms and two patients had *Aspergillus* IgG levels above the cut-off and no symptoms. These are best classified as simple aspergillomas (in the absence of CT imaging) and are not included in our CPA population or analyses.

In the HIV positive subgroup, 50.0% (5/10) of the patients with CPA had cavitation compared to 11.4% (14/123) of the patients without CPA (P=0.005, Fisher exact). Of the patients with CPA, 90.0% (9/10) had a fungal ball compared to 5.7% (7/123) of those without CPA; 60.0% (6/10) of the CPA patients had pleural thickening compared to 14.6%
(18/123) of those without CPA (p=0.002, Fisher exact); and 80.0% (8/10) of the CPA patients had fibrosis compared to 26.0% (32/123) of those without CPA (p=0.001, Fisher exact).

Development of an IgG prediction model

The following eight variables were considered for selection in the logistic regression model for predicting IgG positive: consolidation, fibrosis, cavitation, fungal ball, pleural thickening, HIV status, smear status and blood in sputum (streak or frank blood). From the regression coefficients of the final model, a scoring model was produced as follows:

Score = 4*fungal ball + 2*HIV negative + 1*cavitation

(Where if a patient has a fungal ball it would be replaced with a 1 and if not it would be replaced with a 0. For example, an HIV positive patient with a fungal ball but no cavitation would have a score of 4).

The AUROC for the scoring model was 0.92 (95% CI 0.84-1, figure 3). Table 2 shows the proportion of IgG positive patients for each score. When a cut-off score of 3 was chosen so that any patients with a score of 3 or greater were considered to be IgG positive patients, the sensitivity was 88.2% (15/17, 95% CI 65.7%-96.7%), the specificity was 86.0% (147/171, 95% CI 80.0%-90.4%), the PPV was 38.5% (15/39, 95% CI 24.9%-54.1%) and the NPV was 98.7% (147/149, 95% CI 95.2%-99.6%).

Table 4.2: Proportion of IgG positive patients for each score

<table>
<thead>
<tr>
<th>Score</th>
<th>Number (% of IgG positive patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1/104 (1.0%)</td>
</tr>
<tr>
<td>1</td>
<td>0/13 (0%)</td>
</tr>
<tr>
<td>2</td>
<td>1/32 (3.1%)</td>
</tr>
<tr>
<td>3</td>
<td>2/17 (11.8%)</td>
</tr>
<tr>
<td>4</td>
<td>4/10 (40.0%)</td>
</tr>
<tr>
<td>5</td>
<td>4/6 (66.7%)</td>
</tr>
<tr>
<td>6</td>
<td>2/3 (66.7%)</td>
</tr>
<tr>
<td>7</td>
<td>3/3 (100%)</td>
</tr>
</tbody>
</table>

Fungal ball on its own had an AUROC of 0.86 (95% CI 0.73-0.98), which suggests it was strongly associated with IgG positive in this dataset. Using a cut-off score of 4 or greater
in the scoring model would be equivalent to just using the fungal ball on its own to determine IgG positive patients.

In HIV positive patients, no other variable was significant in a logistic regression model for IgG positive when included alongside fungal ball. The AUROC of fungal ball on its own was 0.91 (95% CI 0.79-1). The sensitivity was 88.9% (8/9, 95% CI 56.5%-98.0%), the specificity was 93.5% (116/124, 95% CI 87.8%-96.7%), the PPV was 50.0% (8/16, 95% CI 28.0%-72.0%) and the NPV was 99.1% (116/117, 95% CI 95.3%-99.8%).

Figure 4.4. A ROC curve of the scoring model used to predict IgG positive patients. The AUROC was 0.92 (95% CI 0.84-1).

Discussion

The African Region accounts for about four out of every five HIV-positive TB cases and TB deaths among people who were HIV-positive [25]. Nigeria is rated fifth in the 22 high burden countries of TB [25] and CPA is a known complication of TB [26]. Even when treated, CPA has a 20–33% short-term mortality and a 50% mortality over 5 years [26]. The estimated prevalence of CPA in TB patients was estimated to be 42.9 per 100,000 in Nigeria, but this estimate was a major extrapolation, that required confirmation [26]. Furthermore, there are no published studies of CPA in HIV positive patients, other than occasional case reports. In this multicenter study, we confirmed the presence of CPA amongst patients being managed for TB in Nigeria with an overall prevalence of 8.7%. However, this overall figure obscures a proportion nearly twice as high in HIV negative
patients (14.5%), compared to HIV positive patients (6.5%). A recent report from Iran
gave a prevalence of 13.7%, very similar to our findings as they excluded HIV-infected TB
patients [27], and similar to data (16.7%) from Japan [28]. A possible explanation for the
marked difference between HIV and non-HIV infected patients is that HIV patients might
not be able to mount a sufficient antibody response during Aspergillus infection,
especially those with low CD4 counts (as seen in this study). This hypothesis is further
buttressed by the fact that none of the HIV-positive control group with CD4+ cell counts
>350 cells/ml had Aspergillus IgG levels above the cut-off. It, therefore, seems most likely
that diagnosable pulmonary aspergillosis does occur more frequently in HIV negative
patients, perhaps on account of the increased frequency of pulmonary cavitation in this
group. Though not statistically significant, it is interesting to note that 14/17 (82.4%) of
the CPA patients were smear negative compared to 91/133 (68.4%) of the non-CPA
patients.

In 2014 in Nigeria, pulmonary TB was reported in 100,000 patients with HIV infection and
470,000 without [25]. However, only 22,000 with HIV infection survived compared to
300,000 who were HIV-infected. Applying the rates of CPA we found, we would
anticipate an annual incidence of ~1,430 HIV positive CPA cases and 43,500 HIV negative
CPA cases. Assuming a 15% annual mortality or lobectomy (cure) rate, we would
anticipate a 5 year period prevalence of 141,619 CPA cases, substantially greater than
the first estimate of 60,383 [26], but close to our more recent estimate of 120,753 [29].
While the HIV patients we studied were not selected for any particular characteristic, we
did explicitly study a number of smear-negative HIV negative patients with continuing
symptoms. Therefore extrapolation to the whole HIV negative population may over-
estimate numbers but conversely, we did omit the 2 patients with probable simple
aspergillomas from this calculation. However, as more HIV positive patients survive TB,
so numbers of CPA cases will rise.

Another important finding is that more than 50% of patients met the criteria of anti-TB
treatment failure (67 were in their last month of treatment and were still symptomatic
and 46 were being retreated for TB). Only 150 patients (of which 105 were smear
negative) had smear/GeneXpert testing done, the remaining 58 patients diagnosis was
radiological. This finding highlights the danger of misdiagnosing ‘TB’ patients and
unnecessary exposure to potentially toxic anti-TB therapy. It also could mean that we have over-estimated the actual CPA rate in Nigeria, by studying more problematic cases.

We measured Aspergillus fumigatus antibodies in this study because Aspergillus fumigatus is the most commonly implicated Aspergillus spp accounting for over 90% of aspergillosis in Europe [30, 31]. However, a recent report from South–Western Nigeria using PCR revealed that A. fumigatus only accounted for 57.1% of clinical and environmental isolates, followed by A. niger 28.6% and A. flavus 7.4% [32]. Earlier studies from Eastern Nigeria showed A. fumigatus 51.9%, A. niger 33.3%, A. flavus 14.8% [33], and from Northern Nigeria, Kwanashie and colleagues found A. fumigatus accounting for 52.4%, A. flavus 21.9%, A. niger 11.4% [34]. It has been suggested that A. fumigatus assays have poor sensitivity for infection with other Aspergillus species [35, 36]. This might account for the three patients (who ended up being classified with CPA) with Aspergillus IgG <40mg/L but with positive sputum cultures for A. niger (n=2) and A. flavus (n=1).

Conventional chest radiography is the imaging modality for initial evaluation of patients with pulmonary complications in LMICs due to its low cost and overall availability. However, it has limited sensitivity and specificity compared to CT scan, since many different infections requiring quite different therapies can present with similar radiological patterns, especially in immunocompromised patients [37]. We found 43.8% of the HIV positive patients to have a normal CXR, despite being managed for tuberculosis; this is similar to the 20-50% of bacteriologically confirmed cases in HIV-infected patients [7,38]. Possibly all abnormal features returned to normal (as we sampled patients towards the end of their anti-tuberculous therapy), or they were virtually normal previously. Presently, CXR often cannot fully distinguish between pulmonary TB and CPA because most often cavities, pleural thickening and fibrosis are seen in both [39, 40]. CT scans are more sensitive in picking up subtle radiological features; this was however not done in this study, a clear limitation. There are no published data documenting the post-therapy CT appearances of pulmonary TB in HIV positive patients, whereas several in HIV-negative patients demonstrate cavitation in 21-23% of Africans [26].

We found 22 patients with fungal balls, including 14 (63.6%) who had CPA. A further 8 patients had an unspecified fungal ball without either symptoms or positive Aspergillus
IgG. This could be due to over-reading of compound shadows in the lung apex, a notoriously difficult location to confidently read, which would be resolved with CT scanning. The presence of an aspergilloma is a late manifestation of CPA in most patients, although some patients have a simple aspergilloma with few symptoms. Bilateral aspergillomas carry a particularly poor prognosis [41] but none were seen in this study although 9 patients had bilateral cavitation, and had a CT scan been done, might have demonstrated one or more aspergillomas. Haemoptysis is a known presentation of pulmonary aspergillomas with associated morbidity and mortality [22]. Frank haemoptysis was observed in nine patients in this study, similar to the frequency found in another study amongst TB patients with aspergillomas [22]. Reports of haemoptysis from aspergillomas have also been documented from Nigerian patients with associated high mortality rates (37.5%) despite surgical intervention [18]. This life-threatening condition could be pre-empted if an earlier diagnosis could be made. While tranexamic acid is helpful, long-term antifungal therapy, lobectomy and/or bronchial artery embolization are more definitive, the last not readily available.

Although chest pain was not significantly associated with CPA in our study, those with CPA were more likely to present with chest pain than those without (58.8% (10/17) vs 36.2% (63/174); p=0.067). This is consistent with a report by Denning and colleagues which documented chest pain as one of the common symptoms in CPA patients [2].

Knowledge of the factors associated with this disease will assist in narrowing down the differential diagnosis and help in deciding the most appropriate confirmatory diagnostics. The challenge of diagnosing CPA in LMICs is daunting since the clinical presentation is non-specific and patients have to pay directly for every aspect of their management in the Nigerian healthcare setting. Nigerian physicians thus minimize cost and tend to treat patient empirically and usually only order laboratory diagnostics when therapeutic measures fail. There is a dire need for quicker and cheaper diagnostics.

There are a number of limitations of this study including the lack of follow up of the study population so serial chest X-rays were not done to document progression or later development of the disease. This was not part of the study design. Also, no CT-scan was done on any of the patients due to lack of funding. Another limitation was the fact only one of the centres at the time of the study had GeneXpert so the other centres had to depend on AAFB smear and radiological features.
We believe there is a need for a predictive model that will help to increase the index of suspicion of CPA amongst clinicians in LMICs and hence maximize the patients’ funds. Clinical prediction rules (CPR) are simple, standardized clinical tools that utilize components of history, physical examination and basic testing to stratify risk, help make a diagnosis, or predict an outcome. With our data, we developed a prediction rule for Aspergillus IgG above 40mg/L, at least in the Nigerian setting. The scoring model had the good discriminative ability; the AUROC was 0.92 (95% CI 0.84-1, figure 3). The ‘best’ cut-off really depends on the importance of sensitivity, specificity, false positives and false negatives i.e. the trade-off between over-treating and missing patients that should be treated. With a cut-off score of 3 in the model, the sensitivity and specificity were both above 85%, though at the expense of possible over-diagnosis as the PPV was just less than 40%. If a score of 4 was used, the model would be equivalent to just assessing fungal ball to determine positive IgG – the sensitivity would be lower (76%) but the PPV would be improved (almost 60%). In the HIV positive group, fungal ball on its own gave a sensitivity of just less than 90% and specificity of over 90%. The PPV of 50% suggests there would still be some degree of over-diagnosis. There is the need for further studies to develop a universal predictive model for low resource settings.

References


Chapter 5


Abstract

Histoplasmosis in Africa has markedly increased since the advent of the HIV/AIDS epidemic but is under-recognised. Pulmonary histoplasmosis may be misdiagnosed as tuberculosis (TB). In the last six decades (1952–2017), 470 cases of histoplasmosis have been reported. HIV-infected patients accounted for 38% (178) of the cases. West Africa had the highest number of recorded cases with 179; the majority (162 cases) were caused by Histoplasma capsulatum var. duboisii (Hcd). From the Southern African region, 150 cases have been reported, and the majority (119) were caused by H. capsulatum var. capsulatum (Hcc). There have been 12 histoplasmin skin test surveys with rates of 0% to 35% positivity. Most cases of Hcd presented as localised lesions in immunocompetent persons; however, it was disseminated in AIDS patients. Rapid diagnosis of histoplasmosis in Africa is only currently possible using microscopy; antigen testing and PCR is not available in most of Africa. Treatment requires amphotericin B and itraconazole, both of which are not licensed or available in several parts of Africa.
Introduction

Inhalation of conidia of *H. capsulatum* leads to histoplasmosis in some people. Hcc is patchily distributed around the world, whereas Hcd is essentially restricted to Africa [1]. Histoplasmosis was first described by Darling in the Canal Zone in Panama in 1906; patients were described as presenting with features suggestive of disseminated TB [2]. The first case of Hcd was described in West Africa in 1943 by Duncan [3].

The true global burden of histoplasmosis is not well documented despite its endemcity and not addressed previously for Africa. Histoplasmosis is not a notifiable disease, thus hard data on the incidence and prevalence, as well as information on its morbidity and mortality, are fragmentary or not available in many endemic areas [4]. Recently, WHO broadened their list of core neglected tropical diseases (NTDs) to include deep mycoses, of which histoplasmosis is one [5]. The greatest attributable risk factor for histoplasmosis is the spread of HIV, although immunosuppressive agents used in transplant patients or chronic inflammatory diseases also contribute to its increase [6]. Disseminated histoplasmosis was classified as an AIDS-defining infection in 1987 [7].

During highly active antiretroviral therapy (HAART), morbidity and mortality due to histoplasmosis remain a public health problem in low- and middle-income countries (LMICs) [4]. Primary infection is extremely common in highly endemic areas based on the prevalence of skin test reactivity, with 23% to 81% and 5% to 50% of the population testing positive in Guatemala and Mexico, respectively [8,9]. In these areas, progressive disseminated histoplasmosis (PDH) can occur in 5% to 20% of patients infected with HIV [1,10]. However, in immunocompetent persons, it is mostly asymptomatic or spontaneously self-limiting [11].

Histoplasmosis is highly prevalent in areas along the Mississippi and Ohio valleys in the United States and in Central and South America [10]. It is also endemic in India and Southeast Asia. In Africa, the most predominant infective agent is Hcc, which can coexist with Hcd. Hcd is primarily found in Central and West Africa and Madagascar, and histoplasmosis caused by this fungus is often referred to as African histoplasmosis, which is a misnomer because African patients can be infected with both variants. Though not endemic in Europe, reports of microfoci of histoplasmosis due to Hcc have been reported in Italy [12].
In the African continent, there are limited incidence cohort data on histoplasmosis despite the burden of HIV disease in sub-Saharan Africa. Surveys of histoplasmin (a mycelial-phase exo-antigen) cutaneous sensitivity have shown that the rate of positive reactors ranges from 0.0% to 28% [13–21], with cross reactivity being demonstrated between Hcc and Hcd in Nigeria [16]. In Nigeria, a higher prevalence of skin test reactivity (approximately 35%) was found in rural populations, especially among farmers, local traders, and cave guides [22].

This review seeks to highlight knowledge gaps regarding the epidemiological, diagnostic (clinical and laboratory), and therapeutic aspects of histoplasmosis in HIV-infected and non–HIV-infected patients in Africa.

Search strategy and selection criteria

Literature searches for publications on histoplasmosis in Africans preceding 30 March 2017, were performed using PubMed, Web of Science, Google Scholar, Cochrane Library, African Journals Online (AJOL), Africa-Wide: NiPAD, CINAHL (accessed via EBSCO Host) databases and grey literature to identify all published papers regarding the topic. Articles published in other languages (e.g., French, German and Portuguese) were considered if they were cited in any of the databases searched. The main search comprised individual searches using detailed medical subject heading (MeSH) terms for histoplasmosis, Africa (also the names of the 54 African countries), and HIV/AIDS combined with terms relevant to histoplasmosis including broad terms diagnosis and management. The Boolean operator ‘AND’ and ‘OR’ were used to combine and narrow the searches. Only reports with patients’ country of origin identified were included. The references in all relevant papers were reviewed for additional publications that may not have been cited elsewhere (‘snow balling’), as well as our own paper files. We did not systematically search all meeting abstracts and other ‘grey literature’, primarily because only a very limited number of scientific conferences related to mycological topics have been held in Africa.

The case definitions employed were based on an international consensus statement by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC/IFICG) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) [27]. Species were distinguished based
on morphological, biochemical and immunological properties. Cases were classified according to their clinical presentations; the demonstration of the fungus from blood, bone marrow, or from one site plus systemic complaints or two non-contiguous sites was considered indicative of disseminated disease; and localised if a single site was involved in the absence of any signs and symptoms indicative of systemic involvement (e.g., fever, weight loss, hepatomegaly, splenomegaly, pancytopenia). Below is a list of all published data of histoplasmosis in Africans.

Our exhaustive literature search revealed a total of 470 documented cases of histoplasmosis reported from the African continent (Table 1) dating from 1952 to 2017 (see S1 Table). Hcd accounted for 247 reported cases and Hcc for 185 reported cases, with some only documented serologically. It is noteworthy that a significant number of the histoplasmosis cases in Africa were reported prior to the outbreak of the HIV pandemic. Hcd was the causative organism in osteolytic lesions in HIV-negative children (S1 Table). HIV coinfection was reported predominantly in Hcc and in adults. In contrast to Hcc, HIV and Hcd coinfection was initially thought to be rare in Africans [34,35]. This was most likely due to a problem of underreporting or under-recognition because recent reports have refuted this; our literature search revealed over 25 reported cases.

Table 5.1: Summary of reported cases of histoplasmosis in Africans (1952-2017)

<table>
<thead>
<tr>
<th>Country</th>
<th>Type of disease and causative agent</th>
<th>HIV status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
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<td>[102]</td>
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</tr>
<tr>
<td>Location</td>
<td>Description</td>
<td>Hcd Status</td>
<td>Reference</td>
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child, cutaneous and subcutaneous in an adult; Hcd

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</table>

**Exposure epidemiology**

Prior to the HAART era, subclinical or symptomatic histoplasmosis occurred in 12/100 person-years at risk in a cohort of HIV-infected patients in the US [27]. The introduction of effective ART has not led to a significant reduction in the incidence of histoplasmosis in HIV-infected patients [28]. No such study has been replicated in Africa.

Gugnani and colleagues. reported a histoplasmin skin sensitivity prevalence of 3.5% and 3.0% in the community and 8.9% and 6.5% in hospitalised individuals for Hcc and Hcd, respectively, in Nigeria [16]. In Eastern Nigeria, a natural reservoir of Hcd was discovered in the soil of caves mixed with bat guano [22]. There were 20 cases of Hcd infection documented in an outbreak amongst cave explorers in Nigeria [29]. Environmental exposure to nitrogen-rich guano soil has been shown to increase the risk of histoplasmosis in the general populace [27,30]. A landmark study analyzing employment and *Histoplasma* exposure in Uganda in the pre-AIDS era showed that those reactive to histoplasmin were mostly sawmill workers [18]. A past history of cave exploration; presence at and/or participation in excavation sites; woodcutting; and exposure to bird roosts, farms, or poultry have also been documented [31]. In a case-controlled study involving HIV-infected patients with histoplasmosis compared to controls without
histoplasmosis, a strong association between histoplasmosis and exposure to chicken coops was reported [31]. Skin-test surveys using histoplasmin showed some exposure throughout Central America and parts of South America as well as Puerto Rico, Dominica, and Mexico in addition to the central US with almost no skin-test sensitivity positivity in Europe apart from Italy and France [10].

**Epidemiology of histoplasmosis in Africa**

Histoplasmosis—including both Hcd and Hcc—has been reported from 32 countries in Africa (Table 1). While Hcc occurs predominantly in Southern and North Africa, Hcd is found primarily in Central and Western Africa. In addition, there have also been five documented cases described from Madagascar (Figure 1) [32,33]. This map shows the distribution of the reported cases across Africa and not necessarily the true distribution of the burden of the problem. Some African countries lack the skilled personnel and facilities to make the diagnosis, and the number of reported cases is likely to be an underestimation because of the fact that there are few skilled personnel and facilities in many areas.
In contrast to Hcc, human immunodeficiency virus (HIV) and Hcd coinfection were initially thought to be rare in Africans.[32,35] This was most likely due to a problem of under-reporting or under-recognition since recent reports have debunked this [13,14]. Our exhaustive literature search revealed a total of 470 documented cases of histoplasmosis reported from the African continent dating from 1952 to 2017 (see 5.1 table). Hcd accounted for 247 cases and Hcc 185 cases reported, with some only documented serologically (Table 5.2). It is noteworthy that a significant number of the histoplasmosis cases in Africa were reported prior to the outbreak of the HIV pandemic. Hcd was the causative organism in osteolytic lesions in HIV-negative children (table 5.1). HIV co-infection was reported predominantly in Hcc and in adults.
Table 5.2: Distribution of Histoplasmosis in Africa

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<td>Western Sahara</td>
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<td>1</td>
<td>17</td>
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<td>17</td>
</tr>
</tbody>
</table>

*HIV testing not done in one case, 15 cases were based on serological testing

***Majority of diagnoses were based on serological data and HIV status not stated in some studies

****1 case was based on serological data (histoplasmin)

*****13 cases were an outbreak with serological diagnosis and typical clinical pattern; all were pulmonary histoplasmosis.
The distribution of the disease across Africa (Fig 1) is far from uniform; Hcd was predominantly reported in West, Central, and East Africa. The majority of the reports of Hcc were in the southern part of Africa; however, Egypt and Morocco were the only two countries in Northern Africa with reports of human histoplasmosis caused by Hcc. It is possible that some of the Hcc cases in South Africa might have been Emergomyces africanus, which is endemic in Southern Africa [36]. Some apparent microfoci of Hcc are reported from the Democratic Republic of Congo (DRC), Ghana, Kenya, Ivory Coast, Benin, and Senegal. Perhaps these cases are due to the mobility and/or emigration of persons in these regions, but probably some microfoci exist. The explanation for the variability and patchy nature of the disease distribution is not clear and could possibly relate to climatic factors, bird migration, and/or contact with bat colonies.

The majority (287 out of 470 [61%]) of the reported cases were in HIV-negative patients. While Hcd accounted for 247 such cases, most were in patients who were immunocompetent (Table 1), and a significant proportion presented as localised cutaneous and/or bone lesions. However, a significant minority presented as disseminated histoplasmosis in AIDS patients. This is consistent with existing data from HIV-infected patients, among which 95% of cases involve disseminated histoplasmosis and 90% of cases involve patients with CD4 counts below 200/mm$^3$ [4]. In Southern Africa, there were 119 cases of Hcc diagnosed, with 80% (95) in HIV-infected patients, which is in keeping with a report from Europe that demonstrated the strong relationship between HIV and Hcc coinfection; histoplasmosis was the AIDS-defining disease in nearly 61% of patients in a review of histoplasmosis in Europe [37].

Of the 470 histoplasmosis cases, West Africa had the highest number of cases (179 [38%]) followed by Southern Africa (150 [32%]), while Northern Africa had the fewest documented cases (18) (Figure 1). In contrast, there are several cases of equine histoplasmosis from Northern Africa [38]. Nigeria had the highest number of reported cases (124 cases) (Table 1). Most cases presented as cutaneous and bone lesions, with only four cases of disseminated Hcd histoplasmosis, which were all HIV-positive patients. These four patients were Nigerian émigrés in the Western world and Saudi Arabia. Nigeria has the second highest number of people living with HIV infection in Africa, an estimated three million [39]. Interestingly, in several of the case reports from Nigeria, patients were first misdiagnosed as malignancies until the histology report showed
otherwise. In contrast, South Africa reported only Hcc cases, with almost an even distribution between HIV-positive and -negative patients. Zimbabwe, which shares a border with South Africa, had 57 histoplasmosis cases reported in one series, all Hcc apart from one Hcd case.

**Clinical presentation**

While Hcc typically presents as an acute respiratory or febrile picture, Hcd rarely manifests as pulmonary disease but more commonly as a subacute infection of skin, lymph nodes, subcutaneous (abscesses), and bone lesions. Disease manifestations vary depending on immune status and the number of fungal particles inhaled. Most cases of acute histoplasmosis in immunocompetent individuals tend to resolve spontaneously [11]. In immunocompromised patients, histoplasmosis accounts for significant morbidity and mortality [6].

*H. capsulatum var. dubosii* infection

A large case series (56 patients) by Cockshott and Lucas gave a detailed clinical presentation of Hcd infection [40]. Cutaneous, subcutaneous, and bone lesions were the most common clinical presentations of this infection and can be localised or disseminated [40]. Cutaneous histoplasmosis is considered to be from haematogenous spread, although occasionally primary inoculation of the skin has been documented [35]. In a series of ‘African histoplasmosis’ patients from Mali, 62% presented with skin diseases, 46% with lymphadenopathy, 21% with bone lesions, 26% with gut disease, and 4% with infection of the lungs [41]. The report of 72 patients with AIDS from Europe revealed skin manifestations in 47.2% of all cases; among the 27 cases acquired from Africa—of which seven were cases of Hcd—skin manifestations were seen in 44% [37]. Localised cutaneous Hcd is seen most predominantly in the immunocompetent individual, while disseminated Hcd is seen in the immunosuppressed. However, disseminated cutaneous disease has been documented in immunocompetent individuals infected with African histoplasmosis in Africans and non-Africans [42]. The spectrum of cutaneous eruption includes polymorphic plaques, papules, pustules, nodules, ulcers, molluscum-like lesions, acneiform eruptions, exfoliative erythroderma, abscesses, and cellulitis irrespective of the immune status [43].
Gastrointestinal histoplasmosis from Hcd has been reported in disseminated disease and the progressive disseminated form. It was found to be more common in the setting of HIV in the review by Loulergue and colleagues [44]. Clinical features include abdominal pain, hematemesis, diarrhoea, dysphagia from peritonitis, gastrointestinal bleeding, and intestinal pathologies such as ulceration and perforation [37,44–46]. Table 2 summarises the presentation of histoplasmosis in Africans.

5.3: Clinical presentation of histoplasmosis in Africans

<table>
<thead>
<tr>
<th>Clinical Classification</th>
<th>Presentations</th>
<th>Details/Complications</th>
<th>Outcome/Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic disease</td>
<td>- Positive skin sensitivity and serology -Skin and lymph nodes affectionation (localized)</td>
<td>No symptoms Seen in areas of low exposure &amp; in the immune competent</td>
<td>Non progressive skin infection (Mainly in Hcd)</td>
<td>[32,35]</td>
</tr>
<tr>
<td>Localized disease</td>
<td>-Skin and subcutaneous -Lymph nodes - Lungs (occasionally)</td>
<td>Cutaneous lesions include ulcers, molluscum-like nodules &amp; papules, non-healing ulcers, tumors, abscesses</td>
<td>Good outcome Clinical remission and clearing Most common feature in Hcd</td>
<td>[36,40,41,85,86]</td>
</tr>
<tr>
<td>Disseminated disease</td>
<td>a. Fever and constitutional symptom b. Pulmonary disease - Acute (APH) - Subacute (SAPH) - Chronic (CPH) c. Lymph nodes d. Extra pulmonary disease CNS Gastrointestinal</td>
<td>A cause of pyrexia of undetermined origin Asymptomatic complications are pericarditis with pleural effusion and pulmonary fibrosis Granulomatous disease and mediastinitis +compression peritonitis, perforation, hepatomegaly and</td>
<td>Good outcome with early diagnosis and institution of HAART - However, presentation is usually late in Africans with initial misdiagnosis for TB or sarcoidosis - Outcome often poor with death and disability (mostly in Hcc)</td>
<td>[48,86–90]</td>
</tr>
</tbody>
</table>

[42–44]
<table>
<thead>
<tr>
<th><strong>Progressive Disseminated Histoplasmosis (PDH)</strong></th>
<th><strong>Features</strong></th>
<th><strong>Some documented in Hcd</strong>&lt;br&gt;Erythema nodosum and Erythema multiforme signifies dissemination</th>
<th><strong>Outcomes</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Very rare&lt;br&gt;Risk factors include:&lt;br&gt;- AIDS&lt;br&gt;- use of corticosteroids&lt;br&gt;- hematological malignancies&lt;br&gt;- Solid organ transplant&lt;br&gt;- Use of TNFα inhibitors</td>
<td>Splenomegaly&lt;br&gt;Features described in localized + Erythema nodosum (EN) &amp; Erythema multiforme (EM) nonspecific symptoms such as recurrent fever, weight loss, anorexia + skin pigmentation asymptomatic as adrenal mass</td>
<td>May or may not cause adrenal insufficiency (mainly in Hcc)</td>
<td>Outcome good with early management</td>
</tr>
</tbody>
</table>

[91–95]

<table>
<thead>
<tr>
<th><strong>Progressive Disseminated Histoplasmosis (PDH)</strong></th>
<th><strong>Symptoms</strong></th>
<th><strong>Outcome</strong></th>
<th><strong>Notes</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Constitutional symptoms (found in Acute and Chronic PDH)&lt;br&gt;Gastrointestinal tract involvement (Subacute PDH)&lt;br&gt;Cardiac</td>
<td>Weight loss, fever, malaise, dyspnoea&lt;br&gt;Diarrhoea and abdominal pain (as in disseminated disease)&lt;br&gt;Valvular disease, cardiac insufficiency, vegetation: dyspnoea,</td>
<td>Very poor outcome (rarely Hcd, mostly Hcc)</td>
<td>Implies</td>
</tr>
</tbody>
</table>

[42–44]
(infliximab & etanercept)

<table>
<thead>
<tr>
<th>CNS</th>
<th>peripheral oedema, angina and fever Headaches, visual, gait disturbance, confusion, seizures, altered consciousness, neck stiffness and pain</th>
<th>chronic progressive disease and diagnostic clue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal affection</td>
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</tbody>
</table>

TB is the closest mimic of histoplasmosis, and limited access to diagnostic facilities may be responsible for some misdiagnoses. Similar to pulmonary TB, chronic pulmonary histoplasmosis (usually Hcc) starts with malaise, fever, fatigue, cough, and sputum. However, sputum production, weight loss, and night sweat are less prominent than TB [47]. Eventually, chronic pulmonary histoplasmosis often results in pulmonary insufficiency and cor pulmonale. It rarely causes death if untreated, unlike TB at the advanced stage [47,48].

In the context of HIV infection, comparative studies of TB and histoplasmosis found that whilst many similar features are noted in the two infections, with most reports detailing information on disseminated Hcc infection, there are also some distinguishing features (Table 5.4) [7]. In the HIV/AIDS context, disseminated histoplasmosis is usual, often with some pulmonary involvement, but other clinical features more prominent., A review of 104 cases from Panama revealed diarrhea or other gastrointestinal symptoms, skin lesions and pancytopenia in AIDS being quite different from TB [49]. Coinfection of TB and histoplasmosis has been documented in 8% to 15% of cases [50]. Other features include reticulonodular lesions, multiple pulmonary nodules, hilar and mediastinal adenopathy, and progressive fibrosis [37,51].
Table 5.4: Summary of major features associated with tuberculosis and Histoplasmosis

<table>
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<th>Parameters</th>
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<th>Histoplasmosis</th>
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</thead>
<tbody>
<tr>
<td>Immune suppression</td>
<td>**</td>
<td>↑↑</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>**</td>
<td>↑↑</td>
</tr>
<tr>
<td>Renal function</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>High liver function tests (AST, ALT, γGT, ALP, LDH)</td>
<td>**</td>
<td>↑↑</td>
</tr>
<tr>
<td>Hepatosplenomegaly (clinical and ultrasound)</td>
<td>**</td>
<td>↑↑</td>
</tr>
<tr>
<td>Inflammatory markers (CRP &gt;70)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Ferritin and triglyceride</td>
<td>**</td>
<td>↑↑</td>
</tr>
<tr>
<td>Systemic involvement (GIT, bone marrow, liver and peripheral blood)</td>
<td>**</td>
<td>↑↑</td>
</tr>
<tr>
<td>Disseminated disease</td>
<td>**</td>
<td>↑↑</td>
</tr>
<tr>
<td>Respiratory system (clinical and laboratory)</td>
<td>↑↑</td>
<td>**</td>
</tr>
<tr>
<td>Central nervous system involvement (clinical and investigation)</td>
<td>--</td>
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</table>

--- Similar features ** Lower frequency ↑↑ Higher frequency

**Diagnosis**

Culture is potentially hazardous for laboratory personnel and requires a level 3 biosafety facility (which is not available in most African countries). It remains the reference method for disseminated histoplasmosis, particularly in HIV-infected patients, although growth requires a one- to six-week incubation, resulting in delay of treatment initiation [52]. Though specificity of the culture method is 100%, sensitivity depends on the fungal load. Bone marrow aspirates yield the highest proportion of positive cultures (70%–90%) [53]. Culture is not very useful in diagnosing primary infections in immunocompetent patients with low fungal load [54]. In disseminated cases, blood cultures using the centrifugation–lysis system or automated blood culture systems have increased sensitivity [55,56].

Bone marrow biopsy for histopathology is a rapid method of establishing a definitive diagnosis of disseminated histoplasmosis [57]. However, this method lacks sensitivity for subacute and chronic forms of the pulmonary histoplasmosis [57]. Another challenge with histology is that the morphology of the *H. capsulatum* yeasts is very similar to other pathogens, and these characteristics can lead to a mistake in identification [58]. Misidentification occurs principally with *Candida glabrata, Talaromyces marneffei,*

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*Pneumocystis jirovecii, Toxoplasma gondii, Leishmania donovani,* and *Cryptococcus neoformans.*

Serology for anti-*Histoplasma* antibodies is particularly useful in cases of low fungal load, as in asymptomatic or chronic pulmonary histoplasmosis [52]. Antibody detection by immunodiffusion or complement fixation is less sensitive in immunocompromised HIV-infected patients than in immunocompetent patients [59,60]. The rise of antibody titres is usually observed two to six weeks after primary exposure [10]. Antibody testing of cerebrospinal fluid (CSF) is critical for suspected cases of neuromeningeal histoplasmosis [61]. Cross-reactions with other fungal pathogens, lymphoma, sarcoidosis, and TB have been reported [62]. A recent study from Brazil using a western blot test strip found a sensitivity of 94.9%, specificity of 94.1%, positive predictive value (PPV) 94.1%, negative predictive value (NPV) 94.9%, and almost perfect precision [63]. Another recent study demonstrated that the Miravista *Histoplasma* antibody (EIA) offers increased sensitivity over other current antibody tests and detects both immunoglobulin G (IgG) and IgM antibodies and complements antigen detection [64]. Therefore, combining antigen and EIA antibody testing provides an optimal method for diagnosis of acute pulmonary histoplasmosis [64].

Diagnosing disseminated histoplasmosis has been significantly facilitated by the development of *Histoplasma* antigen testing. The detection of Hcc circulating antigen has been performed using several EIA methods. Antigen testing of blood or urine in disseminated histoplasmosis is most sensitive in immunocompromised patients and those with more severe illness, with higher titres [65,66]. The antigen level correlates with the severity of the disease [67]. *Histoplasma* antigen cross-reacts in sporotrichosis [68,69], aspergillosis (10%), coccidioidomycosis (60%), paracoccidioidomycosis (80%), and blastomycosis (90%) [66,67]. In spite of this, *Histoplasma* antigen testing still remains the mainstay of diagnosing histoplasmosis in immunocompromised patients. There is no cross-reactivity between *Cryptococcus neoformans* and *Histoplasma* antigen [70,71].

Molecular methods have been reported for the diagnosis of histoplasmosis with inconsistent accuracy [59,71–74]. No single approach based on nucleic acid amplification assays has been established as the dominant method; *Histoplasma* can be detected in tissue biopsies and whole blood, but these methods are not sensitive enough to identify *Histoplasma* in urine or serum [75,76].
In most of the reported cases from Africa, the diagnosis was made by culture and histology; only in five countries (Tanzania, Benin, South Africa, Egypt, and Uganda) was serology reported as being used to make a diagnosis, and in three of the cases, the samples were processed in Western countries.

**Treatment**

The Infectious Diseases Society of America recommends prophylaxis with itraconazole for as long as the CD4 count remains below 150/mm$^3$ in highly endemic areas with an incidence of histoplasmosis of >10 cases per 100 person-years [77]. If efavirenz and itraconazole are given together, itraconazole levels fall by 40%, so higher doses are required.

While waiting for laboratory confirmation of histoplasmosis in a patient with a strong suspicion of histoplasmosis with or without severe symptoms, physicians have the following two choices for treatment induction: intravenous (IV) amphotericin B or oral itraconazole [53]. Although amphotericin B is usually fungicidal and has shown its efficacy in terms of survival, it is also nephrotoxic [78]. Liposomal amphotericin is superior to conventional amphotericin B for disseminated histoplasmosis in AIDS [78]. Itraconazole is also fungicidal for most isolates of *H. capsulatum*, but oral capsules are not always well absorbed in advanced AIDS, and it is associated with many drug–drug interactions, including rifampicin. For these reasons, amphotericin B is preferred for initial therapy of disseminated histoplasmosis in AIDS, but itraconazole is a good choice for subacute disseminated infection [53].

Worryingly, amphotericin B is not licensed and is unavailable in a number of African countries; even where it is available, the cost may be prohibitive [79]. Liposomal amphotericin B is excessively costly and not available in most of Africa. While available in most African countries, itraconazole is prohibitively costly in most [79]. Generic formulations are available, but varying quality is a challenge [80].

Clinical outcomes were variable in the African studies reviewed and depended on a number of factors, such as the type of disease, early/prompt diagnosis, and accessibility to the effective drugs. Most cases of cutaneous lesions and disseminated diseases resolve with amphotericin B and itraconazole, while response to ketoconazole was variable and often poor [81–85]. Loulerge and colleagues reported good responses
 (>50%) and cure in disseminated HIV-positive cases mainly with amphotericin B and high-dose itraconazole [44]. In cases with HIV, outcomes were complicated by other comorbidities associated with immune suppression [46]. In AIDS patients in the US, histoplasmosis-related mortality was around 10% during the HAART era [52].

In conclusion, histoplasmosis is a neglected disease in Africa, a continent that has a significant number of people living with HIV/AIDS. Under-recognition and under-diagnosis are major challenges attributable to the lack of skilled personnel and facilities to make this diagnosis. It is imperative that concerted efforts be made in tackling this. It is also important for physicians outside of endemic regions to recognize this disease and how to manage it. This is particularly important in view of migratory patterns of Africans.

In conclusion, histoplasmosis is a neglected disease in Africa; a continent that has a significant number of people living with HIV/AIDS. Under-recognition and under-diagnosis are major challenges attributable to the lack of skilled personnel and facilities to make this diagnosis. It is imperative that concerted efforts be made in tackling this. It is also important for physicians outside of endemic regions to recognize the circumstances leading to exposure to *Histoplasma*, its clinical manifestations, the approach to diagnosis, indications for treatment, and factors in the selection of antifungal agents. This is particularly important in view of the migratory pattern of Africans.

References


Cockshott BYWP, Lucas AO. B. PETER COCKSHOTT AND ADETOKUNDO O. LUCAS (From the Departments of Radiology and Medicine, University College Hospital, Ibadan, Nigeria) With Plates 35 to 38 IN 1947 the late Dr. J. T. Duncan suggested that there might be an African type of... 1964.


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Abstract

Objectives: Disseminated histoplasmosis is an AIDS-defining illness. Histoplasmosis is commonly misdiagnosed as tuberculosis. Nigeria has the second highest number of people living with HIV/AIDS in Africa. The present study was carried out to investigate the prevalence of skin sensitivity amongst Nigerians to histoplasmin.

Design: A cross-sectional study was conducted in six centers across five geopolitical zones of Nigeria.

Methods: We recruited both healthy non-HIV and HIV-positive adults with CD4 count ≥ 350 cells/mm regardless of their ART status from March to May 2017. Skin tests were performed intradermally; induration ≥5 mm were considered to be histoplasmin positive.

Results: 750 participants were recruited from Lagos (n=52), Yola (n=156), Ilorin (n=125), Calabar (n=120), Ibadan (n=202) and Benin (n=95). 467 (62.3%) were HIV negative, 247 (32.9%) were HIV positive and 36 (4.8%) did not know their HIV status. A total of 32/735 (4.4%) participants had a positive skin test. Study center (p<0.001), education (p=0.002) and age (p=0.005) appeared to be significantly associated with positive skin reactivity at the 0.5% significance level, while sex (p=0.031) and occupation (p=0.031) would have been significant at the 5% significance level. Males had a higher rate of reactivity than females (p=0.031, 7% vs 3%). The highest positive rates were recorded from Benin City (13/86 (15%)) and Calabar (7/120 (6%)) and no positives were recorded in Lagos (p<0.001). HIV status was not statistically significant (p=0.70).

Conclusion: Histoplasmosis diagnostics should be included in the Nigerian HIV guidelines. Epidemiological vigilance of progressive disseminated histoplasmosis should be considered by local health authorities.
Introduction

Histoplasmosis is considered a disease of worldwide distribution, with hyperendemic areas. The presence of the pathogen and clinical disease are described in large geographical areas, including most of Asia and Africa [1]. It is caused by *Histoplasma capsulatum*, a thermally dimorphic ascomycete that has a global distribution. Skin-test surveys using histoplasmin as a reagent, similar to a tuberculin test, showed some exposure throughout Central America and parts of South America as well as Puerto Rico, Dominica, and Mexico in addition to the central USA with almost no skin-test sensitivity positivity in Europe [2]. In Africa, surveys of histoplasmin skin sensitivity have demonstrated positivity rates ranging from 0.0% to 28%, [3-11] with cross-reactivity being demonstrated between *Histoplasma capsulatum var capsulatum* (Hcc) and *Histoplasma capsulatum var duboissii* (Hcd) in Nigeria [6]. In Nigeria, a higher prevalence of skin test reactivity (≈35%) was found in a rural population, especially among farmers, local traders, and cave guides [12] and this was prior to the outbreak of the HIV epidemic.

Given that disseminated histoplasmosis is an AIDS-defining illness with a high mortality [13] and that many cases of histoplasmosis have been reported in Nigeria, it is important to consider if exposure is or is not common in different locations in Nigeria. Immunosuppression is the hallmark of most pulmonary fungal diseases, and Nigeria with an HIV prevalence of 3.7% and an estimated population of 170 million people has the second highest number of people living with HIV in the African continent. In disease-endemic areas, histoplasmosis is the main differential diagnosis for tuberculosis among human immunodeficiency virus (HIV)–infected patients [14]; According to the WHO, the estimated incidence of TB in Nigeria is 322 per 100,000 population with only 15% of the total burden of the disease in the country being documentation with Genexpert and/or smear in 2015 [15]. Data on histoplasmin skin sensitivity screening is more than 3 decades old and was before the advent of the HIV epidemic in Nigeria; it has not been repeated again since. Documentation of histoplasma exposure will assist in tailoring diagnostic testing, especially in HIV-infected patients.

Methods

Both HIV infected patients attending a PEPFAR clinic with CD4 count ≥350 cells/mm
regardless of their ART status and healthy persons from the community (whose HIV status are unknown) were recruited consecutively from six centres across five geopolitical zones of Nigeria (South-eastern Nigeria – Calabar; Northern Nigeria – Yola; North-central Nigeria – Ilorin; South-southern Nigeria – Benin City and South-western Nigeria - Ibadan; Lagos). This was done following a detailed literature search for reports on histoplasmosis in Nigeria. We chose areas where there have been documented reports of cases of histoplasmosis and also areas with no documented cases. Ethical approval was obtained from the institutional Ethics Committee of the study sites’ Health Management Boards. Informed verbal and written consent was obtained from each patient and healthy person after adequate explanation of the study and its objectives. Participants with a previous medical history of histoplasmosis and those who did not return for skin test reading were excluded.

Data collection

A structured questionnaire relating to socio-economic and demographic data; known risk factors associated with *Histoplasma* infection, such as recent (up to a year before) or past (more than a year ago) activities involving soil (gardening, civil construction or agriculture) or visits to farms or caves, or the presence of birds or bats in the home or neighborhood; and travel history was administered to consenting participants. Clinical and laboratory data, such as time of HIV diagnosis, CD4 cell count, ART and antifungal therapy was obtained from the known HIV-infected patients’ records.

Antigen preparation

The *Histoplasma* antigen was prepared by Dr. Conchita Toriello lab at Universidad Nacional Autónoma de México. In brief, *Histoplasma capsulatum* EH53 was obtained from the fungal collection of the Department of Microbiology and Parasitology, School of Medicine, UNAM. Strains were maintained in Sabouraud dextrose agar (Bioxon, Mexico) at 4°C and routinely inoculated in mice to check and regain virulence. Three-week-old mycelia from solid Sabouraud dextrose cultures at 28°C were inoculated into 1L Erlenmeyer flasks with 250 ml of Smith’s asparagine medium [16]. They were maintained in static cultures at 28°C for 2 months. Cultures were checked for their characteristic morphology. Two-month-old cultures of the organism were killed with the addition of 0.05% thimerosal (final concentration) at 28°C for one week. The killing was routinely
checked by a culture of treated mycelia in Sabouraud dextrose agar. Killed cultures were filtrated through a 0.45um Millipore membrane; the filtrate was dialyzed and concentrated 10-fold using an Amicon ultrafiltration system with a PM-10 membrane (Amicon Corp., Lexington, MA). Each filtrate containing crude antigens, including histoplasmin was subjected to further treatment by phenol extraction, ethyl alcohol precipitation, and deproteinization by pronase and Sevag [16]. Purified antigen (PPC-histo) was the products of this last procedure. The purified antigens were adjusted to 2.5mg carbohydrates/mL and 0.5-1.0mg protein/mL [17]

Skin testing

Skin tests were performed by intradermally injecting 0.1 mL of histoplasmin antigen into the inside of the left forearm of each participant. Retractable tuberculin-type syringes were used for each consenting participant. The same investigator using the same measuring instrument performed the intradermal tests and readings. Tests were read at 48/72 hours (because some participants were recruited on a Friday reappeared on the following Monday for reading) after injection and those that produced induration ≥ 5 mm in transverse diameter after 48 or 72 hours were considered to be histoplasmin positive.

Definition

High-risk occupations were classified as agriculture/farming, builders, labourers, factory workers, and those who work with wood i.e. carpenters, furniture makers, wood cutters, wood sellers, and artisans.

Data analysis

Analyses were performed using SPSS Statistics v22.0 and a 5% significance level was used throughout the study unless otherwise specified. Summary statistics were presented using frequencies and percentages or means, standard deviations and ranges, as appropriate. Chi-squared tests and Fisher’s exact tests were used to compare proportions between groups and independent samples t-tests were used to compare means. A 0.5% significance level was used to account for multiple testing in the single variable analysis. An exploratory logistic regression analysis, with skin sensitivity as the outcome, was used to assess whether any variables that were statistically significant in the single variable analysis were independently statistically significantly associated with skin sensitivity.
Results

We identified 124 published clinical cases of histoplasmosis reported from Nigeria (see supplementary document). However 4 cases of disseminated histoplasmosis were in immigrants in Europe and Asia, therefore we could not determine the states in Nigeria they come from; they were thus excluded. So the 120 cases of histoplasmosis reported from Nigeria were mapped (Fig.1) and compared to soil type and vegetation. All were cases of Hcd.

There were 750 participants (who returned after 48/72 hours for test reading) across the 6 centers. Calabar was the only rural setting used for recruitment. The mean age of the participants was 39.2 years (SD 12.2) (range 14-85). Females (540 (72.0%)) outnumbered males (208 (27.7%)) with a 2.6:1 ratio. Of the 750 participants recruited into the study, 467 (62.3%) were HIV negative, 247 (32.9%) were HIV positive and 36 (4.8%) did not know their HIV status. The average age of HIV positive patients was 42.3 (SD 10.0) with a range of 18-72; the average age of HIV negative patients was 37.8 (SD 12.7) with a range of 14-85. Around a third (153/466, 33%) of the HIV negative patients were male, whereas 16% (39/246) of the HIV positive patients were male. While 173 (23%) had a tertiary level of education; 53 (7%) had no formal education.
Figure 6.1: Distribution of reported cases of histoplasmosis in Nigeria in relation to vegetation (A) and soil types (B).
Single variable analysis

A total of 32/735 (4.4%) participants had a positive skin test, defined as a reading ≥5mm (Table 1). Study centre (p<0.001), education (p=0.002) and age (p=0.005) were significantly associated with positive skin reactivity at the 0.5% significance level (p≤0.005), while sex (p=0.031) and occupation (p=0.031) would have been significant at the 5% significance level – due to the issue of multiple testing the stricter 0.5% significance level was used. The highest positive rates were recorded from Benin City (13/86 (15%)) and Calabar (7/120 (6%)) and no positives were recorded in Lagos (p<0.001). Participants with a positive skin test had a higher mean age (45.1 +/- 14.6 years) compared to those without a positive skin test (39.0 +/- 11.9 years) (p=0.005 (independent samples t-test)). The participants in Benin City had the second highest average age (41.8, SD 16.6, range 18-85) of the 6 centers; the average age in Ibadan was 43.1 (SD 10.4, range 19-72), while the other 4 centers all had participants with average ages below 39. Benin City had the third highest rate of participants with no education, 7% (7/95). Of all the risk factors analysed, lack of formal education, 7/50 (14%) (p=0.002), those that lived/worked in areas with lots of fruit trees (p=0.012), 4/51 (8%), or in a high-risk occupation (p=0.031), 6/53 (11%), were more likely to be histoplasmin positive. Males had a higher rate of reactivity than females (p=0.031, 7% vs 3%).

There did not appear to be a significant association with histoplasmin reactivity for: thatched roofs (p=0.59), corrugated roofs (p=0.71), poultry within or around residence (p=0.73), warehouses [home/place of work] (p>0.99), home or place of work in forested regions (p=0.51), contact with hunters (p=0.17), recent travel to an area with caves (p=0.67), living or working in areas with lots of birds (p=0.32), heavy construction sites near workplace or home (p>0.99), smoking (p>0.99), IV drug use (p=0.73), past chest infection (p=0.79), HIV status (p=0.70), prior antifungal therapy (p=0.56), and prior surgery (p=0.46).
Table 6.1: Participants sociodemographic, clinical features in relation to histoplasmin reactivity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reactive (≥5mm)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study centre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lagos</td>
<td>0/52 (0%)</td>
<td>&lt;0.001¹</td>
</tr>
<tr>
<td>Yola</td>
<td>4/151 (3%)</td>
<td></td>
</tr>
<tr>
<td>Ilorin</td>
<td>2/125 (2%)</td>
<td></td>
</tr>
<tr>
<td>Calabar</td>
<td>7/120 (6%)</td>
<td></td>
</tr>
<tr>
<td>Ibadan</td>
<td>6/201 (3%)</td>
<td></td>
</tr>
<tr>
<td>Benin</td>
<td>13/86 (15%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>0.031¹</td>
</tr>
<tr>
<td>Female</td>
<td>17/527 (3%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14/206 (7%)</td>
<td></td>
</tr>
<tr>
<td>Highest education qualification</td>
<td></td>
<td>0.002¹</td>
</tr>
<tr>
<td>None</td>
<td>7/50 (14%)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>7/181 (4%)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>8/322 (2%)</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>9/169 (5%)</td>
<td></td>
</tr>
<tr>
<td>House/workplace has lots of fruit trees around it</td>
<td></td>
<td>0.012²</td>
</tr>
<tr>
<td>No</td>
<td>0/100 (0%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4/51 (8%)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td>0.031²</td>
</tr>
<tr>
<td>Low risk</td>
<td>26/631 (4%)</td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>6/53 (11%)</td>
<td></td>
</tr>
</tbody>
</table>

¹Pearson chi-squared test
²Fisher’s exact test

Multivariable analysis

Variables that were significant in the single variable analysis were included in the exploratory multivariable logistic regression. The analysis is considered exploratory due to the relatively small number of participants with skin reactivity. The fruit tree variable was excluded despite being statistically significant due to a large amount of missing data. To find the variables that were independently statistically significantly associated with skin reactivity, the logistic regression model was run and variables that had a p-value greater than 0.05 were removed, one at a time, until only variables with p<0.05 remained. Only study centre (p<0.001) and education (p=0.002) were statistically significant in the final logistic regression model (see Table 2).
Table 6.2: Final logistic regression model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio for having skin reactivity (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study centre*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benin</td>
<td>1 (-)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calabar</td>
<td>0.47 (0.16-1.37)</td>
<td>0.17</td>
</tr>
<tr>
<td>Ibadan</td>
<td>0.21 (0.08-0.61)</td>
<td>0.004</td>
</tr>
<tr>
<td>Ilorin</td>
<td>0.07 (0.02-0.35)</td>
<td>0.001</td>
</tr>
<tr>
<td>Yola</td>
<td>0.12 (0.04-0.40)</td>
<td>0.001</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (-)</td>
<td>0.002</td>
</tr>
<tr>
<td>Primary</td>
<td>0.17 (0.05-0.59)</td>
<td>0.005</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.11 (0.03-0.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tertiary</td>
<td>0.32 (0.10-0.97)</td>
<td>0.045</td>
</tr>
</tbody>
</table>

*The category for Lagos, where no participant had skin reactivity, had to be removed because the logistic regression model could not converge to a solution with it included.

In an exploratory logistic regression, study center remained statistically significant after accounting for sex, education, occupation, and age, suggesting that these variables may not explain the higher rate of skin sensitivity in Benin.

Discussion

Our finding of histoplasmin reactivity in 4.4% in the studied population is significantly lower than that of the last survey conducted three decades ago by Muotoe-Okafor and colleagues [18] which was 10.6% using 5mm as the cutoff. However, their study was carried out in the vicinity of a natural focus of *Histoplasma capsulatum var duboissii*. In that study, participants included cave guides, traders and farmers examined in the immediate vicinity of the cave, 14/40 (35%) gave a positive skin test. Fifty-five out of 620 (8.8%) reacted positively to histoplasmin in another setting away from bat caves, although in the same state [18]. This location (Ogbunike, Anambra state) was not included in our study. Another plausible explanation is the fact that five out of our six study sites were in urban settings. However, our finding is similar to an earlier survey in Enugu state, South Eastern Nigeria that gave a prevalence of 3.5% [6].

Several environmental conditions have been documented to support the occurrence of *H. capsulatum* in a region [19]. Examples include temperature, high humidity, and soil pH. The growth of *H. capsulatum* in soil is markedly affected by soil pH above 10 and below 5[19]. It is interesting to note that the soil type (pH ≥7) for the areas with high
numbers of reported cases of histoplasmosis and high histoplasmin skin reactivity overlapped (Figure 1b). Benin City (15%) and Calabar (6%) which had the highest rates reported are both in the humid rain forest zone (figure 1a). Participants with houses or workplaces adjacent to numerous fruit trees had a higher rate of skin reactivity than those without fruit trees (8% vs 0%, p=0.012). This result should be treated with caution due to the small number of participants who completed the specific question regarding living in the proximity of fruit trees (n= 151). The skin-test survey also revealed a significantly higher positivity rate among persons working/living around forests, a majority of whom were from rural settings, this finding is consistent with that of a previous report [61]. Because H. capsulatum is found in soil contaminated with bird or bat droppings, farmers, bird handlers, wood cutters, etc are at risk of being exposed to Histoplasma and contracting histoplasmosis. Activities that disrupt the soil during excavation and the construction, demolition, and rehabilitation of buildings generate infected dust; therefore, construction workers in endemic areas should also be considered to be at a high risk of contracting histoplasmosis [20]. The level of education was statistically significant in our study and this is not surprising since most people with no formal or minimal formal education in Nigeria tend to do manual labour or become artisans which are some of the high risk occupations for histoplasmosis, while those with tertiary education most likely will work in offices.

Benin City, though an urban city, is well known worldwide for its traditional crafts - carving, bronze casting and terra cotta sculpture. The city is also noted for her long tradition of woodcraft and furniture making, with the sale of timber contributing significantly to the economy of the state [21]. It is also famous for its forest and rubber plantations [22]. All these activities generate dust and if the soil is infected with Histoplasma then the workers are at risk. Interestingly, there have not been any case reports of histoplasmosis from this area which could be due to lack of skilled personnel and facilities to make this diagnosis. The rate of high-risk occupations, which appeared to be a risk factor for skin sensitivity, was second highest in Benin City (16%), Calabar had a 27% high-risk occupation rate and Yola had 8%, all of the other centers had less than 1%. The 5 participants who worked with furniture or wood were all from Benin City and this is consistent with a report from Uganda that reported high histoplasmin reactivity amongst sawmill workers. [8]. However, an exploratory logistic regression suggested that differences in sex, education, occupation and age between the study centers may not
explain the higher skin sensitivity rate in Benin City as study center remained statistically significant after including these variables in the regression model. Lagos state, a cosmopolitan city, which is the commercial capital of Nigeria, had <2% histoplasmin skin reactivity. This is not so surprising because none of the study participants were involved in the high risk occupations.

As expected, there was no significant association of HIV status with histoplasmin reactivity (p=0.70) in our study, as we selected patients with higher CD4 counts. It is well documented that the greatest attributable risk factor for histoplasmosis is the spread of HIV [23], and thought to be about 100,000 cases annually [24]. Existing data demonstrates that in HIV-infected patients with histoplasmosis, the disease is disseminated in 95% of the cases, and in 90% of the cases it occurs in patients with CD4 counts below 200/mm3 [25]. A recent review showed that in Southern Africa; there were 119 cases of Hcc diagnosed with 80% (95) in HIV infected patients [26]. We suspect that cases are missed in Nigeria in AIDS, because of the lack of a rapid antigen test and facilities for prolonged fungal culture. By identifying localities of risk, we expect that focussed physician education programs and making rapid point of care tests available would aid clinicians in making prompt diagnosis.

In conclusion, we found 4.4% histoplasmin skin reactivity in a country with a high burden of tuberculosis HIV/AIDS. Histoplasmosis is commonly misdiagnosed as tuberculosis. Interestingly, there have been no reports originating from Nigeria on histoplasmosis in the HIV community though it has been documented in Nigerian immigrants/refugees in Europe. There is dire need to re-strategize the management guidelines of this group of patients in Nigeria.

References
Chapter 7


Abstract

Invasive candidiasis has been identified globally as a major cause of morbidity and mortality in neonatal intensive care units (NICU). Systemic candidiasis presents like bacterial sepsis and can involve multiple organs. Identified major risk factors for invasive candida infection (ICI) among neonates include prematurity, prolonged central venous catheterization, and antibiotic use.

Objective: To determine the prevalence of invasive candida infections in the NICU and to identify its associated risk factors.

Methods: The study was a retrospective descriptive study of all cases of culture-proven invasive candida infection in neonates admitted to the NICU over a 4 year period. Study participants were identified from microbiology records of all neonates with a positive candida culture. Medical records of identified neonates were also reviewed, and relevant information obtained.

Results: Over the four years, 2,431 new-borns were admitted into the NICU. From these, 1182 various clinical samples were collected from babies with clinical features suggestive of sepsis and processed in the Medical Microbiology laboratory. Twenty-seven (2.3%) of the cultures yielded fungal organisms which were Candida spp. Fifteen of the candida cultures were from males infants with a male: female ratio of 1.3: 1. Blood stream infection was the most frequent ICI seen in preterm babies (7 out of 12 [58.3%]). Nearly all (91.7%) affected preterm infants with ICI were less than 1500g in weight. All were exposed to invasive procedures and broad-spectrum antibiotics. Neonatal mortality among those with ICI was 18.5% compared to mortality among those without ICI. When the analysis was done in preterm neonates alone, the mortality went up to 33.3%.

Conclusion: There is a significant prevalence in invasive candidiasis in high-risk newborns and the incidence increases with increased practices in risk factors such as invasive procedures and antibiotic use and in lower gestational age babies with VLBW.
Introduction

Worldwide, invasive fungal infections are a major cause of neonatal morbidity and mortality in neonatal intensive care units (NICU). Different species of fungi had been isolated in different studies but the major culprit globally has been the Candida spp which account for more than one-fourth of all microbial infections in the new-born period most especially in the preterm babies [1]. The sources of candidiasis in NICU are often endogenous following colonization of the babies with the fungus. About 10% of these babies get colonized in the first week of life and up to 64% babies get colonized by 4 weeks of hospital stay [1]. It has been shown that Candida species rapidly colonize the skin and mucous membranes of about 40-60% of critically ill infants, and colonization can progress to invasive infection. Invasive candida infection is most often associated with end-organ dissemination of disease and has high mortality [2]. Systemic candidiasis presents like bacterial sepsis and can involve multiple organs such as the kidneys, brain, eyes, liver, spleen, bone, joints, meninges and heart [1].

Major risk factors associated with invasive neonatal candida infections include prematurity, vaginal delivery, prolonged central venous catheterization, and prolonged use of broad-spectrum antibacterial agents [3], uses of H2-receptor antagonists, gastrointestinal diseases (necrotizing enterocolitis [NEC], focal bowel perforation, intravenous nutrition and delayed enteral feeds [4].

It has been observed that many resource-poor centers in developing countries encounter many of the risk factors for invasive Candida infections in their neonatal intensive care units but few consider antifungal prophylaxis in these at-risk neonates. Babies may have been lost due to lack of antifungal prophylaxis protocol or a delay in instituting treatment due to a delay in diagnosis [3]. The diagnosis of invasive Candida infection in our environment depends majorly on microbiologic cultures most especially blood cultures [5-7]. There has not been much data on the prevalence of invasive Candida infections in neonates in Nigeria despite the fact that risk factors for invasive candida infections abound in our daily practice. The present study aims to determine the prevalence of invasive candida infections in the new-borns at the NICU of Lagos University Teaching Hospital (LUTH), Lagos and to identify associated factors.
Methods

The study was a retrospective descriptive study of all cases of culture-proven invasive neonatal candida infection admitted to the NICU of LUTH between January 2012 and December 2015 (4 year period). Ethical approval was obtained from the Health Research and Ethics committee of the institution. The study participants were identified from microbiology records of all neonates admitted to the NICU with a positive candida culture. Medical records of identified neonates were reviewed, and demographic and medical information was obtained.

Definitions

Cases of invasive candida infection were defined as those that fulfilled the following criteria:

1. A clinical picture compatible with fungal sepsis, defined by patients meeting at least 5 of the following parameters [3]: admission to the NICU, history of broad-spectrum antibiotic coverage >5 days, use of a third-generation cephalosporin, negative bacterial blood culture results despite persistent features suggestive of sepsis, need for intubation and mechanical ventilation, severe cardiovascular instability, indwelling central venous catheters, delayed feeding, preterm delivery.

2. A candida pathogen isolated from a sterile site such as blood culture; urine culture; or cerebrospinal fluid (CSF) culture.

Sample collection and processing

Blood, urine and cerebrospinal fluid samples collected aseptically from babies with a clinical diagnosis of suspected sepsis were transported to the clinical microbiology laboratory for immediate processing. Urine samples were collected by supra-pubic tap or sterile catheterization. Blood was cultured in the BACTEC culture system 9050 (Becton Dickinson, New Jersey, US) while urine and cerebrospinal fluid samples were processed according to established standardized protocol. They were cultured on Sabouraud dextrose agar (Oxoid UK) and Mueller Hinton agar (Oxoid UK) to which 5–7% blood had been added. Incubation was in room air for 24 hours at 35–37°C (Oxoid, UK). Gram stain was used to characterize isolates as yeast cells and they were further identified using germ tube test and were categorized as Candida albicans and non albicans spp.
Medical records of identified Candida positive neonates were retrieved and reviewed. Demographic and medical information were obtained and entered in a pre-designed proforma. Data obtained included: gestational age, age at the culture which is also taken as age at diagnosis, sex, procedures done on the baby, antibiotics use, day of commencing enteral feeds, other co-morbidities identified and outcome (survival or death).

Data analysis

Data were entered into Epi Info software version 3.4 (CDC, Atlanta GA, USA). The analysis was done with Statistical Package for Social Sciences (SPSS) software version 22.0 (SPSS Inc., Chicago, IL., USA). Data were presented using descriptive statistics. Discrete data were compared by Chi-square or Fisher exact test. Categorical variables were compared using Pearson’s Chi-square test or Fisher’s exact test. P-values < 0.05 were considered significant for all tests.

Results

Over the four years period, a total of 2,431 new-borns were admitted into the NICU of LUTH. From these, 1182 samples were collected from babies with clinical features suggestive of sepsis and processed in the Medical Microbiology laboratory of LUTH. Eight hundred and twenty-three (69.6%) of these were blood cultures, 252 (21.3%) were cerebrospinal fluid cultures while 107 (9.1%) were urine cultures. There were 711 cultured samples from male and 471 from female neonates. Twenty seven (2.3%) of the cultures yielded fungal organisms and were identified as Candida spp. fifteen of the positive fungal cultures were from males infants with a male: female ratio of 1.3: 1. All the 27 positive Candida cultures were from neonates who met the criteria for invasive candida infection. Thus, the prevalence of ICI in this study was 2.3% of the septic neonates. There were 21 (77%) C.albicans and 6 non-albicans of which 2 were C.krusei. Twelve (44.4%) of the 27 candida positive neonates were preterm. Blood stream infection was the most frequent ICI seen in preterm babies 7(58.3%) followed by urinary tract infection 4(33.3%). Only one (8.3%) CSF culture yielded candida organism (C. krusei) and it was in a preterm baby.

The mean age at diagnosis of ICI was 9.1±6.1days in preterm babies. One baby was 3 days old when ICI occurred. The commonest co-morbidities in preterm babies with ICI
was respiratory distress syndrome (66.7% of affected preterm babies) while surgical conditions were the commonest underlying illness in term infants with ICI (26.7%). These surgical conditions included: Jejunal atresia (1), Imperforate anus (1), Strangulated inguinoscrotal hernia (1) and Eagle-Barrett syndrome (1). Table 8.1 shows the baseline demographic and clinical characteristics of the neonates with ICI. Oral feeds were commenced within three days of life in all the infants. Mean duration of parenteral nutrition was seven days.

Table 7.1: Baseline demographic and clinical characteristics of the neonates with ICI

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Term (n = 15)</th>
<th>Preterm (n= 12)</th>
<th>Total (n= 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (66.7%)</td>
<td>5(41.7%)</td>
<td>15 (55.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>5(33.3%)</td>
<td>7(58.3%)</td>
<td>12 (44.4%)</td>
</tr>
<tr>
<td><strong>Mean Birth weight</strong></td>
<td>2907±341g</td>
<td>1125±300g</td>
<td></td>
</tr>
<tr>
<td><strong>Mean age (days) at diagnosis of ICI</strong></td>
<td>12.8±9.6days</td>
<td>9.1±6.1days</td>
<td></td>
</tr>
<tr>
<td><strong>Co-morbidities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical conditions:</td>
<td>6 (40%)</td>
<td>10 (83.3%)</td>
<td>16 (59.3%)</td>
</tr>
<tr>
<td>Severe perinatal Asphyxia</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td>0</td>
<td>8 (66.7%)</td>
<td></td>
</tr>
<tr>
<td>Features of sepsis</td>
<td>15 (100%)</td>
<td>12 (100%)</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>Features in keeping with ICI</td>
<td>15 (100%)</td>
<td>12 (100%)</td>
<td>27 (100%)</td>
</tr>
<tr>
<td><strong>Source of isolate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>4 (26.7%)</td>
<td>7 (58.3%)</td>
<td>11 (40.7%)</td>
</tr>
<tr>
<td>Urine</td>
<td>11 (73.3%)</td>
<td>4 (33.3%)</td>
<td>15 (55.6%)</td>
</tr>
<tr>
<td>CSF</td>
<td>0 (0.0%)</td>
<td>1 (8.3%)</td>
<td>1 (3.7%)</td>
</tr>
</tbody>
</table>

Treatment and outcomes

All babies with ICI had treatment doses of fluconazole at 6mg/kg/day for at least 21 days. Overall, neonatal mortality in ICI affected babies was 18.5% (5 out of 27 babies) but this value shoots up to 33.3% when mortality was calculated in preterm babies alone. The mortality difference between term (1) and preterm (4) infants was not statistically significant (p 0.08). The only death recorded in term babies with ICI was in a child with jejunal atresia. There was no significant sex predilection for mortality (2 females vs 3 males, p 0.83) and the source of the candidal isolate was also not significant for mortality (2 blood, 2 urine, and 1 CSF cultures; p 0.1). However, the only child with candidal meningitis died.
Associated factors in preterm infants with ICI

Nearly all (91.7%) affected preterm infants with ICI were less than 1500g in weight. All were exposed to invasive procedures such as umbilical venous catheterization; parenteral nutrition and broad-spectrum antibiotics. Table 2 shows the recognized predisposing factors for ICI in the preterm infants.

Table 7.2: Known predisposing factors for ICI in the 12 preterm neonates

<table>
<thead>
<tr>
<th>Predisposing factors</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Need for resuscitation</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>Exposure to broad spectrum antibiotics</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Exposure to invasive procedures</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Weight below 1500g</td>
<td>11 (91.7%)</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>8 (66.7%)</td>
</tr>
<tr>
<td>Length of hospital stay &gt;7 days at diagnosis</td>
<td>7 (58.3%)</td>
</tr>
</tbody>
</table>

Discussion

Neonatal candidaemia is a common, deadly and costly hospital-associated disease [8]. The incidence of ICI in the present study (2.3%) was lower than the 9% and 13.1% documented respectively by Benjamin et al [9] in the USA and Shin et al [10] in Korea. Both studies were of low birth weight babies. A more recent report from South Africa also gave a prevalence of 24% for neonatal candidaemia [8] and still a higher figure (57.4%) was documented from a tertiary hospital in India [11]. Possible explanations for the low prevalence in this study could be due to a number of factors including the fact that most of our patients are term and bigger neonates whereas over 40% of their neonates were preterm with 91.7% being VLBW. It is a well-known fact that earlier gestations and VLBW babies are more prone to fungal sepsis than term babies. Another possible cause may be the increased use of invasive procedures in their patients as they were more likely to have access to mechanical ventilators and central lines for their neonates. Also, fungal sepsis is rarely suspected initially in our settings hence request for fungal cultures may not be optimal. In the present study when the incidence of ICI is considered in septic preterm babies alone, it rose to 44.4% which is considerably higher than most of the reports mentioned above.

Nearly 58% of the Candida isolates in preterm babies in the present study were cultured from blood. This is consistent with other findings which reported candidaemia as the commonest finding in ICI [9]. It is also alarming in septic neonates considering the fact that blood culture (though gold standard) [6, 12] is known to be less than 50% sensitive.
Candida isolation from other sterile sites is a known risk factor and accounts for 41.7% in this study in preterm neonates. Research has shown that isolates of Candida in the blood stream of neonates and the vagina of the mother share a common genotype, which provides direct evidence of the association between congenital candidiasis in the neonate and candida vaginitis in the mother [14]. Unfortunately, this was a retrospective study so we are not able to determine if this was the case here. However, we did document candidemia in a three-day old neonate which highly suggests a congenital origin. Nosocomial candida colonization is the commonest source of candida infection in neonates [8].

The susceptibility profile of the Candida species was not done in this study, it would have been interesting to find out if fluconazole resistance contributed to the mortality since fluconazole is the only licensed antifungal in Nigeria available for treatment of the infection. It was interesting to note that C.krusei which had been previously reported in the study center [15] as being intrinsically resistant to fluconazole was isolated in the CSF of a preterm baby in this study.

Most of the risk factors that had been identified by other researchers [9, 10, 16, 17] for invasive Candidiasis were also noted in our study: low gestational age, the presence of central catheters, invasive procedures and antibiotic use were common predisposing factors in our study population. Yu et al [18] reported that the main risk factors for invasive fungal infections in their study, were third-generation cephalosporin use, peripherally inserted central venous catheters, intubation of >6 days, prior abdominal surgery and neutropenia (<1.5×10⁹ cells/L) during the first week of life. Other studies have investigated the association between antibiotic use and candidaemia [19-21]. The intact gastrointestinal barrier serves a crucial function in preventing Candida species colonizing the gastrointestinal tract and spreading into the blood. The destruction of the gastrointestinal barrier by gastrointestinal surgery is, therefore, a crucial risk factor for candidemia [22]. The only death recorded in the present study in a term baby with ICI was in a child with jejunal atresia.

The crude mortality rate in babies with ICI from our study was 18.5% (5 babies) which was lower than that documented in an Indian study (30.65%) [11]. However, when an analysis was done in preterm neonates alone, the mortality went up to 33.3%. A study done in another center in Nigeria documented mortality from ICI for all patients
admitted to the hospital as 91.7\% and infants made up nearly a third of the population sampled [23]. Most of the deaths in present study occurred in preterm babies emphasizing the vulnerability of this select group and the need to institute proactive preventive measures against ICI. The diagnosis of invasive candida infection depends majorly on microbiologic cultures [5, 6]. The blood volumes required to optimize organism recovery are large and are not easily attainable in neonates. This may cause under-estimation of the incidence of invasive Candida infections. The absence of culture-proven candidal infections may lead to under-diagnosis which in turn may lead to delay in instituting appropriate treatment leading to increased short and long-term morbidity and mortality in affected babies [24]. In view of the fact that laboratory diagnosis of ICI can be insensitive in 50\% of the cases [13] coupled with the fact that the consequences of neonatal ICI are far reaching with neurodevelopmental impairment [24], we recommend instituting an organized antifungal protocol in NICUs for the high-risk neonates. This may lower the incidence of candidaemia and its consequent high morbidity and mortality rates.

In conclusion, our study documented a relatively high rate of invasive candidiasis in the high-risk newborns and noted that the incidence increases with practices in risk factors such as invasive procedures and antibiotic use and in lower gestational age babies with VLBW. Though the retrospective nature of the present study and the small sample size hampered some of the findings made, we believe the study will create awareness in neonatologists in resource-poor settings on the existence of this commonly overlooked disease in our NICUs and also trigger prospective research in ICI in our setting. It is also hoped that the findings from this study will help neonatologists in settings such as ours to have a high index of suspicion for ICI in neonates and aid them in making informed and evidence-based decisions regarding antifungal prophylaxis and treatment in at risk newborns.

References

   Accessed 22/04/2017
Chapter 8

Paper 6 - Fungal opportunistic infections in a cohort of HIV-infected patients in Lagos, Nigeria.

Abstract

Background: Nigeria has a large estimated burden of AIDS-related mycoses such as cryptococcal meningitis (CM). We aimed to determine the proportion of patients with clinically-diagnosed AIDS-related opportunistic infections (OI) at an urban antiretroviral treatment (ART) centre in Nigeria.

Methods: A retrospective cohort study was conducted over a 12-year period (April 2004-February 2016). ART-naïve, HIV-infected adolescents and adults, assessed for ART eligibility at the PEPFAR outpatient clinic at Lagos University Teaching Hospital, Nigeria, were included. Demographic, clinical and laboratory data for each clinic visit were captured in a database. A panel of laboratory tests was performed at baseline and follow-up visits, including CD4+ T-lymphocyte cell (CD4) counts and HIV-1 viral loads (VL). OIs, including fungal infections, were diagnosed clinically.

Results: During the study period, 7046 patients visited the clinic; 4797 (68.2%) were female. The median number of visits was 2 (IQR, 1-3; range 1-15). For 666 patients with a recorded baseline CD4 count, the median CD4 count was 177 cells/µl (IQR, 87-287). A baseline VL was recorded for 702 patients; the median VL was 26850 RNA copies/ml (IQR, 1382-262500). Most patients (6179/7046; 88%) were recorded as having initiated ART. Of 7034 patients, 2456 (34.9%) had a documented OI; 1306 (18.6%) had a fungal OI. The total number of fungal OI episodes was 1632 (some patients had >1 documented fungal OI): oral candidiasis (1473, 90.3%), oesophageal candidiasis (n=118; 8%), superficial mycosis (n=23; 1.6%), Pneumocystis pneumonia (PCP) (n=13; 1%), cryptococcal meningitis (CM) (n=5; 0.4%). One hundred and thirteen patients were known to have died.

Conclusions: Approximately 1 in 5 HIV-infected patients in this retrospective cohort, most of whom initiated ART, were clinically diagnosed with a fungal OI, mostly mucocutaneous. Very few systemic mycoses were diagnosed. Improved access to simple diagnostic tests for CM and PCP should be prioritised for such settings.
Introduction

Opportunistic infections (OIs) have been defined as infections that are more frequent or more severe because of immunosuppression in HIV-infected persons and they remain a leading cause of morbidity and mortality among the HIV-infected population [1–3]. Fungi contribute significantly to opportunistic infections in patients with late-stage HIV infection [4]. Cutaneous/superficial fungal infection the most common group of fungal diseases, are not life-threatening but often lead to considerable social stigma and pain; it has been estimated that HIV/AIDS results in nearly 10 million cases of oral thrush and 2 million cases of esophageal fungal infections annually [5]. *Cryptococcus neoformans* is the most common cause of CNS infection and now accounts for the majority of worldwide deaths from HIV-related fungal infection [5]; while *Pneumocystis jirovecii* is the most common cause of respiratory infection in patients with AIDS across large parts of the world [4]. *Histoplasma capsulatum* (particularly common in parts of the Americas and Africa) and *Talaromyces marneffei* (endemic in South and Southeast Asia) are thermally dimorphic fungi that cause disseminated infections 6. These are AIDS defining infections and are usually lethal unless diagnosed and treated early and correctly [6]. *Coccidioides* spp especially affect patients with AIDS in the Americas and *Emmonsia* sp in South Africa [7,8].

With the increased availability of ART, earlier testing and treatment for HIV, the incidence of opportunistic fungal infections has decreased dramatically in people living with HIV in high-income countries; unfortunately in contrast, in regions with high HIV prevalence, especially sub-Saharan Africa, there is little evidence for a substantial decrease in cases of opportunistic infections [5]. In these settings, many patients present with advanced HIV and with a low CD4 cell. The relationship between OIs and HIV infection is bi-directional. HIV causes the immunosuppression that allows opportunistic pathogens to cause disease in HIV-infected persons. Ols, as well as other co-infections that may be common in HIV-infected persons, can adversely affect the natural history of HIV infection by causing reversible increases in circulating viral load that leads to an accelerated HIV progression and increase transmission of HIV infection [9–11].

Most serious fungal infections demand high-level medical skill, affect all body systems, are usually camouflaged by other well-known diseases, and kill more patients with AIDS than tuberculosis or bacterial infection [4]. There have been enormous advances in
fungal diagnostics and antifungal drug development over the past 20 years, but most of the world’s population (especially in LMICs) has not yet benefited from these advances [4]. In countries with developed health systems, fungal infections are diagnosed and treated, although many are still missed and only identified at autopsy [6]. In LMICs such as Nigeria, the absence of diagnostic tools and antifungal drugs, plus insufficient training of health-care staff, ensures that the morbidity and mortality of fungal infections remains excessively high.

Nigeria has an estimated 3.2million people living with HIV/AIDS with an estimated death rate of 65 per 1000 people living with HIV [12]. With an HIV epidemic history in the country of more than three decades duration [13], an increase in the number of persons at the advanced stage of disease presenting with OIs is expected. This study aimed to determine the proportion of patients with clinically-diagnosed AIDS-related fungi opportunistic infections (OIs) at an urban antiretroviral treatment (ART) centre in Nigeria.

Methods
A retrospective cohort study was conducted over a 12-year period (April 2004-February 2016). ART-naïve, HIV-infected adolescents and adults, assessed for ART eligibility at the PEPFAR outpatient clinic at Lagos University Teaching Hospital (LUTH), Nigeria, were included. The LUTH PEPFAR program was established in close partnership with Harvard and Northwestern comprehensive HIV prevention, treatment and care services. Demographic, clinical and laboratory data for each clinic visit were captured in a database. A panel of laboratory tests was observed to be performed at baseline and follow-up visits, including CD4+ T-lymphocyte cell (CD4) counts and HIV-1 viral loads (VL). Most opportunistic infections, including fungal infections, were diagnosed clinically. A total of fungal opportunistic infections (Oral candidiasis, oesophageal candidiasis, cryptococcal meningitis, pneumocystis pneumoniae and superficial (skin and nail) mycosis) were identified in this study. Laboratory records were accessed from the PEPFAR clinic database. The data was analysed as anonymised data and all patients identifiers were removed so that individual patients could not be identified from these data. CD4 cell counts were chosen from values closest to the period of diagnosing OI.

The study population were adult HIV infected patients, 15years and above. The study was approved by the research and ethics committee of the hospital. This was a purely
record-based study with no ethical issues. OIs in this article will be restricted to fungal infections only.

Definitions

*Mycobacterium tuberculosis* disease: Identification from pulmonary specimen, blood, or other tissue using AAFB microscopy (2004 – 2015) and geneXpert (2015-2016). Some cases were diagnosed clinically as smear negative tuberculosis.

Cytomegalovirus infection: retinitis diagnosed by clinically compatible examination by an ophthalmologist; colitis and esophagitis diagnosed by histopathologic confirmation of cytomegalovirus inclusions.

*Toxoplasma gondii* encephalitis: symptomatic clinical presentation with compatible computed tomographic scan or magnetic resonance image of the brain and response to appropriate therapy; nonresponse to therapy shown by histopathologic evidence of the organism; neurologist-confirmed diagnosis.

We focused on opportunistic diseases that are included in Category C of the AIDS surveillance criteria of the Centers for Disease Control and Prevention [14]. The following diagnoses and criteria were used in our analyses:

1. *Pneumocystis carinii* pneumonia: a) microbiological identification using induced sputum or bronchoalveolar lavage fluid or b) symptomatic presentation with compatible chest radiologic study and clinical response to an appropriate therapeutic regimen.

2. *Candida* esophagitis: symptomatic presentation of dysphagia or difficulty in swallowing with endoscopic evidence of invasive fungi or with clinical response to appropriate therapy.

3. Cryptococcal meningitis: evidence or culture of the organism in the cerebrospinal fluid.

4. Oral candidiasis: symptomatic presentation of white patches (plaque) in the mouth that can often be wiped off leaving behind red areas that may bleed slightly.

5. Superficial mycosis: Skin or nail presentation with microbiological identification using skin/nail scrapings or b) symptomatic relief with appropriate therapeutic regimen.

Data analysis
All analyses were performed on the diagnoses data set which contained a total of 7046 patients. Descriptive statistics were calculated for the categorical variables using frequencies (given as the number of patients and the percentage). Data are presented as mean (±SD) values. Comparisons between the studied groups were done using student’s t-test, with p < 0.05 considered statistically significant. For continuous variables, assessment of normality was carried out. If the variable was found to be normally distributed, the mean, standard deviation and range was given. Non-normally distributed variables were described using their median, range and inter-quartile range. All descriptive statistics were calculated using SPSS 22.0.

**Results**

A total of 7034 patients with HIV were included in the analysis. The majority of patients were female (4797, 68.2%, Table 1). The median number of visits per patient was 2, with an interquartile range of 1-3 and a range of 1-15. A baseline viral load was recorded for 702 patients; the median VL was 26850 RNA copies/ml (IQR, 1382-262500). Most patients (6179/7034; 88%) were recorded as having initiated ART. Of 7034 patients, 2456 (34.9%) had a documented OI. Almost one in five patients were diagnosed as having TB (1381, 19.6%, Table 8.1). The total number of tuberculosis (TB) cases recorded over the 12 year period was 1381 (19.6%) of which 1282 (18.2%) were cases of pulmonary Tb and 182 (2.6%) were cases of extrapulmonary TB. A small number of the patients had HIV encephalopathy (17, 0.2%, Table 9.1). The mean age was 37.3 years (SD 10.0, range 8-78, Table 1). The mean age at diagnosis for males was 41.3 years with a standard deviation of 9.0 (range: 13.0-69.0 years). For females, the mean age was found to be 33.0 years with a standard deviation of 9.7 (range: 8.0 – 78.0 years). There were 113 recorded deaths (1.6%).
Table 8.1: Patient demographics, CD4 counts and RNA measurements.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td></td>
</tr>
<tr>
<td>Mean (Standard Deviation)</td>
<td>37.3 (10.0)</td>
</tr>
<tr>
<td>Minimum</td>
<td>8</td>
</tr>
<tr>
<td>Maximum</td>
<td>78</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2236 (31.8)</td>
</tr>
<tr>
<td>Female</td>
<td>4797 (68.2)</td>
</tr>
<tr>
<td>Age at diagnosis, split by gender (Years)</td>
<td></td>
</tr>
<tr>
<td>Males:</td>
<td></td>
</tr>
<tr>
<td>Mean (Standard Deviation)</td>
<td>41.3 (9.0)</td>
</tr>
<tr>
<td>Minimum</td>
<td>13</td>
</tr>
<tr>
<td>Maximum</td>
<td>69</td>
</tr>
<tr>
<td>Females:</td>
<td></td>
</tr>
<tr>
<td>Mean (Standard Deviation)</td>
<td>33.0 (9.7)</td>
</tr>
<tr>
<td>Minimum</td>
<td>8</td>
</tr>
<tr>
<td>Maximum</td>
<td>78</td>
</tr>
<tr>
<td>TB Patients</td>
<td>1381 (19.6)</td>
</tr>
<tr>
<td>HIV Patients</td>
<td>17 (0.2)</td>
</tr>
<tr>
<td>CD4 Count</td>
<td></td>
</tr>
<tr>
<td>First Count:</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>184</td>
</tr>
<tr>
<td>Minimum</td>
<td>1</td>
</tr>
<tr>
<td>Maximum</td>
<td>1657</td>
</tr>
<tr>
<td>First Quartile‡</td>
<td>85</td>
</tr>
<tr>
<td>Last Quartile‡</td>
<td>328</td>
</tr>
<tr>
<td>Last Count:</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>418</td>
</tr>
<tr>
<td>Minimum</td>
<td>1</td>
</tr>
<tr>
<td>Maximum</td>
<td>2096</td>
</tr>
<tr>
<td>First Quartile‡</td>
<td>221</td>
</tr>
<tr>
<td>Last Quartile‡</td>
<td>612</td>
</tr>
<tr>
<td>RNA Measurement</td>
<td></td>
</tr>
<tr>
<td>First Measurement:</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>51259</td>
</tr>
<tr>
<td>Minimum</td>
<td>0</td>
</tr>
<tr>
<td>Maximum</td>
<td>16,100,000</td>
</tr>
<tr>
<td>First Quartile‡</td>
<td>2314</td>
</tr>
<tr>
<td>Last Quartile‡</td>
<td>283617</td>
</tr>
<tr>
<td>Last Measurement:</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>200</td>
</tr>
<tr>
<td>Minimum</td>
<td>0</td>
</tr>
<tr>
<td>Maximum</td>
<td>16,100,000</td>
</tr>
<tr>
<td>First Quartile‡</td>
<td>25</td>
</tr>
<tr>
<td>Last Quartile‡</td>
<td>8597</td>
</tr>
<tr>
<td>Mortality</td>
<td>113 (1.6)</td>
</tr>
</tbody>
</table>

†Unless otherwise specified, values are number of patients (%).
‡ First quartile=25th percentile; third quartile=75th percentile.
Frequency and distribution of fungal OIs

The total number of patients with an AIDS-related fungal opportunistic infection was 1306 (18.6%). Of these patients, 1284 (98.3%) had an oral candidiasis, however there were 1473 cases in this 1284 patients. 110 (8.4%) patients had oesophageal candidiasis with 118 incidences of the disease diagnosed. 13 (1.0%) patients had 13 cases of PCP diagnosed. 5 (0.4%) patients had cryptococcal meningitis and 21 (1.6%) patients were diagnosed with superficial (skin and nail) mycosis, there were 23 incidence cases. See table 8.2 below.

Table 8.2: AIDS-related fungal opportunistic infections

<table>
<thead>
<tr>
<th>Infection</th>
<th>Number</th>
<th>% of total population</th>
<th>% of those with AIDS-related fungal OIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any AIDS-related fungal OI</td>
<td>1306</td>
<td>18.6</td>
<td>-</td>
</tr>
<tr>
<td>Oral candidiasis</td>
<td>1284</td>
<td>18.3</td>
<td>98.3</td>
</tr>
<tr>
<td>Oesophageal candidiasis</td>
<td>110</td>
<td>1.6</td>
<td>8.4</td>
</tr>
<tr>
<td>PCP</td>
<td>13</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Cryptococcal meningitis</td>
<td>5</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Superficial mycosis</td>
<td>21</td>
<td>0.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Thus, there were 1632 incidences of fungal OIs amongst the 1306 patients who had an OI. Most patients (1197/1306, 91.7%) had only one of the five types of OIs, 99 patients had 2 different OIs, 6 patients had 3 different OIs and 4 patients had all five types of OIs described above. Of the patients who had a fungal OI, most only had one OI (1066/1306, 81.6%) during the study period. The remaining 240 patients had between 2 and 7 incidences of OIs. The majority of the 1632 incidences of OIs were candida oral infections (1473, 90.3%, Figure 9.1). Around 8% (118) of the OIs were candida oesophagus infections. PCP pneumonia, mycosis and cryptococcal meningitis all made up less than 1.5% of the total number of OIs reported.
Figure 8.1: Distribution of fungal opportunistic infections

There was no significant difference between the age of patients who had an OI and those who did not have an OI (38.1, SD 10.7 vs 37.1, SD 9.8; p=0.30) see table 8.3. Male patients were significantly more likely to have an OI than female patients – 22.3% (498/2236) of males had an OI compared to 16.8% (807/4797) of females (p<0.001).

Table 8.3: Relationship of OIs to age and gender

<table>
<thead>
<tr>
<th>Infection</th>
<th>Number</th>
<th>Number (%) Male</th>
<th>Mean Age (SD), Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any AIDS-related fungal OI</td>
<td>1306</td>
<td>498/1305 (38.2%)</td>
<td>38.1 (10.7), 11-68</td>
</tr>
<tr>
<td>Candida oral</td>
<td>1284</td>
<td>489/1283 (38.1%)</td>
<td>37.9 (10.5), 11-68</td>
</tr>
<tr>
<td>Candida oesophagus</td>
<td>110</td>
<td>46 (41.8%)</td>
<td>39.2 (9.0), 27-60</td>
</tr>
<tr>
<td>PCP pneumonia</td>
<td>13</td>
<td>3 (23.1%)</td>
<td>58.5 (9.2), 52-65</td>
</tr>
<tr>
<td>Cryptococcal meningitis</td>
<td>5</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Superficial mycosis</td>
<td>21</td>
<td>9 (42.9%)</td>
<td>38.6 (14.8), 13-55</td>
</tr>
</tbody>
</table>

Relationship with CD4 count

The median value for CD4 was 205 (range: 4-1739). Patients who had an OI during the study period and patients who never had an OI during the study period were compared in their CD4 counts at their first and last visits. There was a statistically significant different in the CD4 counts at both the first visit of a patient and at the last visit of a patient (both p<0.001). At their first visit, patients who had an OI during the study period had a lower CD4 count compared to those who did not have an OI during the study period (with OI: median 112, IQR 46-242, range 1-1193 vs without OI: median 199, IQR
99-343, range 1-1657). There appeared to be a slightly larger difference in CD4 count at a patient’s last visit between those who did and those who did not have an OI during the study period (with OI: median 248, IQR 82-474, range 1-1863 vs without OI: median 442, IQR 253-628, range 2-2096). The median CD4 count from the visit that was closest to the date of the earliest OI (within 90 days) was 197 (IQR 69.5-377.5, range 4-1739).

Trends of fungal OIs from 2004 to 2016

Of the diagnosis that had their date of diagnosis included (1403 out of 1632); the distribution of OIs over the years demonstrated the highest incidence cases to have occurred in the year 2015 (185 cases) followed by 2014 (166 cases); 2005 and 2006 (164 cases each). The year 2004 had the lowest recorded cases (11) followed by 2012 (24 cases) and 2016 (33 cases). The trend for oral candidiasis closely follows this. (see Table 4). Two cases of PCP were diagnosed in the year 2006, 2007, 2014 and 2015 respectively; and one cases each in 2005 and 2013. 2 cases of cryptococcal meningitis were reported in 2015. See table 8.4 and Figure 8.2.

<table>
<thead>
<tr>
<th>Year of OI</th>
<th>Candida Oral</th>
<th>Candida Oesophagus</th>
<th>Superficial Mycosis</th>
<th>PCP Pneumonia</th>
<th>Cryptococcal Meningitis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>2005</td>
<td>150</td>
<td>11</td>
<td>3</td>
<td>1</td>
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Figure 8.2: Analysis of total OIs by Year; 2004 -2016

Discussion

Of all people living with HIV globally as of 2013, 9% of them live in Nigeria [15]. This study demonstrates that approximately 1 in 5 HIV-infected patients in this retrospective cohort, most of whom had initiated ART, were clinically diagnosed with a fungal OI. Majority (68%) of the study population were female, this is in keeping with previously published data from other Nigerian studies which gave rates of 59.0 -65.8% [16,17]. Approximately thirty five percent of the studied population had an opportunistic infection; this is higher than the 22.4% reported from Eastern Nigeria [17] and 26% from Central Nigeria [16] but lower than 68.6% reported from North Central Nigeria [18]. It is pertinent to note that the high rate from North Central Nigeria was prior to the HAART (highly active antiretroviral therapy) era; however our data span both pre-HAART and HAART era. A study from Bahrain gave a prevalence of 34.5% [19], while a recent Uganda study reported 291,168 incidences of OIs in 108,619 patients over a 12 years period [20].

OIs are associated with reversible increases in circulating viral load, and these increases
could lead to accelerated HIV progression or increased transmission of HIV [1]. Interestingly, the Millennium Development Goal 6 was halting and beginning to reverse the HIV epidemic by 2015.

There is extensive evidence that low baseline CD4 cell count and low CD4 cell count during ART are major risk factors for OIs both in high income countries and low middle income countries [21]. Our study found that there was a statistically significant different in the CD4 counts at both the first visit of a patient and at the last visit of a patient (both p<0.001). Our findings are consistent with the Bahraini study that had similar findings [19]. This study revealed that male patients were significantly more likely to have an OI than female patients (p<0.001) and this is similar to a report from Bahrain [19] but in contrast to findings from Uganda [20]. A possible explanation for this is the fact the ‘pick up’ rate of HIV in Nigeria in higher for women because of the screening policy for pregnant women (keeping in view that highest prevalence is in the reproductive age group), so they are more likely to start ART earlier unlike in men, who most times present with AIDS defining infections; there is also the challenge of stigmatisation that prevents men from accessing screening programs. Our socio-cultural and religious beliefs also hinder accessing screening.

Although not a life-threatening condition in its self, oral and oesophageal candidiasis significantly lowers the quality of life of people living with HIV/AIDS. It tends to manifests with local discomfort and altered taste sensation. Severe cases can interfere with administration of medication and nutritional intake. Oesophageal candidiasis presents with dysphagia and retrosternal pain. Candidiasis accounted for majority of cases of fungal OIs in this study, this finding is consistent with previous reports [5,22,17,23,24].

A South African post mortem study on the cause of death in HIV infected patients within the first months of ART revealed that fungal OIs accounted for 21% of cases [25]. Very few systemic opportunistic fungal infections were diagnosed in the present study. Only 5 cases of cryptococcal meningitis were diagnosed over a 12-year period, this is alarming because in the same centre of study, a recent study demonstrated a prevalence of 8.9% [26] of cryptococcal antigenaemia, a similar study in Mid-Western Nigeria revealed a rate of 12.7% [27] with an estimated 57,894 annual cases (37.4/100,000) [28]. These 5 cases were diagnosed by culture method. Interestingly, a study from North Central Nigeria gave a hospital based frequency of cryptococcal meningitis of 36% in HIV infected
patients presenting with neurological symptoms [29]. Another study from same centre on hospitalised HIV infected patients demonstrated that cryptococcal meningitis accounted for 8.8% of all OIs with a 12% in hospital deaths [30]. PCP accounted for 1.0% of AIDS related mycotic infections in the present study; this is lower than 12.6% reported from a study in Western Nigeria [31]. It is pertinent to note here that diagnosis in the present study was made clinically based on radiology and symptoms while in the study cited above it, PCP was diagnosed using PCR assay.

The distribution and pattern of fungal OIs in the studied population did not reveal any significant change in trend over the years. In the early years of HAART in Nigeria (2005-2009); there appears to be a slight and steady decrease in reporting of fungal OIs (majorly mucocutaneous candidiasis), however the rates subsequently picked up in 2013 with the highest incidence in 2015. We are not too sure if this is due to better reporting modalities or improved clinical acumen or as a result of better ‘retention in care’ or if this might be as a result of virological failure. However we do know that the rates of tuberculosis (a major opportunistic infection in HIV patients) reporting increased over the same period.

Limitations in this study included the lack of diagnostics and skilled personnel to make appropriate diagnosis, so our findings might not reflect fully the picture on ground. Most of the diagnoses were made clinically. Patients/relative pay from pocket for test and treatment that is not covered by the PEPFAR/APIN program and infections (outside of Hepatitis B and C) diagnostics is not covered, no fungal infection is covered. Hospitalised HIV-infected patients in LUTH were not captured in this data. Fluidity of patient affects retention in care. However, this is the largest retrospective study from Nigeria and could form the basis for further surveillance programs.

Opportunistic fungal infections remain a significant cause of morbidity and mortality in the HIV infected population [5]. While superficial fungal infections (skin and nail) in most patients with HIV infection follow a normal pattern, atypical presentations and more severe forms are common in patients with AIDS. Diagnosis of AIDS defining invasive fungal opportunistic infections such as cryptococcal meningitis has been made cheaper, easier and quicker using the CrAg LFA which is 100% sensitivity in serum and cerebrospinal fluid [32]. Unfortunately despite an estimated cost of just $4 per test, no centre in Nigeria, a high HIV burden country with a PEPFAR/APIN funded ART program
performs this test. PCP diagnosis has also been made faster and easier with PCR and beta glucan assays; however these are not feasible and affordable in a resource limited setting such as Nigeria.

In conclusion, this study highlights the lack of diagnostics for serious fungal infections which are a significant cause of morbidity and mortality in an HIV high burden country. There is dire need for improved access to simple diagnostic tests for AIDS defining fungal OIs such as CM, PCP and histoplasmosis and this should be prioritised for such settings. This is to enable us achieve the sustainable developments goals by 2030.

References

Chapter 9

Summary/conclusion

The general aim of this research was to investigate the epidemiology of serious fungal infections in Nigerians. The emphases were on chronic pulmonary aspergillosis, cryptococcosis, histoplasmosis, and fungal infections in critically ill patients in our (Nigerian) setting. The studied population were some of the at risk groups; tuberculosis patients being managed for smear negative or treatment failure TB, HIV-infection patients, healthy Nigerians (for histoplasmin skin sensitivity survey) and patients admitted in ICU and NICU. Also a twelve-year retrospective inventory of diagnosis of fungal infections in a cohort of HIV-infected patients in a PEPFAR program was conducted and reported.

The estimate of the burden of serious fungal infections amongst Nigerians by Oladele and Denning (2014) predicted an estimated 57,866 annual cases of cryptococcal meningitis; 75,000 patients with AIDS cases are expected to develop Pneumocystis pneumonia annually (40% incidence rate in children) and 19,000 new cases of chronic pulmonary aspergillosis annually with a 5-year period prevalence of 60,377 cases [1]. From our data, the estimated burden of cryptococcal meningitis is 57,894 annual cases (37.4/100,000) and applying the rates of CPA we found, we would anticipate an annual incidence of ~1,000 HIV positive CPA cases and 43,500 HIV negative CPA cases. Assuming a 15% annual mortality or lobectomy (cure) rate, we would anticipate a 5-year period prevalence of 140,264 CPA cases, substantially greater than the prior estimate of 60,377.

This final chapter describes the major conclusions and proposed future work from the research describe in Chapters 3-9.

10.1 Chapter 3

This study has been published in Open Forum of Infectious Diseases. This study revealed an overall prevalence of cryptococcal antigenemia of 8.9% amongst HIV-infected outpatients with CD4+ counts <250 cells/mm3, irrespective of their ART status, This is particularly unsettling because WHO recommends screening for asymptomatic CrAg should be limited to ART-naive patients with CD4 counts <100 cells/mm3 [2]. This study highlighted the beneficial effect of ART adherence in preventing clinical cryptococcosis
infection despite cryptococcal antigenemia. It also revealed that patients were being
given suboptimal doses of fluconazole. Drug exposure is an important factor for the
emergence of resistance. This is an issue for concern because several reports of
fluconazole resistance in Cryptococci spp are in the literature [3-7]. With the new
UNAIDS 90-90-90 targets, the Nigeria National guidelines for HIV prevention treatment
and care advocates for Serum or plasma CrAg screening in ART naïve adults followed by
pre-emptive antifungal therapy to reduce the development of disease should be
considered in patients with CD4+ cell count less than 100cells/mm [8]. This has however
not been implemented anywhere in the country, instead preference is being given for
prophylaxis with fluconazole instead of the screen and pre-emptive treatment approach.
This approach is burdensome given the issue with selective pressure of antimicrobials,
the cost of this approach, challenges with misdiagnosis etc. Screening for serum CrAg has
been demonstrated to be highly sensitive, specific and cost effective in preventing death
in HIV-infected patients with CD4 counts of <100 cells/ mm3 [9]. There is thus need for a
future study to establish the burden of cryptococcal antigenaemia nationwide
 prospectively and determine the cost-effectiveness of implementing CrAg screening
using the CrAg lateral flow assay in Nigeria compared to current practice without
screening. A decision-analytic model will be constructed to compare two strategies for
cryptococcal prevention among people living with HIV with CD4 < 100 in Nigeria. Primary
outcomes will be expected costs, DALYs, and incremental cost-effectiveness ratios
(ICERS). This is particularly important because there is limited accessibility and availability
of first line drugs (Amphotericine B and flucytosine) in Nigeria [10]. It is also pertinent to
note that no Centre, to the best of my knowledge does therapeutic lumbar punctures in
Nigeria due to unavailability of sterile lumbar puncture trays.

Sample Size Determination of proposed study:

With a desired margin of error of less than 2.5% and a 95% confidence interval our
formula for sample size calculation is:

\[ ME = \frac{z \sqrt{p(1-p)}}{n} \]

Where ME = margin of error, in this case 2.5%
z = z score for a 95% confidence interval, which is 1.96
p = our working prevalence value of 9% [11].
n = sample size to be calculated
The value of \( n = 500 \).

To allow for 10\% non-response rate (attrition error), minimal sample size = 550.

**Study Design**

It will be a prospective cross-sectional study. Blood samples will be obtained by randomized sampling from eligible participants, after an informed and signed consent is obtained.

Each participating hospital will recruit 100 participants belonging to 3 groups:

- 40 ART naïve with CD4 count less than 200 cells/\( \mu \)l.
- 40 ART experienced with CD4 count less than 200 cells/\( \mu \)l.
- 20 clinical stage three or four patients.

Hence, a total of 900 blood samples will be collected from the 9 participating hospitals nationwide.

**Inclusion Criteria**

All consenting adult retroviral disease patients with a CD4 count less than 200 cells/ul registered at PEPFAR clinics (either on outpatient care or on admission at the tertiary hospitals earlier mentioned).

**Exclusion Criteria**

- Retroviral disease patients with CD4 count above 200 cells/ul.
- CM symptomatic patients with CD4 < 200
- Participants who do not give consent for the study.
- CM asymptomatic patients with CD4 < 200 but with sub-clinical disease

**Data analysis**

All data will be entered into an excel spreadsheet. All statistical analysis will be performed on SPSS version 21.0.0. An economic model will be designed and analysed by
a social scientist based on the pricing data collated.

Funding is presently being sourced for this study, we hope to commence next year.

10.2 Chapter 4

This study has been published in the International Journal for Tuberculosis and Lung Disease.

CPA is a known complication of TB [12]. Even when treated, CPA has a 20–33% short-term mortality and 50% mortality over 5 years. To the best of my knowledge as at the time of this publication, there were no published studies of CPA in HIV positive patients, other than occasional case reports. The major findings from this study were:

1. CPA is a sufficiently common problem to be considered a public health issue in Nigeria.

2. CPA is a neglected disease in Nigeria, with an annual incidence of ~1,430 HIV positive CPA cases and 43,500 HIV negative CPA cases, most matching the WHO diagnostic criteria for ‘smear-negative’ tuberculosis.

3. CPA is more common in non-HIV TB patients (almost twice as high) than in HIV-infected TB patients.

4. Using our data, we developed a prediction rule for *Aspergillus* IgG above 40mg/L, at least in the Nigerian setting.

Currently available commercial *Aspergillus* specific IgG kits measure *Aspergillus fumigatus* antibodies because *Aspergillus fumigatus* is the most commonly implicated *Aspergillus* spp accounting for over 90% of aspergillosis in Europe [13, 14]. However, data from Nigeria shows that *A.fumigatus* accounts for slightly over 50% of environmental isolates followed by *A.flavus* and *A.niger* [15-17]. Although it has been suggested that *A. fumigatus* assays have poor sensitivity for infection with other *Aspergillus* species [18, 19]; further studies will be needed to validate this and to develop *A. flavus* and *A.niger* assays. Fungal cultures of sputum specimen will also help in isolation, identification and determining the susceptibility pattern of the *Aspergillus spp*; it would also aid in making identifying other fungal that could be misdiagnosed as tuberculosis such as *Histoplasma capsulatum*. Radiological findings demonstrated fungal balls in some patients that had
*Aspergillus* IgG level <40mg/L. It will be interesting to examine all fungal balls removed at surgery to determine the specie of *Aspergillus* involved.

In this study, the *Aspergillus* IgG cut-off level used (>40mg/AL) is what was developed based on European data, we do not know if this is truly applicable to Africans and Nigerians in particular, this is will be part of my future studies. Below is brief description of the proposed study;

Study type and sample size determination of the standardisation of *Aspergillus* IgG for Nigerians

17 positive *Aspergillus* IgG (identified using IMMUNOCAP) samples are presently stored at -80°C in APIN central laboratory in LUTH from the study in this chapter [20]. The present study will be a multicentre observational prospective cohort study aimed at recruiting 400 healthy blood donors. This will facilitate the calculation of the optimal diagnostic cut-offs for each ELISA assay in conjunction with the test used in diagnosing CPA among the LUTH cohort.

Cut-off values for aspergillus IgG titre will be determined using data from the healthy patients and those with CPA. The 95% confidence interval (CI) for the sensitivity of the aspergillus IgG cut-off (proportion of the 17 CPA patients correctly identified by the IgG cut-off) will have a width of around 38% if the sensitivity is 80% i.e. the 95% CI would be around 61-99%. If the sensitivity is 70%, the expected 95% CI would be around 48-92%.

Since we are determining normative range/reference values at least 120 participants is required to power the study [21]. We will categorise the patients along their ages and three age groups will be used, thus a minimum of 360 study participants are to be recruited. We plan to recruit 400 healthy blood donors across 8 centres nationwide.

Inclusion criteria

Consenting adults aged 18years and above

Study participants for each centre will be recruited amongst these age groups.
Table 9.1: Proposed participants recruitment targets

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<td>41 – 60</td>
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Exclusion criteria

1. Seriously immunocompromised patients e.g. prolonged use of corticosteroids > 3 weeks, HIV infected patients

2. Participants with pulmonary symptoms and underlying pulmonary diseases

3. Antifungal drug use within 3 months of screening.

4. Pregnancy or lactation.

5. Diabetics

6. Those unwilling to provide written informed consent.

Funding is being sourced to conduct this study.

10.3 Chapter 5 and Chapter 6

The review in Chapter 5 has been accepted for publication in PlosOne Neglected Tropical Diseases and Chapter 6 has been submitted and is under review in PlosOne.

It is well documented that the greatest attributable risk factor for disseminated histoplasmosis is the spread of HIV, with an estimated 100,000 cases annually [22]. Existing data demonstrates that in HIV-infected patients with histoplasmosis, the disease is disseminated in 95% of the cases, and in 90% of the cases it occurs in patients with CD4 counts below 200/mm³ [23]. Subsaharan Africa has the highest number of people living with HIV/AIDS, though ART coverage and retention in care has improved it is still significantly lower than in the Western world.

Chapter 5 highlighted the following:

1. The fact that histoplasmosis is a neglected diseases in Africans.

2. Histoplasmosis can be misdiagnosed as tuberculosis
3. That West Africa had the highest numbers recorded cases of histoplasmosis (179), and that the majority (162 cases) were caused by *Histoplasma capsulatum* var. *dubosii* (Hcd) with Nigeria accounting for approximately 70% of cases.

4. Diagnosing disseminated histoplasmosis has been significantly facilitated by the development of *Histoplasma* antigen testing.

5. In most of the reported cases from Africa, the diagnosis was made by culture and histology; only in 4 countries was serology used to make a diagnosis and in 3 of those the samples were processed in Western countries.

6. Amphotericin B which is the drug of choice is not licensed in and unavailable in number of African countries and even where it is available the cost may be prohibitive

Under recognition and under diagnosis are major challenges attributable to the lack of skilled personnel and facilities to make this diagnosis. Future studies are needed using histoplasmin antigen testing to determine the true burden of the problem amongst Africans especially in the HIV-infected population where it is an AIDS defining disease. Most EIA need equipment requiring electricity and skilled laboratory personnel to perform them, this is a challenge in most of Africa and in Nigeria in particular where electricity supply is epileptic. There is need to develop point of care assays like the ‘pregnancy test strips’ or the CrAg LFA for histoplasmin antigen to bridge this gap.

Chapter 6 was a six-center study that revealed an overall 4.4% prevalence of prior subclinical histoplasmosis using histoplasmin skin testing in Nigeria. There was no significant association of HIV status with histoplasmin reactivity (p=0.70), as we selected patients with higher CD4 counts. However high-risk occupations, which exposes individuals to *Histoplasma* was significant and environmental factors such as soil type and humidity was associated with positive histoplasmin skin sensitivity. It is therefore imperative that Histoplasmosis diagnostics should be included in the Nigerian HIV guidelines and local health authorities should consider epidemiological vigilance of progressive disseminated histoplasmosis. I hope to disseminate this result to the relevant authorities to advocate for inclusion in Nigerian HIV and TB guidelines.

An epidemiological study on the incidence of histoplasmosis amongst HIV-infected
patients clinically diagnosed with tuberculosis or bacterial pneumonia is being designed.

10.4 Chapter 7 and Chapter 8

The ICU and NICU studies highlight the peculiar challenges of a lack of diagnostics for serious fungal infections. The centre where these studies were conducted happens to be amongst the best tertiary hospitals in Nigeria. Yet there are no facilities for serological or molecular diagnosis of serious fungal infections commonly seen in ICU settings such as invasive aspergillosis (one of the most commonly missed cause of death found at post mortem in ICU patients) [24] and invasive Candida infections. This is further compounded by the fact that patients/ their relatives pay out of pocket for their healthcare in our setting. In Nigeria, BAL samples are not routinely collected so there is significant room for misdiagnosis. Blood culture which only available test to diagnose candidaemia in Nigerians for identification of candidaemia is only 50% sensitive and takes time [25]. Most serological test currently available (such as BD, Candida albicans germ tube antibody and intravenous immunoglobulins) are not sufficient to make confirmatory diagnosis on their own of ICI [26]. In 2014 the US Food and Drug Administration gave marketing approval for the first direct blood test for detecting Candidemia (T2 Candida); though the speed and sensitivity of this test is very good it is however requires instrumentation and it is expensive [27].
Nigeria is amongst the 15 “focus countries” of PEPFAR. PEPFAR supports training and salaries for personnel (including clinicians, laboratorians, pharmacists, counsellors, medical records staff, outreach workers, peer educators, etc.), renovation and refurbishment of health care facilities, updated laboratory equipment and distribution systems, logistics and management for drugs and other commodities. This is intended to ensure the sustainability of PEPFAR services in host countries, enabling long-term management of HIV/AIDS. The PEPFAR funded laboratory now has skilled personnel. Interestingly, despite fungal diseases being a leading cause of morbidity and mortality among the HIV-infected population there is no mycology bench/laboratory in the PEPFAR/APIN laboratory in Nigeria. Not a single centre uses the point of care CrAg LFA. No single mycological test is done in their laboratory that caters strictly for HIV-infected patients. The 12 years retrospective study is an ‘eye opener’. It highlights the infrequency of life-threatening fungal infections, probably because of a lack of diagnostics and dependence on just clinical acumen to diagnose fungal infections in this vulnerable population. Most cases of PCP are diagnoses of exclusion, where clinicians rely on ‘ground glass’ appearance of chest x-rays, thus missing mild, moderate cases and atypical presentations of PCP. There is also the unnecessary exposure of these patients to a potentially toxic drug at high doses – cotrimazole.

Despite the huge burden and high mortality rates of fungal infections in HIV-infected patients, these diseases remain understudied and underdiagnosed compared with other infectious diseases [28]. A high index of suspicion with early diagnosis and institution of appropriate therapy has been shown to reduce the morbidity and mortality associated with invasive fungal infections. These are dependent on the knowledge and skill of the managing clinician. The author and colleagues has evaluated of knowledge and awareness of invasive fungal infections amongst trainee doctors in Nigeria using a self-administered questionnaire and found only 2 (0.002%) out of the 1046 respondents had a good level of awareness of IFIs and many/most/some had major misconceptions related to important fungal pathogens. Part of this data was presented at ECCMID 2014 [29], and the manuscript is presently being peer-reviewed for publication. It is thus not surprising that there is a high likelihood of missed diagnosis of these serious fungal infections in Nigerians. Focused physician training programs are urgently needed to
address the many knowledge gaps. A revision of the Nigerian medical curriculum to reflect the rising importance of invasive fungal infections is also recommended.

With the preliminary data from this research, the author saw the need for intervention and in November and December 2015 organized a training program on two sites in Nigeria (Lagos and Calabar). The targeted audience was specialist doctors that routinely managed the patient groups at risk of serious fungal infections. Pfizer pharmaceuticals supported the program and Prof Denning delivered several comprehensive lectures and answered questions extensively online. The feedback from the doctors was very encouraging; however, we noted that diagnosis was still a challenge. In February 2017, we organized an onsite 3 days extensive training program with hands on training in radiological and laboratory diagnosis of invasive fungal infections. Prof David Denning and Prof Malcolm Richardson were facilitators. At the conclusion of the training, which was attended by 35 doctors from across Nigeria, a Society for Medical Mycology in Nigeria was formed under the supervision of Prof Richardson. The society will focus on research and step down training; they will also be involved in disseminating research findings, advocacy and health policy development. Prof Denning on behalf of GAFFI also met with representatives of the Minister of Health, the Director General of Federal Ministry of Health, Nigeria and Director General of the National Agency for Food and Drug Administration and Control (NAFDAC) to advocate for diagnostics and therapeutic modalities of serious fungal infections.

There are multiple opportunities to improve care for patients with fungal disease in Nigeria. Making an accurate diagnosis of opportunistic infection is paramount to reducing the current mortality from AIDS in Nigeria is 160 000 deaths (110 000 – 230 000) [30]. In Nigeria, a country with a population of over 170 million people with 3.2 million people thought to be living with HIV/AIDS there is no mycology reference laboratory and most tertiary centers do not have a mycology bench in their routine medical microbiology laboratory. No non-culture based tests for fungal disease are done in Nigeria: cryptococcal and Aspergillus antigen, Aspergillus IgG and IgE and other fungal antibody tests, molecular diagnosis for Pneumocystis being obvious and necessary examples Part of the reason for this is that these tests are (or are perceived to be) too expensive for Nigerians where patients pay out of pocket for healthcare. There is also the challenge of frequent power outages in Nigeria (these laboratory equipment needs a
constant power supply), lack of skilled laboratory personnel and lack of political will. There is thus dire need for affordable, rapid point-of-care diagnostic tests (such as have been developed for cryptococcosis) for CPA, IA, pneumocystosis, and probably histoplasmosis. Additionally, antifungal drugs, including amphotericin B, liposomal amphotericin B, and flucytosine, need to be much more widely available. Such measures, together with continued international efforts in education and training in the management of fungal disease, have the potential to improve patient outcomes substantially. I must add here that though the immY CrAg test is relatively cheap and affordable, it is not currently being used in any facility in Nigeria, thus the problem also includes a major knowledge gap on the part of the clinicians and this must be addressed.

Diagnostic capacity is the cornerstone of the healthcare system; a qualitative standard of fungal disease diagnosis would be best achieved by combining clinical and microbiological expertise of mycology with a comprehensive laboratory testing portfolio. A mycology reference laboratory, as an integral part of the clinical laboratory network, should ideally be present for every 5–10 million people [27]. There is therefore a need to establish a reference mycology laboratory in at least three regions in the country, as well as making cryptococcal antigen testing widely available. This will serve both for diagnostics and capacity building. Social and political will is needed for this. Even when accurate diagnoses have been made, and when there are readily available and accessible the appropriate drugs for treatment, therapeutic drug monitoring has to be available also. This is best done (at least in the early phase) in the reference laboratory where the trained personnel and facilities for doing this will be located. Long term however, there will be step down training using designed laboratory training modules. These courses will be three weeks to four months long and all participants will receive a proficiency and training certificate. The training program will aim to deliver at least one skilled laboratory personnel per 250,000 populations in Nigeria. This might sound ambitious but it is the long term plan.

Disseminating the results of this research locally in Nigeria is necessary to raise awareness and drive clinical practice change amongst the clinicians that manage the at risk groups. Doctors tend to resist change as has been demonstrated in simple hand hygiene practice![31-33]. There is thus need for sustained continuing medical education on serious fungal infections. Copies of the published results will also be presented to the
different faculties of the National Postgraduate Medical College of Nigeria to help substantiate the need for curriculum review and also for use in ‘train the trainer’ programs. Nationwide training program will also be useful using materials developed by LIFE (Leading International Fungal Education- www.LIFE.org). One such program, which has been scheduled to start in February 2018, is the ‘Cryptococcal Screening and Treatment Program for Healthcare Providers in Nigeria’. The program aims to drive awareness of the high burden of the problem in the country, the use of point of care diagnostic such as the IMMY LFA and the role of therapeutic lumber puncture as stated in the 2017 updated WHO Advanced HIV guidelines [34].

This research has only addressed the burden of cryptococcal meningitis, CPA and histoplasmosis; ABPA, SAFS, IA, fungal keratitis, Pneumocystosis (especially in HIV-infected children) and mucormycosis are yet to be addressed and will be the ground for future research.

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33. Erasmus V, Daha TJ, Brug H, Richardus JH, Behrendt MD, Vos MC, van Beeck EF.
Appendix 1


Abstract

Background: Infections are common complications in critically ill patients with associated significant morbidity and mortality. Aim: This study determined the prevalence, risk factors, clinical outcome and microbiological profile of hospital-acquired infections in the intensive care unit of a Nigerian tertiary hospital.

Materials and Methods: This was a prospective cohort study, patients were recruited and followed up between September 2011 and July 2012 until they were either discharged from the ICU or died. Antimicrobial susceptibility testing of isolates was done using CLSI guidelines.

Results: Seventy-one patients were recruited with a 45% healthcare-associated infection rate representing an incidence rate of 79/1000 patient-days in the intensive care unit. Bloodstream infections (BSI) 49.0% (22/71) and urinary tract infections (UTI) 35.6%
(16/71) were the most common infections with incidence rates of 162.9/1000 patient-days and 161.6/1000 patient-days respectively. Staphylococcus aureus was the most common cause of BSIs, responsible for 18.2% of cases, while Candida spp. was the commonest cause of urinary tract infections, contributing 25.0% of cases. Eighty percent (8/10) of the Staphylococcus isolates were methicillin-resistant. Gram-negative multidrug bacteria accounted for 57.1% of organisms isolated though they were not ESBL-producing. Use of antibiotics (OR = 2.98; p= 0.03) and surgery (OR= 3.15, p< 0.05); in the month preceding ICU admission as well as urethral catheterization (OR= 5.38; p<0.05), and endotracheal intubation (OR= 5.78; p< 0.05) were risk factors for infection.

Conclusion: Our findings demonstrate that healthcare-associated infections are a significant risk factor for ICU-mortality and morbidity even after adjusting for APACHE II score.

Introduction

The prevalence of ICU-acquired infections is significantly higher in developing countries than in industrialized countries, varying between 4.4% and 88.9% [1]. A recently published World Health Organisation (WHO) review revealed that “In low- and middle-income countries the frequency of ICU-acquired infection is at least 2 – 3 higher than in high-income countries; device-associated infection densities are up to 13 times higher than in the USA” [2]. While critical care medicine is a science in the developed world, it is just emerging in Nigeria with a population of over 170 million people. Only 10 of 36 states of the federation have ICU beds. A study conducted in Jos, Nigeria has estimated the prevalence of nosocomial infections is approximately 6% and a disproportionate 20% of these occur in critically ill patients in intensive care units [3]. Another study from Northern Nigeria demonstrated 37 (30.8%) positive bacterial isolates from samples collected from ICU patients [4].

A recent European multicentre study posted that the proportion of infected patients in intensive care units can be as high as 51%; most of these are healthcare-associated [2]. Endotracheal intubation with mechanical ventilation increases the risk of nosocomial pneumonia by 6 to 21 times [5]. Central venous catheterization accounts for 97% of all nosocomial blood stream infection [6]. Urinary catheterization is the most important risk
factor for acquisition of nosocomial urinary tract infections. Nasotracheal intubation is the most significant risk factor for acquisition of nosocomial sinusitis [7]. Other established risk factors include co-morbidities.

Critically ill patients with severe sepsis in intensive care units (ICUs) require lengthy and expensive management, with an associated high mortality, with rates ranging from 30% to 50% [8]. ICU-acquired infections have been found to be an independent risk factor for ICU-mortality even after adjusting for possible co-morbidities [9,10,11]. Strategies to reduce these rates are dependent on accurate and adequate data however, there is a paucity of local data on ICU-acquired infections in our setting, and thus, there is an overdependence on data from other climes which do not necessarily reflect the local realities. Also, healthcare-associated infections (HAI) are known to vary in terms of aetiology, resistance pattern and risk factors even in different units in the same hospital setting.

To initiate necessary policies that are critical to effective treatment of ICU-acquired infections and prevent antibiotic resistance development, there should be surveillance of bacterial aetiologies and infection patterns. This study determined the prevalence, risk factors, clinical outcome and microbiological profile of hospital-acquired infections in the intensive care unit of a Nigerian tertiary hospital.

**Methods**

This was a prospective and observational study. Patient recruitment was conducted in the ICU of the Lagos University Teaching Hospital, Nigeria (LUTH), from September 2011 to July 2012. LUTH is a 761-bed tertiary hospital with a six-bed ICU that admits critically ill patients from all specialties. The ICU admits approximately 220 patients annually. This study was approved by the Health Research and Ethics Committee of the Lagos University Teaching Hospital, Lagos.

All patients that were fifteen years of age and above whose surrogates gave informed written consent over the study period were recruited into the study. Exclusion criteria were those patients whose anticipated stay in the ICU would be less than 48 hours or those unwilling or whose surrogates did not give consent. All written consent forms were stored in a locked filing cabinet as approved by the ethics committee. A structured proforma was used to collect patients’ relevant information. The proforma was divided
into 4 sections: socio-demographic, medical and drug history, possible risk factors, and outcome. The severity of underlying diseases was assessed within 24 hours of admission using the Acute Physiological and Chronic Health Evaluation index (APACHE II score) [12]. A score of twenty and above was taken as a severe premorbid state where the patient has about 40.0% probability of dying. The endpoint of the study was patient discharge from the ICU or death.

Definitions

Health-care associated infections (HAIs) are defined as an infection developing >48 hours after hospital admission or within 30 days after discharge from a hospital [13].

Patient-days is the total number of days that patients were in the ICU during the period of study.

Multi-drug resistance (MDR) was defined only for gram-negative bacteria, as resistance to three or more groups of antibiotics [14]

Sample collection and processing

Urine, blood, endotracheal aspirate, cerebrospinal fluid, wound aspirate and stool samples were aseptically collected from 71 recruited patients on the first day of admission into the ICU (no bronchoalveolar lavage specimen was collected). Repeat samples were taken after 48 hours of admission. Further samples were collected whenever there was clinical suspicion of infection; otherwise, they were collected weekly. All samples were transported to the clinical microbiology laboratory for immediate processing. Blood was cultured in the BACTEC culture system 9050 (Becton Dickinson, New Jersey, US). The other samples were processed according to established standardized protocol. Anaerobic cultures were not done. Isolates were identified to the species level using MicroBact® (Oxoid, UK). Quality control was done using E. coli (ATCC 25922) and S. aureus (ATCC 25923).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was determined by Kirby-Bauer disc diffusion method [15] on Mueller-Hinton agar (MHA) plates according to CLSI guidelines [16]. The tests were controlled with E. coli (ATCC 25922), S. aureus (ATCC 25923), P. aeruginosa (ATCC 27853). All Staphylococci were screened, according to Clinical Laboratory Standards
Institute guideline [16]. An isolate was described as methicillin-resistant Staphylococcus aureus (MRSA) or methicillin-resistant coagulase-negative Staphylococcus (MRCO NS) if the zone of inhibition was ≤22mm around a cefoxitin (30µg) disc. The positive control strain was S. aureus (ATCC 25923) for positive control.

Antimicrobial resistance testing

The presence of ESBLs was suspected if an isolate of K. pneumoniae or E. coli demonstrated resistance to one or more of the indicator beta-lactam antibiotics – ceftriaxone, cefotaxime or cefepime [17]. The screening and confirmation of ESBLs production by the gram-negative bacteria E. coli and Klebsiella spp. was carried out using CLSI criteria [16]. For the confirmatory test (phenotypic), a double-disc diffusion synergy test was performed. An extended zone of inhibition toward the disc containing clavulanic acid (dumb-bell shape) was interpreted as synergy, indicating the presence of an ESBL. The quality control strain used was E. coli (ATCC 25922).

All Staphylococcus spp. isolated were subjected to testing which was performed according to the CLSI guidelines [16]; 15µg erythromycin and 2µg clindamycin discs were placed 15-26 mm apart on MHA plate. After incubation for 16-18 hours at 35 ±2ºC, a flattening of the zone of inhibition of the clindamycin disc adjacent to the erythromycin disc (referred to as D-zone), or presence of hazy growth within the zone of inhibition around clindamycin even without the classical D-zone, was reported as “clindamycin resistance”.

All Enterococcus spp. isolated were tested for vancomycin resistance using E-test (AB, Biodisk, Solna, Sweden), manufacturer’s instructions were strictly adhered to. The plate was incubated at 37ºC for 24 hours. Minimum Inhibitory Concentration (MIC) result was interpreted according to CLSI guideline [16]. Isolates with MIC breakpoint of >16µg/ml were considered resistant to vancomycin resistant.

Data analysis

Data were entered into Epi Info software version 3.4 (CDC, Atlanta GA, USA). The analysis was done with Statistical Package for Social Sciences (SPSS) software version 19.0(SPSS Inc., Chicago, IL., USA). Categorical variables were compared using Pearson’s Chi-square test or Fisher’s exact test. P-values < 0.05 were considered significant for all tests.
Multivariate logistic regression analysis was employed to determine the independent contribution of clinical variables to the prediction of acquisition of ICU infections in the hospital as dependent variables. The same statistical test was used to determine independent predictors of ICU mortality except that APACHE II-adjusted model was used. Variables that had a value of $P \leq 0.2$ on univariate analysis were entered into a forward stepwise logistic regression model. Goodness-of-fit was evaluated by Hosmer-Lemeshow test. Two-tailed $p$ values were reported. The association between the independent determinants of ICU-acquired infections and hospital mortality were estimated using odds ratios and 95% confidence intervals. For outcome analysis, patients were distributed into two subgroups according to survival status (died or discharged).

**Results**

During the study period, 139 patients were admitted into the ICU, but only 71 patients were eligible for the study. Among the 68 patients that were excluded, 39 were admitted for less than 48 hours in ICU, 23 were children under the age of 15 years and 6 declined. The male to female ratio was 1:0.8 with an age range of 15 years to 69 years, with a mean of 38.7 ($\pm 14.9$). Length of stay (LOS) in the ICU ranged from 2 to 38 days with a median of 5 and Interquartile range (IQR) of 5-12 days. All patients had urinary catheters inserted for urine output monitoring and 35 (49.3%) had Central venous catheter (CVC) in-situ. Thirty-seven (52.1%) of the patients were either transferred from the ward or another hospital while 34 (47.9%) came via the Accident and Emergency unit (Table 7.1). Early-onset infections (infection developing between two and seven days on ICU admission) were described for 25 (78.1%) infections, while 7 (21.9%) had late-onset infections.

Table 1. Baseline demographic and Clinical characteristics of Patients admitted in the Intensive Care Unit of Lagos University Teaching Hospital, Lagos

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>frequency</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>45.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>54.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Age | 71 | 38.7(±14.9)
---|---|---
15-40 | 43 | 60.6
41-69 | 28 | 39.4
Apache II Score | 71 | 21.2 (± 8.07)
<20 | 35 | 49.3
≥20 | 36 | 50.7
Length of ICU stay | 71 | 9.1 (± 7.3) 7 (5-12)
2-7 | 41 | 57.7
8-38 | 30 | 42.3
Duration of antibiotic administration in ICU (days) | 69 | 8.0 (±6.4)
Locations before ICU admissions
Hospital | 37 | 52.1
Home | 34 | 47.9
Wards admitted in hospital before transfer to ICU
Outside hospital | 20 | 28.2
Accident and Emergency | 34 | 47.9
Surgical ward | 8 | 11.3
Medical ward | 3 | 4.2
Obstetrics and Gynaecology | 3 | 4.2
Neurosurgery ward | 3 | 4.2

Clinical characteristics of patients

Thirty-five (49.3%) of the patients had an APACHE II score of less than 20 while 36 (50.7%) had a score of 20 and above, the mean APACHE II score was 21.2 (±8.07). Admission diagnoses were respiratory failure 19 (26.8%); severe sepsis 11(15.5%); eclampsia 5(7.0%) and others 21 (29.6%) and these included post-surgical patients, patients with chronic renal failure and malignancies) (Figure 7.1). A total of 45 laboratory-confirmed infections were identified in 32 patients, representing a prevalence rate of 45.1%, and an incidence rate of 79/1000 patient-days (Incidence rate was derived
by dividing the number of new nosocomial infections acquired in a period by the total number of patient-days for the same period x 1000). The total patient-days in the study was 405. The most common infection was bloodstream infections accounting for 49.0% (22/45) of all infections. Two patients had 3 episodes and 3 patients had 2 episodes of BSI which were catheter associated. Eleven patients had a total of 16 (35.6%) urinary tract infections (UTI); 1 patient had 3 episodes and 3 patients had 2 episodes each of UTI. A total of 4 (8.9%) skin-soft tissue infections (SSTI) were reported amongst 3 patients, one had 2 episodes of SSTI. Three (6.7%) RTIs were observed in three patients (Figure 7.2).

Figure 1: Admitting diagnosis of Patients into the ICU

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eclampsia</td>
<td>7.0%</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>8.5%</td>
</tr>
<tr>
<td>Multi-trauma</td>
<td>12.7%</td>
</tr>
<tr>
<td>Sepsis</td>
<td>15.5%</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>26.8%</td>
</tr>
<tr>
<td>Others</td>
<td>29.6%</td>
</tr>
</tbody>
</table>
Microbiological profile of pathogens identified

Twenty different species of pathogenic microorganisms were identified in the 45 infections recorded. Proteus mirabilis, Staphylococcus aureus, and Coagulase-negative Staphylococci were the most frequently isolated pathogens accounting for 5 (11.1%) each respectively. Staphylococcus aureus was the most common cause of bloodstream infection, accounting for 4 (18.2%) cases, followed by Coagulase-negative staphylococcus and Klebsiella pneumoniae with 3 (13.6%) isolates each. Non-Candida albicans 4 (25.0%) and Coagulase-negative staphylococci (12.5%) were the commonest cause of urinary tract infections (Figure 7.3).
Eleven gram-positive bacteria were isolated, S.aureus 5(45.5%), Coagulase negative staphylococcus 5(45.5%), and one Enterococcus feacalis. Four (80%) of the Staph aureus were Methicillin resistant staphylococci (zones of inhibition were <21mm), Only one (20%) of S. aureus (MRSA) isolates showed resistance to clindamycin, gentamicin, and levofloxacin respectively. However, all S. aureus isolates were susceptible to ciprofloxacin. Four (80%) of the Coagulase-negative staphylococci were Methicillin resistant (MRCoNS); three (3) were resistant to clindamycin and gentamicin respectively, one displayed resistance to ciprofloxacin but none to levofloxacin. The only Enterococcus Feacalis isolated was vancomycin resistant (VRE) breakpoint of >16µg/ml was used. The resistance profile of the gram-negative bacteria revealed that 16(57.1%) were MDR (Table 7.2).

Table 7.2: Resistance profile of Gram negative isolates from Intensive Care Unit infections in Lagos University Teaching Hospital, Lagos.
### Resistance to antibiotics (R/S for single isolates; %R for n ≥ 2 isolates)

<table>
<thead>
<tr>
<th>Isolates</th>
<th>n</th>
<th>SAM</th>
<th>FEP</th>
<th>CRO</th>
<th>CIP</th>
<th>GEN</th>
<th>LVX</th>
<th>MEM</th>
<th>TZP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>5</td>
<td>60</td>
<td>0</td>
<td>60</td>
<td>100</td>
<td>60</td>
<td>20</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>3</td>
<td>33.3</td>
<td>66.7</td>
<td>66.7</td>
<td>66.7</td>
<td>33.3</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>3</td>
<td>33.3</td>
<td>0</td>
<td>100</td>
<td>66.7</td>
<td>66.7</td>
<td>33.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>3</td>
<td>NA</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td><em>Burkholderia pseudomallei</em></td>
<td>2</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacterbaumannii</em></td>
<td>1</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td><em>Pantoea agglomerans</em></td>
<td>1</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td><em>Enterobacter gergoviae</em></td>
<td>1</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>1</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><em>Proteus rettgeri</em></td>
<td>1</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><em>Providentia stuartii</em></td>
<td>1</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><em>Serratia rubidaea</em></td>
<td>1</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>1</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
</tbody>
</table>

**KEY:** SAM=Ampicillin-sulbactam; FEM= Cefepime; CRO= Ceftriaxone; CIP= Ciprofloxacin; GEN= Gentamicin; LVX= Levofloxacin; MEM= Meropenem; TZP= Piperacillin-tazobactam; R= Resistance; S= Sensitive; NA= Not applicable

Risk factors and outcome of HAIs in the ICU

Five factors were identified as statistically significant regarding HAIs in the ICU using univariate analysis: Use of antibiotics one month before ICU admission (OR= 0.334; p= 0.03), surgery one month before admission (OR= 0.181, p< 0.001); urethral catheterization (OR= 5.38; p<0.05), endotracheal intubation (OR= 5.78; p< 0.05), and patients’ location before ICU admission (OR= 0.11; p< 0.05) (Table 7.3).
Table 3. Univariate analysis of risk factors for Intensive Care Unit-acquired infections in Lagos University Teaching Hospital, Lagos

<table>
<thead>
<tr>
<th>Factors</th>
<th>Infected n(%)</th>
<th>Non-Infected n(%)</th>
<th>Odds ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of antibiotic one month before hospital admission</td>
<td></td>
<td></td>
<td>0.334</td>
<td>0.03</td>
</tr>
<tr>
<td>Yes</td>
<td>20(62.5)</td>
<td>14(35.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12(37.5)</td>
<td>25(64.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery one month before admission</td>
<td></td>
<td></td>
<td>0.181</td>
<td>0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>21(65.6)</td>
<td>10(25.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>11(34.4)</td>
<td>29(74.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urethral catheterization</td>
<td></td>
<td></td>
<td>5.38</td>
<td>0.03</td>
</tr>
<tr>
<td>Yes</td>
<td>32(100.0)</td>
<td>33(84.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0(0.0)</td>
<td>6(15.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endotracheal intubation</td>
<td></td>
<td></td>
<td>5.78</td>
<td>0.02</td>
</tr>
<tr>
<td>Yes</td>
<td>29(90.6)</td>
<td>13(33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3(9.4)</td>
<td>26(66.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location before admission</td>
<td></td>
<td></td>
<td>0.11</td>
<td>0.001</td>
</tr>
<tr>
<td>Hospital wards</td>
<td>13(59.1)</td>
<td>4(13.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accident/Emergency unit</td>
<td>9(40.9)</td>
<td>25(86.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age(years)</td>
<td></td>
<td></td>
<td>3.28</td>
<td>0.17</td>
</tr>
<tr>
<td>≥60</td>
<td>2(6.25)</td>
<td>7(17.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;59</td>
<td>30(93.75)</td>
<td>32(82.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.44</td>
<td>0.086</td>
</tr>
<tr>
<td>Female</td>
<td>18(56.25)</td>
<td>14(35.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14(</td>
<td>25(64.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Septicaemic patients had higher mortality rates than non-septicaemic patients (75.0% vs 25.0%). Five (5) factors were significantly associated with this severe outcome. These factors were ICU-acquired infection (OR =8.2; p= 0.04); endotracheal intubation (OR= 5.7; p= 0.04); urethral catheterization (OR= 7.5; p= 0.04); acquisition of infection within seven days of admission (OR= 4.9; p= 0.05) and an APACHE II score value greater or equal to 20 (OR=9.04; P <0000) (Table 4). After controlling for the effect of the APACHE II score on the clinical outcome of infections in ICU, using a forward stepwise multivariate logistic regression method, only ICU-acquired infection was found to be statistically significant for ICU mortality (Table 4).
Table 7.4. Result of Univariate and Multivariate regression analysis of risk factors for ICU mortality.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>p-value</td>
</tr>
<tr>
<td>ICU-aquired infection</td>
<td>8.2</td>
<td>0.004</td>
</tr>
<tr>
<td>LOS &lt; 7days before infection</td>
<td>4.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Endotracheal intubation</td>
<td>5.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Urethral catheterization</td>
<td>7.5</td>
<td>0.004</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>9.04</td>
<td>0.000</td>
</tr>
<tr>
<td>Location before admission</td>
<td>1.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Age</td>
<td>1.7</td>
<td>0.28</td>
</tr>
<tr>
<td>Gender</td>
<td>0.63</td>
<td>0.32</td>
</tr>
<tr>
<td>Use of antibiotic one month before admission</td>
<td>0.77</td>
<td>0.58</td>
</tr>
<tr>
<td>Surgery one month before admission</td>
<td>0.50</td>
<td>0.24</td>
</tr>
<tr>
<td>Malignancy</td>
<td>1.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Nasogastric intubation</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>LOS &gt; 7 days</td>
<td>0.81</td>
<td>0.66</td>
</tr>
</tbody>
</table>

KEY: LOS= Length of ICU stay; OR= Odds ratio; CI= Confidence interval

Discussion

The 45% prevalence rate of HAIs reported is very high, it demonstrates the inadequacy of the infection control processes in place in our ICU. This rate is significantly higher than the pooled prevalence of 35.2% from ICUs in LMICs [2]. A number of reasons could be attributable to this including but not limited to; lack of an antibiotic stewardship program in Centre of study [2,18], lack of trained ICU nurses [19,20, 21], frequent turnover of ICU nurses, poor nurses to patient ratio [20,21], no established infection control policy [2],...
poor hand hygiene due to irregular water supply in centre of study, high bed occupancy rates, patients relatives are required to pay from pocket for every item used in managing these patients, high level of human traffic in ICU (relatives, students, HCWs) etc. Antibiotic stewardship programs aim to optimize appropriate antibiotic treatment while minimizing antibiotic resistance thus improving patient safety, a recent review by Ramsamy et al (2016) emphasized this [22].

BSIs (49.0%) were the most documented infections in our ICU and this is contrary to previous findings [2,24,24] which documented hospital-acquired pneumonia as the most common type of HAIs in ICU settings. This finding can easily be explained by the fact that BAL samples which are a more representative sample are not routinely collected in the centre of study and also there was only one functional ventilator during the study period so fewer patients developed ventilator associated pneumonia. Also in the index ICU, use of a pair of sterile gloves was the best of barrier precautions practiced during catheterization. The common practice of using femoral veins for central venous catheterization instead of subclavian vein could have contributed to this high BSI rate, as there are increased associated infections risks with the later [25]. Due to challenges with financial constraints and need for access for intravenous drugs, most times patients were treated with antibiotics instead of changing catheters. CA-UTIs at 35.6% were also high and the readily apparent reason is that all patients are catheterized for monitoring. In the ICU studied, the local (institutional) protocol was to change urinary catheter (latex) within 5-7days however we found that catheters were in place for more than 10 days in some patients due to financial constraints. Candida spp. were the most prevalent agents of urinary tract infection in our study and this is probably due to poor catheter care which allows this organism which is part of skin flora to colonize the catheter and eventually become pathogenic following migration into the bladder [26,27]. This finding compares well with that of Rosenthal et al. [27] and that of a more recent study [28].

Intensive care unit (ICU)-acquired infections are a challenging health problem worldwide, especially when caused by multidrug-resistant (MDR) pathogens. Twenty different species of microorganisms were involved in 45 episodes of ICU infections in this study. While Staphylococcus aureus, coagulase-negative Staphylococcus (CoNS), and Proteus mirabilis were the most frequently isolated organisms causing ICU-acquired infections. MDR gram-negative bacteria were responsible for about two-thirds of the total infections
recorded. This trend contrasts with findings from studies done in developed countries where the prevalent cause of healthcare-associated infections is switching over to gram positive organisms [29,30]. These findings are however comparable to another study where Enterobacteriaceae, Staphylococcus aureus, and CoNs were the most common isolates associated with BSI [27]. The increase in BSIs due to CoNS in our study is consistent with a report by Hidron and colleagues [31]. The reasons for this new trend seem not readily available, however, epidemiological variables and changes in risk factors (e.g., increased use of invasive procedures in ICU especially presence of CVC lines in situ) may offer plausible explanations.

The most worrisome finding in this study was that almost all the gram-positive bacterial agents of BSIs were MDR, including the only Enterococcus spp. Previous studies have reported a steady rise of resistant pathogens from patients in ICU [32,33]. The degree of antimicrobial resistance among key pathogens in our hospital’s ICU as revealed by this study was striking, though, a previous similar study conducted in ICUs in developing countries revealed similar high antibiotic resistance rates [26]. The wide use of broad-spectrum antibiotics, prolonged administration of antibiotics, extremely sick patients, and cross-transmission of pathogens via hands and materials, especially where there is a low nurse-to-patient ratio as we had in the index ICU, may all have contributed to the high antibiotic resistance rates in this study [34,35].

The highest rates of antibiotic resistance displayed by the bacterial strains were to amoxicillin-clavulanate and ceftriaxone. During the time of the study, these two drugs were the commonest drugs given empirically to ICU patients. This practice might have built up selective pressure that had led to the evolution of resistant strains which clonally expanded over time. This is indeed a notable finding, which addresses one of the key objectives of this study that is: determining the microbiological profile of the index ICU to guide in developing an antibiotic policy for it. A recent ‘surviving sepsis’ publication by Ramsamy et al (2016) noted that ‘unnecessary administration of antimicrobial therapy not only impacts on the individual patient but also on those patients in the same ICU environment’ and that ‘knowledge of inherent flora and their antimicrobial susceptibility patterns are crucial’ [22].

Our findings revealed a high rate of multi-drug resistant (MDR) gram-negative bacilli (57.1%). A similar study demonstrated comparable high rates of MDR (51%) in gram-
negative bacilli among patients in Afghanistan [36]. Discordantly, much lower MDR rates in gram-negative bacilli - 5.9% in P. aeruginosa, 1.2% in E. coli and 0.9% in K. pneumonia - were obtained in ICU in Canada [37]. The explanation for this very high rate of MDR (57.1%) with gram-negative bacteria is not clear especially when none of the species was positive for ESBL-production, as most MDR gram-negative bacterial strains are ESBL-producers [38]. However, lack of antibiotic stewardship programs resulting in irrational antibiotic use in developing countries such as Nigeria when compared to Canada can be might account for this variation. Another plausible explanation could be that those gram-negative bacteria expressed both ESBLs and AmpC-like beta-lactamases. The AmpC beta-lactamase-producing strains do not demonstrate lowering of the MIC when combined with clavulanic acid and so are positive on the initial screen test, but negative on the phenotypic confirmation test. So, if such gram-negative strains are tested, AmpC beta-lactamase-producing effect will not allow phenotypic expression of the ESBLs [39]. Unfortunately, we did not test for AmpC beta-lactamase-production in this study.

Another significant finding was the high rate (80.0%) of methicillin resistance among the Staphylococcus spp, which is close to the 84.0% prevalence rate obtained by Rosenthal and colleagues [26].

Other studies have independently associated acquisition of MDR-ICU-acquired infections with risk factors such as the use of antibiotics one month prior to ICU admission, surgery one month before admission, urethral catheterization and endotracheal intubation [40,41,]. In our study, only the use of antibiotics one month before ICU admission was independently associated with the acquisition of ICU infections and by extension, other multidrug-resistant organisms. Contrary to previous observation [40,41], being severely ill (assessed by APACHE II score), and prolonged length of ICU stay seemed not to be risk factors for ICU-acquired infections (MDR by extension) among patients analyzed in this study.

Limitations of this study include the relatively small size of study population which is a reflection of the limited ICU beds available and the fact that healthcare generally is out of pocket in the study environment. We speculate that this could have contributed to the study’s lack of power to detect some significant relationships from our data. Secondly, the convenience sampling technique we used helped us to access our study participants
easily; however, it could have introduced sampling bias, distorting good representation of the entire population.

In conclusion, ICU-acquired infections remained a significant risk factor for ICU mortality even after adjusting for APACHE II score. There is dire need to develop and entrench an antibiotic stewardship policy in our ICU and set up a national surveillance program to monitor infections in our Nigeria. A robust infection control program is also a matter of urgency in our setting.

References


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34. Ho PL, for the Hong Kong intensive care unit antimicrobial resistance study (HK-ICARE) group carriage of methicillin-resistant staphylococcus aureus, ceftazidime-resistant gram negative bacilli and vancomycin resistant enterococci before and after intensive care unit admission. Critical Care Medicine 2003; 31: 1175-1182.


Appendix 2: Informed consent forms

Form 1: Prevalence of cryptococcal antigenemia in antiretroviral naïve and antiretroviral experienced HIV infected patients with CD4+ count below 250 cells/mm3 in Lagos, Nigeria.

Participant Information Sheet

You are being invited to take part in a research study as part of a PhD student work. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for taking the time to read this.

Who will conduct the research?

Dr Rita Oladele
Faculty of Biology, Medicine and Health
School of Biological Sciences, Division of Infection, Immunity & Respiratory Medicine
The University of Manchester, Second Floor Education and Research Centre,
Wythenshawe Hospital, Southmoor Road, M23 9LT
Department of Microbiology CMUL, University of Lagos/LUTH, Idi-Araba, Lagos.

What is the purpose of the research?

This study is designed to evaluate the exposure to a microbial organism known as cryptococcus in Nigeria. This microbe causes life-threatening infections in persons of low resistance to infection. The results will help develop guidelines for managing patients of low resistance to infection that are at risk of developing the infection.

Why have I been chosen?

You have been selected because there have been documented cases of the infection in persons of low resistance to infection like you.

What would I be asked to do if I took part?

In the course of this study, you will be asked questions in the form of questionnaires to obtain information from you, it is not compulsory to answer any question you are not comfortable with. About 5mls of blood will be collected from you for testing, like normally done at some of your
The skin prick will produce a little pain or discomfort, otherwise there are no minimal risks associated with the study procedures. You are free to share any concerns you have with the doctor giving you this form. If your test result is positive and you are attending the US President's Emergency Plan For AIDS Relief (PEPFAR) clinic, your result will be communicated to the doctor treating you there.

What happens to the data collected?

These questionnaires are posted to University of Manchester, the information will be transcribed unto an a secure computer folder stored on the University of Manchester computer and analysed using a computer software. The data will be analysed and the results published in academic journals, but you will not be identified by name.

How is confidentiality maintained?

The information obtained will be treated in absolute confidence. Your name will not be put on the questionnaire, you will only be identified by a unique number. Your information shall not be divulged to anyone except the researchers of this study. This data will be kept for 15 years.

What happens if I do not want to take part or if I change my mind?

It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw, without giving a reason and without detriment to yourself.

Will I be paid for participating in the research?

A voucher to the value of N700 naira will be given to you for your time spent each time you come.

What is the duration of the research?

It will take approximately 30mins to fill the questionnaire and collect the blood sample.

Where will the research be conducted?

PEPFAR clinic in the teaching hospital.

Will the outcomes of the research be published?

The results will be published in scientific journals, medical journals and medical conferences.

Who has reviewed the research project?

This project has been reviewed by the University of Manchester Research Ethics Committee 5. It has also been reviewed by the Lagos University Teaching Hospital Research Ethics committee.

What if something goes wrong?

If you ever have any questions about this study, or if you have a research-related harm/reaction, you should contact the researcher who’s name and details are given below. If you are distressed we can support you or refer you to a clinical psychologist/psychiatrist if necessary.

What if I want to make a complaint?
Minor complaints

If you have a minor complaint then you need to contact the researcher(s) in the first instance.

Dr. Rita Oladele
Dept. of Microbiology & Parasitology
L.U. T. H., Lagos
08171570142
drritaoladele@yahoo.com  rita.oladele@postgrad.manchester.ac.uk

If you are still not satisfied or have a major issue you can contact:
The Chairperson,
LUTH Health Research and Ethics Committee
Room 107, Administration Block,
Lagos University Teaching Hospital,
Lagos, Nigeria.

Formal Complaints

If you wish to make a formal complaint or if you are not satisfied with the response you have gained from the researchers in the first instance then please contact the Research Governance and Integrity Manager, Research Office, Christie Building, University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing: research.complaints@manchester.ac.uk or by telephoning 0161 275 2674 or 275 2046.

What Do I Do Now?

If you have any queries about the study or if you are interested in taking part then please contact the researcher(s)

Dr. Rita Oladele
Dept. of Microbiology & Parasitology
L.U. T. H., Lagos
08171570142
drritaoladele@yahoo.com  rita.oladele@postgrad.manchester.ac.uk
This Project Has Been Approved by the University of Manchester’s Research Ethics Committee [16393].
INFORMED CONSENT

(To be explained to the enrollee in the language they understand best)

Mr/Mrs/Chief/Alhaji/Alhaja/Dr.-----------------------------------------------

Whose address is-----------------------------------------------------------------

Hereby give consent for my participation in the research titled: Prevalence of cryptococcal antigenemia in antiretroviral naïve and antiretroviral experienced HIV infected patients with CD4+ count below 250 cells/mm³ in Lagos, Nigeria.

The research has been explained to me and I am aware that the tests to be carried out will not harm me and that the information obtained from me will be kept confidential.

I am also aware that I have the right to withdraw from the study at any point if I so wish.

All other terms of this consent have been explained to me in a language that I understand.

Sign/ thumbprint---------------- Sign------------------------

Date-------------------------- Date------------------

Interviewer

Witness

-----------------------------------------------------------------

Sign Date

Informed consent form 2: Chronic Pulmonary Aspergillosis as a cause of smear negative TB and / or anti-TB treatment failure in HIV infected Nigerians.

Participant Information Sheet

You are being invited to take part in a research study as part of a PhD student work. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for taking the time to read this.

Who will conduct the research?
Dr Rita Oladele

Faculty of Biology, Medicine and Health

School of Biological Sciences, Division of Infection, Immunity & Respiratory Medicine

The University of Manchester, Second Floor Education and Research Centre,

Wythenshawe Hospital, Southmoor Road, M23 9LT

Department of Microbiology CMUL, University of Lagos/LUTH, Idi-Araba, Lagos.

What is the purpose of the research?

To evaluate Chronic Pulmonary Aspergillosis as a cause of smear negative TB and / or anti-TB treatment failure in HIV infected Nigerians which is a life threatening disease that is treatable.

Why have I been chosen?

You have been chosen because you have been diagnosed with tuberculosis and you are starting treatment.

What would I be asked to do if I took part?

You will be required to fill two questionnaires, there are no ‘right’ or ‘wrong’ answers to these questions, we just want to know what you think., This will take approximately 15mins of your time. Then a spoonful of blood will be collected through a syringe from you every three months. It is the same procedure that will be used by your doctors to check if you suffere from possible infection. You will experience some mild pain at the site of the needle prick (minimal pain). If you have productive cough, you will be asked to bring an early morning sputum sample. A chest X-ray will also be done free for you. The result of the test and the chest x-ray will be given to you in a letter to give to your doctor who is treating you for TB at the clinic you are attending.

What happens to the data collected?

The questionnaire will not have your name or address on it, it will however have a unique number to identify you. These questionnaires are posted to University of Manchester, the information will be converted to computer file atUniversity of Manchester r and analysed using a computer software. The results are also stored in the secure electronic file in a folder. The data will be analysed and the results published in academic journals, but there will be nothing in the publication to identify you. The data may also be used in future studies.

How is confidentiality maintained?

The information obtained will be treated in absolute confidence. Your name will not be put in the questionnaire, you will only be identified by a unique number. No part or whole of your information shall be divulged to anyone except the researchers. We keep your records absolutely confidential. This data will be kept for ten-15 years

What happens if I do not want to take part or if I change my mind?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take
part you are still free to withdraw at any time and we will not keep your data if you decided to withdraw before the test is done. If you withdraw after the test is done and we have already collected you data, we will keep your data (anonymised) and will be analysed unless you explicitly request for your data to be destroyed. If you wish to withdraw you do not need to give a reason and your decision will not affect the treatment you receive from your own doctor. Will I be paid for participating in the research?

The sum of N700 naira will be given to you for inconvenience caused to you while travelling to the clinic and for your time spent each time you visit the clinic for this study.

What is the duration of the research?

The study duration is 12 months. You will be coming for this study at the beginning of your treatment, 3 months into your treatment, end of your treatment, three month after end of treatment and 6 month after end of treatment. The time that you will commit to this research will include time for giving the consent, filling in the questionnaires which is approximately 15 mins and collection of 5mls of blood (i.e venous blood collection)

Where will the research be conducted?

In the TB clinic you are attending.

Will the outcomes of the research be published?

Yes in academic journals, but there will be no means to identify you in the publication

Who has reviewed the research project?

This project has been reviewed by the University of Manchester Research Ethics Committee 5. It has also been reviewed by the Lagos University Teaching Hospital Research Ethics committee.

What if something goes wrong?

If you ever have any questions about this study, or if you have a research-related harm/reaction, you should contact the investigator. Name, address, and phone number are given below. We are ready to hold on to see if you want to carry on or withdraw. And if you are distressed we can counsel with you or refer you to a clinical psychologist/psychiatrist if necessary.

What if I want to make a complaint?

Minor complaints

If you have a minor complaint then you need to contact the researcher(s) in the first instance.

Dr. Rita Oladele
Dept. of Microbiology & Parasitology
L.U. T. H., Lagos
08171570142
drritaoladele@yahoo.com  rita.oladele@postgrad.manchester.ac.uk

If you are still not satisfied or have a major issue you can contact:
The Chairperson,
LUTH Health Research and Ethics Committee
Room 107, Administration Block,
Lagos University Teaching Hospital,
Lagos, Nigeria.

Formal Complaints

If you wish to make a formal complaint or if you are not satisfied with the response you have gained from the researchers in the first instance then please contact the Research Governance and Integrity Manager, Research Office, Christie Building, University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing: research.complaints@manchester.ac.uk or by telephoning 0161 275 2674 or 275 2046.

What Do I Do Now?

If you have any queries about the study or if you are interested in taking part then please contact the researcher(s)
Dr. Rita Oladele
Dept. of Microbiology & Parasitology
L.U. T. H., Lagos
08171570142
drritaoladele@yahoo.com  rita.oladele@postgrad.manchester.ac.uk
This Project Has Been Approved by the University of Manchester’s Research Ethics Committee [16393]

INFORMED CONSENT

(To be explained to the enrollee in the language they understand best)

Mr/Mrs/Chief/Alhaji/Alhaja/Dr.---------------------------------------------

Whose address is-----------------------------------------------------------------

Hereby give consent for my participation in the research titled: Evaluation of Chronic Pulmonary Aspergillosis as a cause of smear negative TB and / or anti-TB treatment failure in HIV infected Nigerians

The research has been explained to me and I am aware that the tests to be carried out will not harm me and that the information obtained from me will be kept confidential.

I am also aware that I have the right to withdraw from the study at any point if I so wish.

All other terms of this consent have been explained to me in a language that I understand.

Sign/ thumbprint-------------    Sign---------------------------

Date-----------------------------    Date-----------------------------

Interviewer

Witness

-----------------------------------------------------------------

Sign     Date

Form 3: Histoplasmin skin sensitivity survey in Nigeria

Participant Information Sheet

You are being invited to take part in a research study as part of a PhD student work. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for taking the time to read this.

Who will conduct the research?

Dr Rita Oladele
Faculty of Biology, Medicine and Health
School of Biological Sciences, Division of Infection, Immunity & Respiratory Medicine
The University of Manchester, Second Floor Education and Research Centre,
Wythenshawe Hospital, Southmoor Road, M23 9LT
Department of Microbiology CMUL, University of Lagos/LUTH, Idi-Araba, Lagos.

What is the purpose of the research?
This study is designed to evaluate the exposure to a microbial organism known as histoplasma in Nigeria. This microbe causes life-threatening infections in persons of low resistance to infection. The results will help develop guidelines for managing patients of low resistance to infection that are at risk of developing the infection.

Why have I been chosen?
You have been selected because there have been documented cases of the infection in your region.

What would I be asked to do if I took part?
In the course of this study, you will be asked questions in the form of questionnaires to obtain information from you, it is not compulsory to answer any question you are not comfortable with. You will be given a small injection intradermally (through the skin), just like BCG vaccine (for TB) or tests called mantoux test (for TB). We will ask you to come back to enable us to look at the site of the injection after 48-72hours to measure the swelling or skin raised at the site of injection.. The skin prick will produce a little pain or discomfort, otherwise there are no minimal risks associated with the study procedures. You are free to share any concerns you have with the doctor giving you this form. If your test result is positive and you are attending the US President’s Emergency Plan For AIDS Relief (PEPFAR) clinic, your result will be communicated to the doctor treating you there.

What happens to the data collected?
These questionnaires are posted to University of Manchester, the information will be transcribed unto an a secure computer folder stored on the University of Manchester computer and analysed using a computer software.. The data will be analysed and the results published in academic journals, but you will not be identified by name.

How is confidentiality maintained?
The information obtained will be treated in absolute confidence. Your name will not be put on the questionnaire, you will only be identified by a unique number. your information shall not be divulged to anyone except the researchers of this study.. This data will be kept for 15 years.

What happens if I do not want to take part or if I change my mind?
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw, without giving a reason and without detriment to yourself.

**Will I be paid for participating in the research?**

A voucher to the value of N700 naira will be given to you for your time spent each time you come. You are coming first to do the test and 2/3 days later to read it.

**What is the duration of the research?**

The first day you will spend 10mins to fill the questionnaire and less than 2 minutes for the injection. 48/72 hours later you will come back the clinic to enable us look at the site of the inject and measure the size of the skin swellings around the injection site called (reading the test) and this will take another 2mins.

**Where will the research be conducted?**

In the primary healthcare centre or PEPFAR clinic in the teaching hospital.

**Will the outcomes of the research be published?**

The results will be published in scientific journals, medical journals and medical conferences.

**Who has reviewed the research project?**

This project has been reviewed by the University of Manchester Research Ethics Committee 5. It has also been reviewed by the Lagos University Teaching Hospital Research Ethics committee.

**What if something goes wrong?**

If you ever have any questions about this study, or if you have a research-related harm/reaction, you should contact the researcher who’s name and details are given below.. If you are distressed we can support you or refer you to a clinical psychologist/psychiatrist if necessary.

**What if I want to make a complaint?**

**Minor complaints**

If you have a minor complaint then you need to contact the researcher(s) in the first instance.

Dr. Rita Oladele

Dept. of Microbiology & Parasitology

L.U. T. H., Lagos
If you are still not satisfied or have a major issue you can contact:

The Chairperson,
LUTH Health Research and Ethics Committee
Room 107, Administration Block,
Lagos University Teaching Hospital,
Lagos, Nigeria.

**Formal Complaints**

If you wish to make a formal complaint or if you are not satisfied with the response you have gained from the researchers in the first instance then please contact the Research Governance and Integrity Manager, Research Office, Christie Building, University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing: research.complaints@manchester.ac.uk or by telephoning 0161 275 2674 or 275 2046.

**What Do I Do Now?**

If you have any queries about the study or if you are interested in taking part then please contact the researcher(s)

Dr. Rita Oladele
Dept. of Microbiology & Parasitology
L.U. T. H., Lagos

08171570142
drritaoladele@yahoo.com  rita.oladele@postgrad.manchester.ac.uk
This Project Has Been Approved by the University of Manchester’s Research Ethics Committee [16393].

INFORMED CONSENT

(To be explained to the enrollee in the language they understand best)

Mr/Mrs/Chief/Alhaji/Alhaja/Dr.-----------------------------------------------

Whose address is---------------------------------------------------------------

Hereby give consent for my participation in the research titled: Histoplasmin skin sensitivity survey in Nigeria.

The research has been explained to me and I am aware that the tests to be carried out will not harm me and that the information obtained from me will be kept confidential.

I am also aware that I have the right to withdraw from the study at any point if I so wish.

All other terms of this consent have been explained to me in a language that I understand.

Sign/ thumbprint------------------  Sign-------------------------

Date------------------------  Date------------------------

Interviewer

Witness

-----------------------------------------------

Sign  Date