DIAGNOSIS AND EPIDEMIOLOGY OF SERIOUS FUNGAL INFECTIONS IN GHANA

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ABPA	Allergic bronchopulmonary aspergillosis
AFST	Antifungal susceptibility testing
AH	African histoplasmosis
AHD	Advanced HIV disease
AN	*Aspergillus* nodules
APH	Acute pulmonary histoplasmosis
ASSURED	Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users
BAL	Bronchoalveolar lavage
BDG	Beta-D glucan
BSL	Biosafety level
CA	Cryptococcal antigenemia
CCPA	Chronic cavitary pulmonary aspergillosis
CDC	Centre for Disease Control and Prevention
CGB	Canavanine glycine bromothymol blue
CGD	Chronic granulomatous disease
CH	Classical histoplasmosis
CNPA	Chronic necrotizing pulmonary aspergillosis
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CPA	Chronic pulmonary aspergillosis
CPH	Chronic pulmonary histoplasmosis
CrAg	Cryptococcal antigen

CXR	Chest x-ray
DH	Disseminated histoplasmosis
EDL	Essential diagnostic list
EIA	Enzyme immunoassay
FFPE	Formalin-fixed paraffin-embedded
FPPL	Fungal priority pathogens list
GAFFI	Global Action for Fungal Infections
GDP	Gross domestic product
GM	Galactomannan
GMS	Grocott-Gomori methenamine silver
GVHD	Graft-versus-host disease
HAART	Highly active antiretroviral therapy
Hcc	*Histoplasma capsulatum* var. *capsulatum*
Hcd	*Histoplasma capsulatum* var. *duboisii*
HIV	Human immunodeficiency virus
IA	Invasive aspergillosis
ICT	Immunochromatographic test
ICU	Intensive care unit
Ig	Immunoglobulin
IRIS	Immune reconstitution inflammatory syndrome
IV	Intravenous
KOH	Potassium hydroxide
LAT	Latex agglutination test
LFA	Lateral flow assay
LFD	Lateral flow device

LMIC	Low- and middle-income country
NHIS	National health insurance system
NTMLD	Non-tuberculous mycobacterial lung disease
PAS	Per-iodic acid Schiff
PCR	Polymerase chain reaction
PJP	*Pneumocystis jirovecii* pneumonia
PTB	Pulmonary tuberculosis
QoL	Quality of Life
SAFS	Severe asthma with fungal sensitization
SAIA	Sub-acute invasive aspergillosis
SFI	Serious fungal infection
SGRQ	St. George Respiratory Questionnaire
SSA	Sub-Saharan Africa
TAT	Turn-around-time
WHO	World Health Organization

ABSTRACT

In many resource-limited settings, advances in diagnosis and epidemiology of serious fungal infections (SFIs), which generally excludes superficial fungal infections, are yet to be realized. However, there are significant number of patient groups at risk for SFIs, and the geography and socio-economic conditions are more favourable for SFIs in the general population. In these settings, SFIs are commonly diagnosed by histopathology, because antigen-antibody tests are less accessible. Recent introductions of fungal rapid diagnostics tests (RDTs) are revolutionizing SFI diagnosis globally. The aim of this research was to update the epidemiology of SFIs in Ghana, by using RDTs to aid the diagnosis of key SFIs among common at-risk patient groups and retrospectively evaluating histopathologically diagnosed SFIs.

The research comprised five studies. Study 1: Patients living with HIV were screened for cryptococcosis and histoplasmosis using cryptococcal antigen (CrAg) lateral flow assay (LFA), *Histoplasma* antigen (Histo Ag) enzyme immunoassay (EIA) and LFA followed by confirmation with laboratory and medical imaging methods (*n* = 150); Study 2: Patients being investigated for new or relapsed pulmonary tuberculosis (PTB) were screened for chronic pulmonary aspergillosis (CPA) using *Aspergillus*-specific antibody LFA (Asp Ab LFA) in combination with medical imaging and culture (*n* = 183); Study 3: Patients with confirmed PTB, receiving anti-TB regimen were screened with Asp Ab LFA at the end of PTB treatment (T_1) and six months post-treatment (T_2) to detect CPA (*n* = 47); Study 4: Patients with haematological malignancy were screened for invasive aspergillosis (IA) using the *Aspergillus* galactomannan LFA, computed tomography scan and culture (*n* = 56); Study 5: Histopathology reports from 2012-2021 were reviewed to evaluate the spectrum of fungal infections (*n* = 107) and confirm aetiological agents with molecular methods.

The prospective studies revealed the following: CrAg and Histo Ag prevalence rates were 2.7% (95% CI, 0.1 – 5.3%) and 4.7% (95% CI, 0.7 – 8.7%), respectively, with disease confirmed in all antigen-positive cases; CPA prevalence was 9.7% (95% CI, 5.0 – 14.4%) overall, but was 50% (95% CI, 28 – 72%) in patients with previous PTB and 3.7% (95% CI, 0.5 – 6.9%) in those with no PTB history; the rate of new CPA development was 3.0% and 7.4% at T_1 and T_2, respectively with an overall incidence of 10.7% (90% CI, 1.1 – 20.3%) over 12 months; 5.4% (90% CI, 0.4 – 10.4%) of patients with haematological malignancy met the criteria for IA diagnosis. The retrospective study identified 107 cases of diverse fungal infections, including previously unreported infections.

In conclusion, this thesis demonstrated that SFIs occur at significant rates among the major at-risk patient groups and diverse fungal infections are diagnosed in Ghana. Importantly, access to RDTs and histomolecular assays, improves case detection and diagnostic accuracy respectively. The key priority for further research is to undertake targeted large-scale studies to confirm the reported rates and cost-effectiveness of routine fungal testing.

DECLARATION

The author declares that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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DEDICATION

This thesis is specially dedicated to all patients affected by serious fungal infections in Ghana, that are often misdiagnosed or undiagnosed due to inadequate awareness, low index of suspicion, poor local epidemiology, and insufficient access to essential (fungal) laboratory tests.

ACKNOWLEDGEMENT

First, I am extremely thankful to God for taking me through the entire PhD journey successfully.

I am eternally appreciative of the CARIGEST SA and the University of Manchester, for funding my studentship, research work, trainings, and meetings attendance throughout the PhD duration. I am particularly grateful for the 3-month extension period proffered me due to unavoidable delays caused by the COVID-19 pandemic. These institutions gave life to my dreams of postgraduate education to acquire the relevant knowledge, skills, and network to champion the efforts of improving the practice, training, and research of medical mycology in Ghana. My profound appreciation goes to my supportive supervisors, Prof David Denning, and Dr Chris Kosmidis. This work was a reality because of their dedication, guidance, contributions, understanding and patience. I also thank my PhD advisor, Dr William Whittaker for the emotional support and second opinions regularly proffered. I thank the Administrative Officers of the Division of Evolution, Infection and Genomics and Division of Immunology, Immunity to Infection and Respiratory Medicine particularly Marian Halfpenny, Amanda Connaghan, Jillian Doyle, and Carolyn Glynn for promptly processing my expense refunds, ensuring that I mostly had funds for research expenditure.

My sincere gratitude also goes to the lead local collaborators in Ghana, mainly at the Korle-Bu Teaching Hospital (KBTH) and University of Ghana Medical School (UGMS), Drs Isabella Asamoah, Jane Afriyie- Mensah, Solomon Quayeson and Peter Puplampu; Profs Japheth Opintan and Yvonne Dei-Adomakoh; Dr Frederick Hobenu (37 Military Hospital), and Prof Agyeman Badu Akosa (Pathology Consultants Organization/Ghana Standard Authority) for their support, oversight supervision and commitment to having the various studies successfully implemented in their respective departments or units. Despite their busy clinical practice, academic and administrative work schedules, they opened their offices to me at their least available free time.

I wish to also express my sincere gratitude to the staff that were directly and indirectly involved in the five studies, particularly, those who acted as study coordinators and assistants, namely, Dr Vincent Ganu, Mr John Mensah, and Mesdames Gifty Akoni and Perfect Dzandu (HIV Clinic); Mrs Nelly Eshun, and Messr Charles Domotey, Godfrey Adams, Michael Annor and Emmanuel Tetteh (TB Clinic); Mr Abraham Lamptey and Mrs Rose Offei-Agyeikum (Haematology Day Care); Mr Daniel Potakey (Department of Pathology, KBTH), Mr Leonard Okine (Cellular Pathology Division, Ghana Standard Authority) and Mrs Irene Padi (37 Pathology Laboratory) and Messr Prince Pappoe-Ashong and Isaac Sraku (UGMS Clinical Virology Laboratory). All studies were successfully

implemented simultaneously because you spared time beyond your routine work to assist with the various studies. I also thank the staff of the HIV Laboratory, TB Laboratory and Haematology Research Laboratory (all from the KBTH) and Medical Microbiology Research laboratory and Clinical Virology Laboratory (both of UGMS).

I thank the staff of the following laboratories: Mycology Reference Centre Manchester, Manchester University NHS Foundation Trust; Mycology Laboratory, St. John's Institute of Dermatology at the Guy's and St. Thomas NHS Foundation Trust, London; and Mycology Division, Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh, for honing my practical laboratory knowledge and skills in fungal diagnostics which was helpful for my laboratory work and data analysis. Special thanks go to the Manchester Fungal Infection Group for their direct and indirect contributions to the implementation of this research project especially Dr Sara Gago for her assistance with purchase of laboratory items. Also, I appreciate the Immuno-Mycologics (IMMY) Diagnostics, LDBio Diagnostics and Optimum Imaging Diagnostics (OIDx) for subsidizing the price of the test kits used in the studies. I thank Drs Maria Buitrago and Ana Alastruey Izquierdo (Instituto de Salud Carlos III, Madrid) for their advice on histo-molecular analysis.

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Many thanks to you all!

REFLECTIONS OF THE AUTHOR

The author of this thesis, Bright Katey Ocansey, BSc., graduated from the University of Ghana School of Biomedical and Allied Health Sciences (SBAHS) in 2016 with a Bachelor of Science degree in Medical Laboratory Sciences, as the best graduating student in his programme. The author developed an interest for fungal infections, particularly, laboratory diagnostics, during an undergraduate lecture in the final year by Dr George Antempim Pesewu (Senior Lecturer, SBAHS). The lecturer bemoaned the general absence of significant epidemiological data on fungal infections in Ghana due to the persistent inadequate awareness and insufficient diagnostic incapacity. Dr Pesewu shared an online course on 'Fungal Microscopy and Histology' (sanctioned by the University of Manchester) with the class and encouraged us to take the course. This was the genesis of the propulsion to drive an improvement of the status quo in medical mycology in Ghana. Bright, following the lecture, challenged himself, and developed a very ambitious idea to champion efforts to improve medical mycology education, practice, and research in Ghana. To start this journey, he had attempted to do a project in mycology, specifically, on vulvovaginal candidiasis. A project balloting policy at the Department of Medical Laboratory Sciences, however, attached him to haematology; he, thus, had to undertake his project work in haematology. After failed efforts to change to microbiology/mycology, he succeeded with a swap for histopathology, which generally has a closer link to diagnosis of fungal infections. His dissertation was on 'Histological staining potential of Teak leaf extract as a counterstain (a natural alternative for synthetic eosin)'. Bright continued gradually by posting about fungal infections on his social media platforms and engaging researchers, academicians, and practitioners in the field of laboratory medicine on how to develop a career in medical mycology.

After school, the author registered with the Allied Health Professions Council, Ghana, as a Medical Laboratory Scientist (MLS) and had his one-year mandatory internship at the Family Health Hospital, Accra. He went on to work as an MLS and laboratory demonstrator for microbiology at the Family Health Hospital and Family Health Medical School respectively. He started studying the status of medical mycology in different countries both developed and developing, and how he can form partnerships or collaborations to develop this neglected field of medicine in Ghana. Through such searches online, he chanced upon Prof David Denning, then as President (now immediate past Chief Executive) of the Global Action for Fungal Infections (GAFFI) via LinkedIn. Apparently, Prof Denning was on a similar journey at the global level with a goal of reducing morbidity and mortality from fungal infections by increasing access to essential diagnostics and antifungal drugs, particularly, in low-and middle-income countries. The aftermath of the initial discussion with Prof

Denning resulted in the author leading a survey that estimated the burden of serious fungal infections in Ghana, which highlighted the scarcity of epidemiological data, particularly, on invasive infections. This survey was an eye opener and provided the foundation to stimulate further research to answer the underlining knowledge, epidemiological, diagnostic, and therapeutic gaps. Following this survey, Bright received a funding award from the Fungal Infection Trust, UK, to conduct three gap analysis surveys to appreciate the magnitude of the existing gaps on awareness, diagnostic, and therapeutic capacity. These surveys revealed final year health sciences students (including medical, pharmacy, medical laboratory science students) knew very little about fungal infections, sub-optimal laboratory diagnostic capacity for detecting fungal infections and insufficient access to essential antifungal agents. The inadequate awareness observed among the students was confirmed to be similar among practicing healthcare professionals by the author through his engagements with them, especially during the doctoral research. Additionally, the checks by the author revealed that there were no functional Medical Mycology Departments/Units/Divisions at universities, hospitals, and medical research centres/institutes in Ghana.

To pursue the agenda of improving awareness, diagnosis, and management, particularly, among healthcare practitioners, researchers, and academicians, Bright founded a non-governmental organisation, the Fungal Infections Kare Initiative (FIKI) Ghana (formerly Fungal Infections Care). FIKI Ghana, has since been organising and supporting sensitization programmes on fungal infections. Again, after identifying the laboratory diagnostics challenges, Bright conceived an idea of establishing a fungal laboratory supply start-up to provide easy access to reagents, test kits and culture media for fungal diagnostics. Bright then engaged some laboratory managers about implementation of these fungal tests in their laboratories. The major concerns raised by the managers were inadequate or scanty epidemiological data on the fungal infections which the assays will be used to diagnose, the generally low index of suspicion among clinicians, and the fact that the utility of these assays has not been evaluated in clinical settings in Ghana. The efforts to find answers to these legitimate concerns by exposing Ghanaian clinicians to essential fungal tests, utilizing these assays in clinical settings, and generating local epidemiological data, prompted and motivated the need to undertake this doctoral research. Bright subsequently applied for the PhD Medical Mycology programme with the University of Manchester inspired by Prof Denning and Prof Rita Oladele (Prof Denning's former PhD student from Nigeria). The combined findings of the burden estimate and gap analyses surveys, and the assay implementation concerns largely influenced the concept and design of this research.

Prior to starting his PhD, Bright was a MLS at the New Hope Specialist Hospital, Aflao, Ghana. With the gradual growing interest of healthcare professionals, researchers, and academicians in fungal infections because of the activities of FIKI Ghana, Bright led the formation of the Ghana Medical Mycology Group. The aim was to bring on board healthcare professionals, academics and researchers with similar interests and synchronize various contributions in increasing awareness and improving prevention, diagnosis, treatment, research, and training. The Group was later formally registered as the Ghana Medical Mycology Society and has since been involved in organising educational and training meetings and supporting members to attend international meetings to expand their knowledge in key medical mycology areas. Throughout the PhD period, the author has received specialized fungal diagnostics training from the following laboratories: Mycology Reference Centre Manchester, Manchester University Foundation NHS Trust; Mycology Laboratory, St. John's Institute of Dermatology, Guy's and St. Thomas NHS Foundation Trust, London; Mycology Division, Postgraduate Institute of Medical Education and Research, Chandigarh, India and Loyola Medical Centre, Chicago, USA (through the Fungus Testing Laboratory Consortium).

The author plans to establish a clinical fungal testing laboratory in Ghana to provide diagnostic services and engage in research and training. Recently, a University of Manchester Researcher2Innovator Pitching price was awarded to the author to support the establishment of this start-up after his PhD. The successful operation of this private or public-private laboratory is anticipated to act as the framework that will drive the establishment of mycology laboratory departments/units in public health institutions, at least in tertiary hospitals and research centres. Bright has an available position as a Lecturer at the Department of Medical Microbiology, University of Ghana Medical School. With this opportunity, Bright hopes to establish what will be the first ever functional Medical Mycology Unit at the University and support other universities in establishing same through collaborations.

In addition to the five published papers in this thesis, Bright has authored several publications, contributed to a book chapter, and given oral and poster presentations at international and national conferences, seminars, workshops, and symposiums. The author was recently presented the Best Outstanding Output award at both the Faculty of Biology, Medicine and Health Doctoral Academy and Manchester Doctoral Academy Excellence Awards in June 2023.

THESIS STRUCTURE

This thesis is submitted in the journal format. This format was selected because portions of the literature review and findings from the implemented studies, have been either published or drafted for submission for publication in a peer-reviewed journal. An overview of the structure of the thesis and publications emanating from it, is illustrated by the table below and further described thereafter.

An overview of the thesis structure and publications

Chapter	Chapter description	Publication feature	Paper title	Publication status
1	Introduction and Literature Review	Portions of Literature Review on 'Histoplasmosis'	Histoplasmosis in Africa: Current perspectives, knowledge gaps, and research priorities	Published in *PLOS Neglected Tropical Diseases* (2021)
2	Research framework, methods, ethics, and contributions	N/A	N/A	N/A
3	Study 1: HIV-associated cryptococcosis and histoplasmosis	Published paper from Study 1	Cryptococcal and *Histoplasma* Antigen Screening Among People with Human Immunodeficiency Virus in Ghana and Comparative Analysis of OIDx *Histoplasma* Lateral Flow Assay and IMMY *Histoplasma* Enzyme Immunoassay	Published in *Open Forum Infectious Diseases* (2022)

4	Study 2: Aspergillosis in presumed TB patients	Published paper from Study 2	Chronic pulmonary aspergillosis is common among patients with presumed tuberculosis relapse in Ghana	Published in *Medical Mycology* (2022)
5	Study 3: Aspergillosis in confirmed TB patients receiving anti-TB treatment	Published paper from Study 3	Importance of *Aspergillus*-Specific Antibody Screening for Diagnosis of Chronic Pulmonary Aspergillosis after Tuberculosis Treatment: A Prospective Follow-Up Study in Ghana	Published in *Journal of Fungi* (2022)
6	Study 4: Aspergillosis in haematological malignancy	Published paper from Study 4	Invasive Aspergillosis among Haematological Malignancy Patients in Ghana: A Pilot Study at the National Referral Hospital	Published in the *West African Journal of Medicine* (2023)
7	Study 5: An overview of serious fungal infections diagnosed in Ghana	Drafted manuscript from Study 5 based on partial molecular data	Trend, spectrum, and aetiology of fungal infections in Ghana: a 10-year retrospective study of histopathologically diagnosed cases	Planned to be submitted to *Mycopathologia* for publication after obtaining complete molecular data
8	Summary and Conclusion	N/A	N/A	N/A

Chapter One provides an introduction of SFIs in the global, African, and Ghanaian perspective, reviews the literature on the SFIs of research interest, with a focus on their pathogens, epidemiology, clinical, diagnosis and management details among at-risk groups and finally outlines the rationale and aims of the research. Portions of the literature review on

'Histoplasmosis' was re-written and modified into a suitable publication material and published (Paper 1) in the *PLOS Neglected Tropical Diseases* DOI: 10.1371%2Fjournal.pntd.0010111

Chapter Two details the framework of the research and the methods and materials that were employed in conducting all the studies. The chapter was organized into the five studies and presented by blending the protocols separately approved by the institutional review boards of the hospitals involved in the research in Ghana and the UREC of the University of Manchester in UK. Additionally, the methods of the two terminated two studies are included. Contributions of the external research collaborators were also summarized.

Chapter Three (Paper 2) sums up the first study in this thesis. The study was conducted among newly diagnosed HIV patients and people with HIV returning to care at the national HIV referral clinic, and the patients were screened for cryptococcosis and histoplasmosis using cryptococcal antigen and *Histoplasma* antigen assays respectively, and subsequently, confirmed with culture and histopathology. Secondarily, the performance of a newly introduced, simple *Histoplasma* lateral flow assay (LFA) was compared to the recommended *Histoplasma* antigen enzyme immunoassay (EIA). Histoplasmosis was showed to be probably more common than cryptococcosis among people living with HIV in Ghana and the LFA performed well in detecting histoplasmosis cases. This paper has been published in *Open Forum Infectious Disease*. DOI: 10.1093/ofid/ofac277

Chapter Four (Paper 3) describes the second study, conducted among patients presumed to have pulmonary tuberculosis (PTB). This included those with previous PTB and those without previous PTB, who were being investigated for PTB relapse and new PTB respectively. Patients were recruited while reporting to the national TB referral clinic, for *Mycobacterium tuberculosis* (MTB) testing and were screened for chronic pulmonary aspergillosis (CPA). CPA was found to be common in the recruited patients, particularly, in patients who were being investigated for PTB relapse. The study demonstrated that CPA was a significant cause of PTB-like symptoms in Ghana and that routine *Aspergillus*-specific antibody screening could reduce misdiagnosis and prevent inappropriate treatment. This study has been published in *Medical Mycology*. DOI: 10.1093/mmy/myac063

Chapter Five (Paper 4) contains the third study, which was a 12-month prospective longitudinal study. Patients from the second study above, who had a positive MTB test and placed on anti-TB treatment regimen were identified, followed up and screened for CPA at the end of their treatment and 6 months after completing treatment. At each of the time points, at least one patient in the

cohort developed CPA. Diagnosis was initiated by the detection of *Aspergillus*-specific antibodies, which then indicated performing CT scan for confirmation. This study has been published in the *Journal of Fungi*. DOI: 10.3390/jof9010026

Chapter Six (Paper 5) details the fourth study in this thesis. The study aimed to generate preliminary epidemiological data, particularly, on prevalence rates of invasive aspergillosis (IA) among patients with haematological malignancy managed at the national haematology referral centre in Ghana using the *Aspergillus* galactomannan LFA, culture and CT scan in an international diagnostic algorithm. Probable and possible cases of IA were identified among enrolled patients. The role of the LFA in IA diagnosis, as well as the relationship of IA cases to neutropenia and antifungal prophylaxis were also evaluated. This paper has been published in the *West African Journal of Medicine*. PMID: 37390225/

Chapter Seven (Paper 6) describes the fifth study, which was a retrospective study that evaluated histopathology reports spanning from 2012 to 2021, from three major histopathology laboratories including the national pathology reference laboratory, to obtain a snapshot of the trend and spectrum of fungal infections diagnosed in Ghana. Aetiological agents were confirmed with molecular analysis. Diverse fungal infections were reported including endemic and rare fungal infections, with some cases confirmed by molecular analysis. This study has been written up and will be updated with the completed molecular data and submitted for publication in a peer reviewed journal.

Finally, Chapter Eight summarizes the thesis, inter-relate the research questions with the main findings and reflect on the contributions and impact of the study in Ghana and beyond. The chapter, also describe the limitations of the study and suggest recommendations, future works, and implications for healthcare policy and practice of clinical mycology in Ghana.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

'Improving diagnosis and updating the epidemiology of serious fungal infections in African settings: How fungal rapid diagnostic tests can enhance case detection and histomolecular analysis can ensure accurate diagnosis and ultimately both generating local epidemiology and transforming patient care.'

The assertion above is the foundation of this thesis, which involved: (i) exploring the use of fungal rapid diagnostic tests (RDTs) in relevant diagnostic algorithms to screen and detect key serious fungal infections (SFIs) among patients with common underlying conditions for SFIs, that is, human immunodeficiency virus (HIV), tuberculosis (TB), and haematological malignancy, (ii) reviewing histopathologically-diagnosed fungal infections to evaluate the trend of the number of diagnosed cases and spectrum of infections, and (iii) applying histomolecular analysis to identify the aetiologies of histopathology-diagnosed fungal infections.

1.0 SERIOUS FUNGAL INFECTIONS

Fungi describe a large and diverse group of organisms, belonging to the fungal kingdom and exist as saprophytes in the environment. They fundamentally occur as either a chain of tubular filament-like cells termed hyphae or single cell termed yeast. There are over 100,000 named species of fungi, but only about 500 have been reported to be pathogenic to humans and associated with human fungal infections (1). In relation to initial site of infection and degree of tissue penetration, fungal infections are broadly classified into superficial, mucocutaneous infections, subcutaneous and deep or invasive fungal infections. Superficial and mucocutaneous infections are limited to the outermost layers of the skin, hair, nails, and mucous membranes, while subcutaneous infections affect the dermis, subcutaneous tissues, and adjacent bones. Both classes of fungal infections are usually not life-threatening, but affect the quality of life, and mostly occur in apparently healthy and immunocompetent individuals. Conversely, deep, or invasive fungal infections, describe infections that invade and develop in deep tissues, usually originating from the lungs and spreading to other internal organs, with occasional local extensions and dissemination through cutaneous manifestations. Invasive infections, are often associated with high morbidity and mortality, and usually affect immunocompromised individuals (2,3). Additionally, the term SFIs, are commonly used to describe invasive fungal infections, as well as chronic and complicated non-invasive infections and often exclude superficial and muco-cutaneous infections.

Historically, the majority of SFIs, particularly invasive infections, are not well-recognized, receive little attention, are difficult to diagnose and manage, and are often accompanied with poor clinical outcomes. The major risk factors for SFIs include HIV infection, pulmonary tuberculosis (PTB), chrsonic obstructive pulmonary disease (COPD), asthma, haematological malignancy, transplant (organ and stem cell), intensive care unit (ICU) admissions, and use of immunomodulator or immunosuppressive drugs (Figure 1.1). More recently, the Coronavirus Disease 2019 (COVID-19) pandemic has added up to the ever-expanding list of at-risk patient groups for SFIs (4).

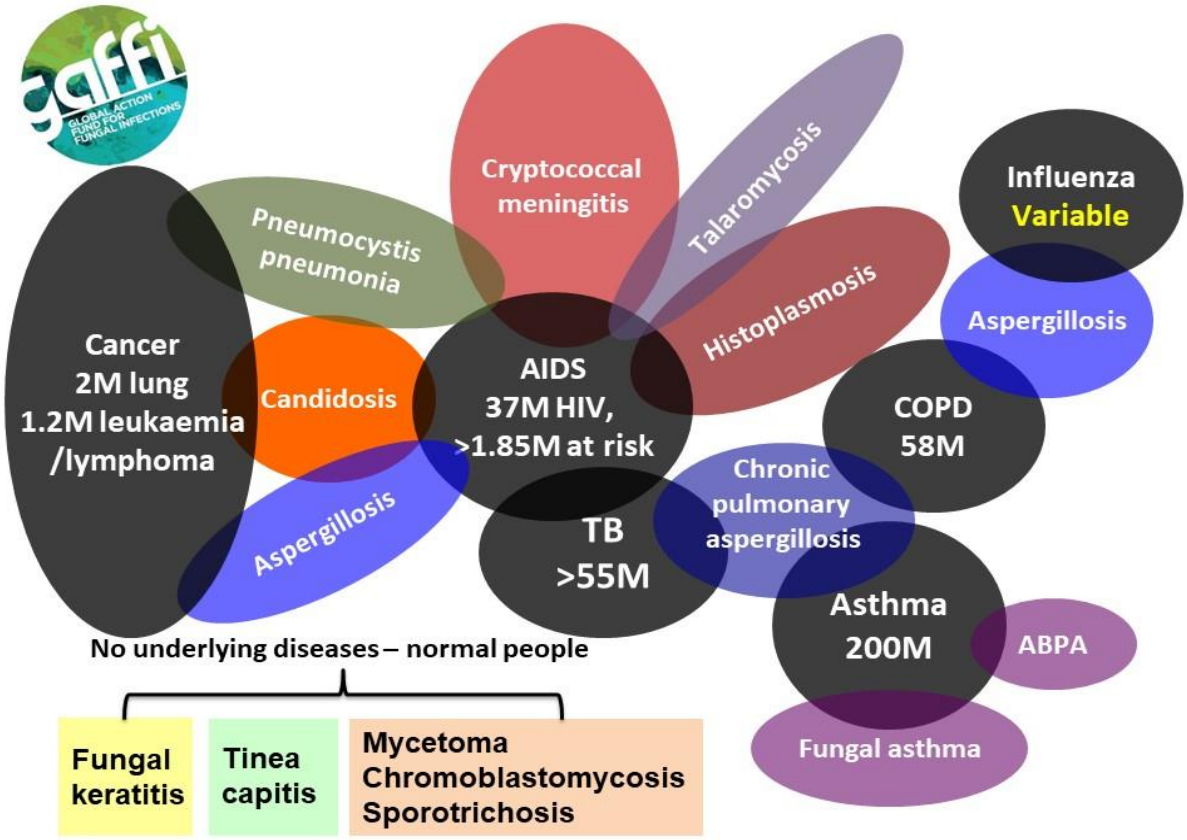


Figure 1.1: Populations and patient groups at-risk for SFIs

(Adapted from GAFFI, https://gaffi.org/why/fungal-disease-frequency/)

However, in the past few decades, SFIs are gaining attention globally due to the expanding number of the at-risk patient groups and the increasing size of their population, as well as improving diagnostics and therapeutics. Fungal diagnostics have greatly evolved and advanced, with introduction and expansion of non-culture- based tests that are associated with high clinical performance, and the anti-fungal pipeline also continues to broaden with the discovery of new classes and novel adjunct treatments are continuously exploited (5–7). Nevertheless, these gains have not been largely realized in resource-limited settings where the at-risk patient group and population is probably highest, and the geography and socio-economic conditions are more

favourable for SFIs. Patients with HIV and PTB are probably the most common at-risk patient groups and are associated with TB-like fungal infections and HIV-related opportunistic fungal infections (Figure 1.1, Table 1.1) (8). To harmonize the gains made in medical mycology and prioritize fungal pathogens globally, the World Health Organisation (WHO) recently, through a worldwide systematic effort, released the first-ever fungal priority pathogens list (FPPL) with the aim of driving research and policy interventions, to strengthen the response to the threat of fungal infections and antifungal resistance. This lead to the categorization of fungal pathogens into critical, high and medium group priorities (Figure 1.2) (9).

Table 1.1: Major at-risk groups and key associated SFIs

At-risk group	**Key Associated SFI**	**Common affected sites**
HIV	Cryptococcosis	Central nervous system (CNS)
	Histoplasmosis	Pulmonary, cutaneous, disseminated
	Pneumocystosis	Pulmonary
	Invasive aspergillosis	Pulmonary, sinonasal
TB	Chronic aspergillosis	Pulmonary
	Acute/chronic histoplasmosis	Pulmonary
	Chronic mucormycosis	Pulmonary
Haematological malignancy	Invasive aspergillosis	Pulmonary, CNS, sinonasal
	Invasive candidiasis	Blood
	Invasive mucormycosis	Pulmonary, CNS, sinonasal
Transplant (haematopoietic stem cell or organ)	Invasive aspergillosis	Pulmonary, CNS, sinonasal
	Invasive candidiasis	Blood
	Invasive mucormycosis	Pulmonary, CNS, sinonasal
ICU admissions/critically ill	Invasive aspergillosis	Pulmonary
	Invasive candidiasis	Blood, abdomen
COVID-19	Invasive aspergillosis	Pulmonary
	Invasive candidiasis	Blood
	Invasive mucormycosis	Sinus, CNS

CNS – central nervous system

(Adapted from Bongomin *et al.* 2017 and Hoenigl *et al*. 2022) (4,10)

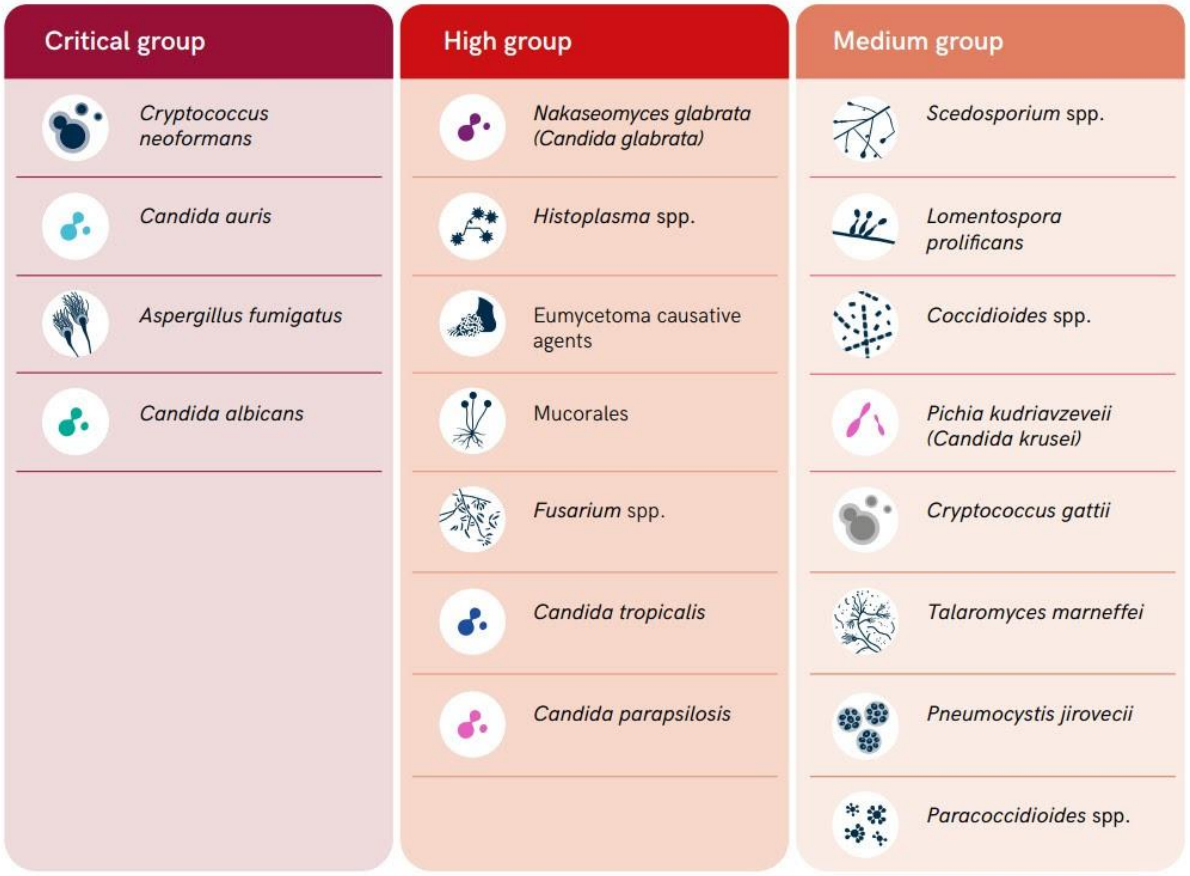


Figure 1.2: The WHO Fungal Priority Pathogens List

(Adapted from WHO, 2022) (9)

Globally, the burden of SFIs is estimated at approximately 11.5 million, resulting in about 1.5 million deaths annually (Figure 1.3) (3). In 2020, the estimated deaths from the top ten SFIs, mainly invasive infections, were comparatively similar to those from TB (11) and many more than malaria (12), highlighting the public health importance of SFIs.

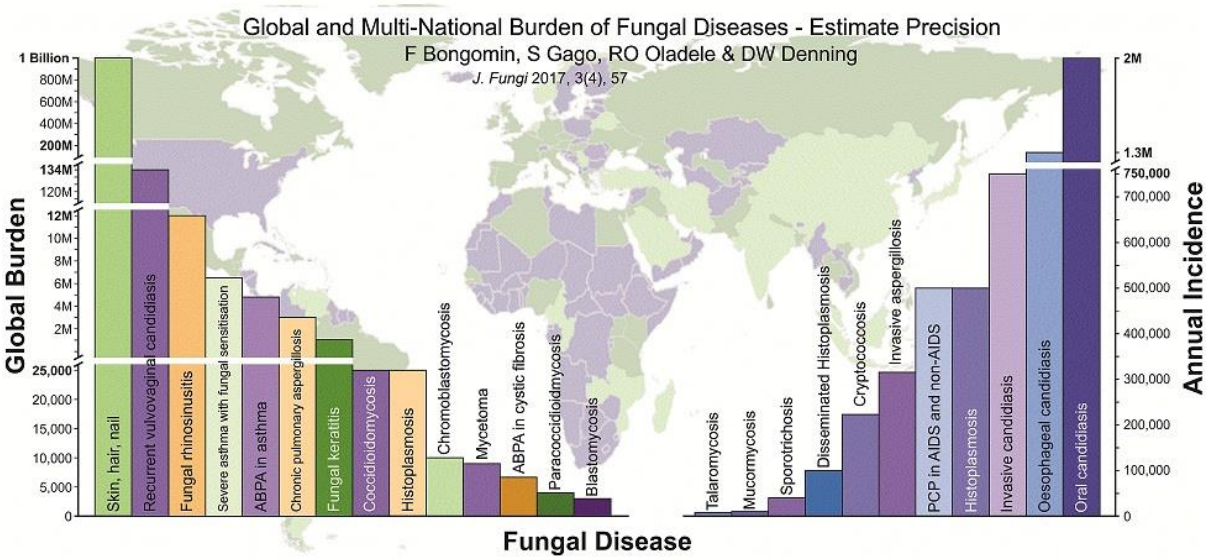


Figure 1.3: Global and Multi-National Burden of Fungal Infections

(Adapted from Bongomin *et al*. 2017)

In Ghana, a 2019 modelling study estimated that about 4% of the population is affected by SFIs, and this includes over 35,000 affected by invasive infections annually (Table 1.2) (13). Unfortunately, the regular association between SFIs and other common underlying conditions or infectious diseases, either through mimicking them or occurring as a co-morbidity or a sequel, places the focus mostly on the underlying conditions and rarely on the fungal infection. Despite the expansion of the group of patients at risk for SFIs, HIV, PTB, and haematological malignancies remain the major drivers of SFIs globally (10). There are many diverse SFIs that may be associated with one or more at-risk groups, but some specific spectrums of SFIs are more frequently reported and connected to a particular at-risk patient group (Table 1.1).

Table 1.2: Estimated Burden of Selected SFIs in Ghana

SFI	Underlying conditions	Incidence (Annual)	Prevalence
Cryptococcal meningitis (CM)	HIV	6,275	
Pneumocystis jirovecii pneumonia (PJP)	HIV	12,610	
Disseminated histoplasmosis (DH)	HIV	724	
Invasive aspergillosis (IA)	Cancer, HIV	1,254	
Mucormycosis	None	58	
Chronic pulmonary aspergillosis (CPA)	TB, COPD		12,620
Candidemia	Critical care, cancer	1,446	
Candida peritonitis	Critical care, post-surgery	217	

COPD – chronic obstructive pulmonary disease

(Adapted from Ocansey *et al*. 2019)

In many African settings, there is a high prevalence of HIV, and thus, the propensity for SFIs is high (10,14). The specific SFIs associated with HIV include, cryptococcal meningitis (CM), *Pneumocystis jirovecii* pneumonia (PJP), invasive aspergillosis (IA) and disseminated histoplasmosis (DH). Except for IA, they are all presently described as advanced HIV disease (AHD) infections. IA was removed from the list in 1984 for being relatively uncommon and because neutropenia, which is the most significant host factor for IA, is not frequent in HIV patients (15,16). Nearly half of HIV deaths are reportedly caused by opportunistic fungal infections, essentially invasive infections (3). Many of these deaths are recorded in resource-limited settings, due to the inadequacy of early HIV diagnosis, and the little attention to SFIs in HIV care with no or limited access to screening or diagnosis facilities. CM is estimated to cause 15-20% of all HIV-related deaths in sub-Saharan Africa (SSA), an observation that has not seen an improvement in tandem with the recent decline of estimated absolute burden of HIV-associated CM (17–19). Detection of cryptococcal antigen (CrAg) in peripheral blood, termed cryptococcal antigenaemia (CA), precedes manifestation of CM (20), and pre-emptive treatment with fluconazole is a crucial intervention to prevent culminating in CM. An average of 7.2% seroprevalence of CA have been reported from studies in SSA and 4.4% globally (19,21). The CrAg lateral flow assay (LFA) is the current mainstay of detection of CA and CM, although isolation of *Cryptococcus* spp from cerebro-spinal fluid (CSF) remains the gold standard. Human histoplasmosis can be described in two forms according to the causative organism. Classical histoplasmosis (CH), which is caused by *H. capsulatum* var. *capsulatum* (hcc), primarily affecting the lungs or sometimes disseminated, has emerged as an important AHD-related infection and is, at least, as common as TB in Latin America (22,23). A review of histoplasmosis in Africa, revealed substantial case reports of CH, although often neglected on the continent and believed to be under-diagnosed (24,25). CH is frequently linked to PTB and even surpassed the burden of the latter among HIV patients in areas of Latin America (26). Two recent studies from Nigeria suggest that histoplasmosis probably also has a link with TB in SSA (27). In contrast to CH, African histoplasmosis (AH) caused by *H. capsulatum* var. *duboisii* (hcd) is sparsely linked to HIV, and seldom affects the lungs, but rather, is characterized by lesions on skin, subcutaneous tissues, lymph nodes, and bones. The cases of AH reported in HIV are mostly disseminated and associated with poor clinical outcome (28–30). Until recently, the diagnosis of histoplasmosis has mainly relied on conventional techniques (direct microscopy, histopathology, and culture). Detection of *Histoplasma* antigen, particularly, on enzyme immunoassays (EIA) platforms, has now strongly gained relevance in the diagnosis of histoplasmosis and incorporated in the WHO Essential Diagnostics List (EDL) and diagnostic algorithms (26,31,32).

TB, particularly, PTB, is a major underlying condition commonly associated with a group of fungal lung infections, mainly chronic pulmonary forms of aspergillosis. Other forms include pulmonary histoplasmosis, cryptococcosis and mucormycosis. These fungal lung infections may often mimic PTB, and often described as TB-like fungal infections. Chronic pulmonary aspergillosis (CPA) is the commonest and is largely associated with PTB, especially, in high TB burden countries (33–37). CPA often occurs as a post-TB treatment complication in patients who develop cavities (33,38,39). However, it is also reported to occasionally occur in patients with active TB infection or during anti-TB treatment (40,41). A study in Uganda revealed that CPA complicates 4.9–6.3% of all treated PTB cases (39). Another study in Nigeria estimated that about 80% of CPA cases occurred in smear-negative PTB patients with treatment failure (36). Increased levels of *Aspergillus*-specific antibodies is key in the diagnosis of CPA and automated EIA platforms such as ImmunoCAP or Immulite are traditionally considered the superior (42,43).

Patients with haematological malignancies represent another significant at-risk patient group for SFIs, with more epidemiological, diagnosis, and management data emanating from developed countries. The clinical outcomes of these patients are negatively affected by SFIs. IA is the commonest SFI among these patients, followed by invasive mucormycosis and candidiasis (44). The major host factor for IA is the depth and duration of neutropenia (45,46). IA is acquired by inhalation of spores of *Aspergillus* species, and over 70% constitute pulmonary disease (47). Globally, the estimated incidence of IA among individuals with haematological malignancy is between 4% and11% (48). The case fatality rate attributed to IA can be as high as 80% (49). The very few studies from Africa, are mostly from North Africa (45,50). Implementing current diagnostic algorithms in many African settings may be difficult. For example, the *Aspergillus* galactomannan (GM) EIA used in aiding IA diagnosis is not accessible (26,51–53).

Fortunately, the landscape of antigen-antibody detection for SFIs is moving from EIAs to immunochromatographic RDTs in the form of LFA or lateral flow device (LFD). Comparatively, these RDT platforms are suitable and easy to implement in resource-limited settings. Indeed, many of the fungal RDTs meet the WHO's ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users) criteria. They are cheap, have considerable performance, can be used as point-of-care tests, and deliver rapid results. They also have stable reagents, and the test kits can be stored at room temperature. Additionally, they have a long shelf-life (up to 2 years), and are easy to perform, with no need for processing of samples or specialized laboratory equipment.

Presently, the clinical use of the fungal RDTs is limited in several African settings. This could be an attributable factor for the scarcity of epidemiology data on SFIs in these settings where diagnosis of SFIs is largely driven by histopathological investigations. Histopathology, particularly, plays a relevant role when the availability and accessibility of other techniques (such as fungal culture, antigen-antibody detection and molecular analysis) are inadequate and in many cases, is the only available technique utilized in aiding SFI diagnosis (52,54,55). Indeed, histopathology is critical to defining a proven case of invasive fungal infections according to several international guidelines (26). Unfortunately, most SFIs are diagnosed post-mortem, as even in developed countries, pre-mortem diagnosis is difficult (56–58).

Analysing laboratory data is a common approach employed in evaluating the epidemiology of infectious diseases. The role of histopathological laboratory data analysis in this regard for SFIs, has been occasionally exploited and reveals relevant epidemiological data, mostly in Africa (probably due to absence of other laboratory methods for SFI diagnosis) (59–63). Additionally, these analyses allow for the assessment of the index of suspicion of clinicians requesting histopathological investigations (63). For instance, reviewing the epidemiological data on SFIs in Ghana, reveals case reports and case series mainly diagnosed by histopathology (Table 1.3).

Table 1.3: Overview of studies on SFIs in Ghana

No.	Disease type	Study type	Laboratory method	Reference
1	Pulmonary aspergillosis (chronic)	Case report	None (only medical imaging, CT scan)	(64)
2	Small intestine mucormycosis	Case report	Histopathology /post- mortem	(65)
3	Disseminated cryptococcosis	Case report	Histopathology /post- mortem	(66)
4	Disseminated aspergillosis	Case report	Histopathology/post- mortem	(67)
5	Cryptococcal antigenemia	Cross-sectional (*n* = 92)	Latex agglutination	(68)
6	Fungal keratitis	Cross-sectional (*n* = 199)	Direct microscopy, culture	(69)
7	Fungal keratitis	Cross-sectional (*n* = 290)	Direct microscopy, culture	(70)
8	Cryptococcal meningitis	Cross-sectional (*n* = 53)	Direct microscopy, culture, CrAg LFA	(71)
9	Cryptococcal meningitis	Cross-sectional (*n* = 84)	Direct microscopy, culture, CrAg LFA	(72)
10	Cryptococcal meningitis	Cross-sectional (*n* = 4955)	Culture	(73)
11	Basidiobolomycosis	Case report	Histopathology	(74)

12	Basidiobolomycosis	Case series (*n* = 4)	Histopathology	(75)
13	African histoplasmosis	Case report	Histopathology	(76)

CT – computed tomography

Ghana is a tropical country in SSA, located in the West African sub-region between Latitudes 4" and 11" N of the equator. The country shares border with Togo in the east, Burkina Faso in the north, Cote D'Ivoire in the west, and the south bounded by the Gulf of Guinea. Ghana covers a land area of 238,533 km² and has 16 administrative regions. Accra is the capital city and situated in the Greater Accra Region, which is the most populous. The 2021 National Population and Housing Census reported the Ghanaian population to be 30.8 million, with 58.2% adults (18 years and older) and 50.7% females. The rural population was 43.3%. The recent (2019) gross domestic product (GDP) per capita for Ghana according to the World Bank is US$ 2,363. The official language is English, and the main religions are Christianity (71.2%) and Islam (17.6%) (77). Ghana's healthcare system is administered by the Ministry of Health and Ghana Health Service. The health expenditure per capita for Ghana was US$ 75 in 2019. The healthcare services are funded by the Government, internal generated fund, and international donors. About 59% of the population is insured by either the public national health insurance scheme (NHIS) or private health insurance (78). The healthcare system has five levels of providers: 1) Community-based Health Planning and Service compounds or health posts, 2) health centres and clinics, 3) district hospitals, 4) regional hospitals, and 5) tertiary or quaternary hospitals. There were about 350, 000 people living with HIV and about 10, 000 HIV-related deaths recorded in 2021. In the same year, the total TB incidence was 45,000 and 13,401 total new and relapse notifications. The notified cases consist of 93% pulmonary and 75% bacteriologically confirmed cases. Deaths from TB were reported to be 15,700 (79). The estimated annual new cancer cases in 2021 were 24,009 (including about 2,000 haematological malignancy cases) and 15,802 deaths (80).

Generally, in Ghana, awareness on SFIs is low and the index of suspicion among clinicians of fungal infection is insufficient. There is limited training in the diagnosis and management of SFIs with much of the focus on superficial and mucocutaneous fungal infections, which have some epidemiological data, and their diagnostic and therapeutic tools are mostly available and accessible across Ghana (81). Meanwhile, presently the essential fungal diagnostics and antifungal drugs, and expertise in the diagnosis and management for SFIs are very inadequate (52,54,81). Specifically, direct examination of clinical specimens is performed at almost all levels of laboratory, although many rural laboratories lack reagents such as potassium hydroxide (KOH). Skin, hair, nails, and vaginal samples are all often analysed where reagents mainly KOH are available, whilst

CSF examination with India ink is occasionally performed. Some key specimens commonly analysed for fungi such as skin biopsy, bronchoalveolar lavage (BAL), corneal scraping and lumbar puncture are not routinely collected or performed except at tertiary hospitals, due to cost and insufficient trained personnel (54,81). Histopathology and fungal culture are mostly restricted to tertiary and some private hospitals and medical laboratories. This is mainly due to insufficient laboratory equipment and infrastructure and specifically lack of technical expertise for fungal culture. For instance, processing of specimens and handling of fungal growth are conducted under at least biosafety level (BSL)-2 cabinets which were not available in many laboratories until recently due to the COVID-19 laboratory testing investment. Testing for CD4 T lymphocytes or helper T cells (CD4 cells) counts among HIV are important in identifying relevant candidates to screen them for HIV-associated SFIs. However, CD4 cells counts tests are presently not routinely available in public health facilities but from private health facilities or laboratories. Regarding antigen-antibody detection assays, critical assays listed on the WHO EDL such as the CrAg, *Histoplasma* antigen, *Aspergillus* antigen, *Aspergillus* antibody tests are all almost not available in Ghana except in few private medical laboratories, where these tests are sent for analysis at their partner laboratories outside Ghana, in South Africa, United Kingdom, India and Germany (81). Advanced medical imaging such as CT and magnetic resonance imaging (MRI) scans plays a critical role in the diagnosis of SFIs, especially when the lungs, CNS, and sinonasal areas are affected. Although both are available in several secondary and tertiary health facilities, they may be expensive for most patients and not covered by the NHIS. The consequential effects of these diagnostic challenges are that, essential antifungal drugs for SFIs, including liposomal amphotericin B, flucytosine, posaconazole, are mostly unavailable or inaccessible by patients who may need them (81).

1.1 ASPERGILLOSIS

1.1.1 *Aspergillus* and Aspergillosis

Aspergillus was discovered by Italian biologist Pier Antonio Micheli in 1729, describing it as a spore-bearing structure (82). *Aspergillus* is a fungal genus consisting of ubiquitous organisms that causes an infection termed aspergillosis. There are over 446 known species of *Aspergillus*, but only a few are reported to cause disease in humans (83). *Aspergillus fumigatus* is the commonest pathogen, but other species such as *A. flavus, A. niger, A. terreus,* and *A. nidulans* are also frequently reported in cases of aspergillosis (84). There is a broad genetic variation among species of *Aspergillus* (85). *Aspergillus* produce conidia or spores as part of asexual reproduction that are released in the environment and easily inhaled by humans (86). *Aspergillus* can be found in a wide range of

environmental niches such as soil, dust, decaying vegetation, and compost globally. Agricultural activities, domestic cleaning and construction works may disperse and contaminate the immediate environment with *Aspergillus* conidia. Characteristically, *Aspergillus spp* have the ability to survive and grow in several environmental conditions and the small size of their spores (2-3 µm) enables penetration into tissues after inhalation (87,88). Species of *Aspergillus* usually possess characteristic morphologies that allow for their identification when grown on media by macroscopic and microscopic analysis. However, advanced molecular techniques may be required to identify rare species. In a study by Alcazar-Fuoli *et al*. (89), deoxyribonucleic acid (DNA) sequencing was used to correctly identify some uncommon species previously misidentified as *A. fumigatus* by conventional methods. The correctly identified species had antifungal susceptibility patterns different from *A. fumigatus*.

1.1.2 Clinical spectrum of Aspergillosis

The first case of aspergillosis in a human was reported in 1789 from France and affected the maxillary sinus (90). Aspergillosis constitutes a spectrum of disease emanating from the interaction between the *Aspergillus* spores and the host. The immunological status of the host is a critical contributor to the response to *Aspergillus* spp and determines subsequent disease development, clinical characteristics, course and prognosis (Figure 1.4) (91). Among severe immunocompromised hosts, acute, lethal, and invasive forms of aspergillosis are experienced while immunocompetent hosts present with chronic or non-invasive forms. On the other hand, allergic reactions may occur in people with identified hypersensitivity when exposed to *Aspergillus* spores (92). Beyond immunological status, genetic factors are reported to contribute to the susceptibility to different spectrums of aspergillosis (93–95). The most common site of aspergillosis is the lungs and may manifest as either allergic reactions, passive colonization, germination or angioinvasion (96). Pulmonary aspergillosis is thus classified into allergic bronchopulmonary aspergillosis (ABPA), severe asthma with fungal sensitization (SAFS) caused by *Aspergillus* spp, CPA and invasive pulmonary aspergillosis (IPA). Nevertheless, these forms of infection and reaction mechanisms to *Aspergillus* can occasionally occur in other body sites such as the paranasal sinuses and also disseminate from the lungs to many extra-pulmonary sites including brain, heart, kidney, liver, spleen, thyroid (97,98).

ABPA and SAFS collectively termed as 'fungal asthma', are relatively rare conditions that represent the allergic inflammatory reactions to *Aspergillus* spp colonization or exposure. They mainly complicate poorly controlled asthma and cystic fibrosis (92). SAFS can result in worsening of asthma exacerbations while ABPA may deteriorate and result in chronic lung disease. CPA is a

slow, progressive and destructive lung disease that mimics and complicates respiratory disorders that cause structural defects of the lungs, such as cavities (99). IA is a life-threatening opportunistic disease usually associated with severe immunosuppression and caused by angioinvasion of tissue by *Aspergillus*. IA most frequently manifests as IPA but may spread from the lungs to other internal organs and cause tissue damage. There is another syndrome, subacute invasive aspergillosis (SAIA) (previously chronic necrotizing pulmonary aspergillosis (CNPA)), that appears to be an intersection of CPA and invasive pulmonary aspergillosis (IPA), by demonstrating both features of CPA and IPA usually occurring in patients with mild to moderate immunosuppression, including patients with diabetes mellitus and those on corticosteroids (100). SAIA is a locally invasive disease, presenting as a slowly progressive cavitary lung disease associated with chronic respiratory symptoms.

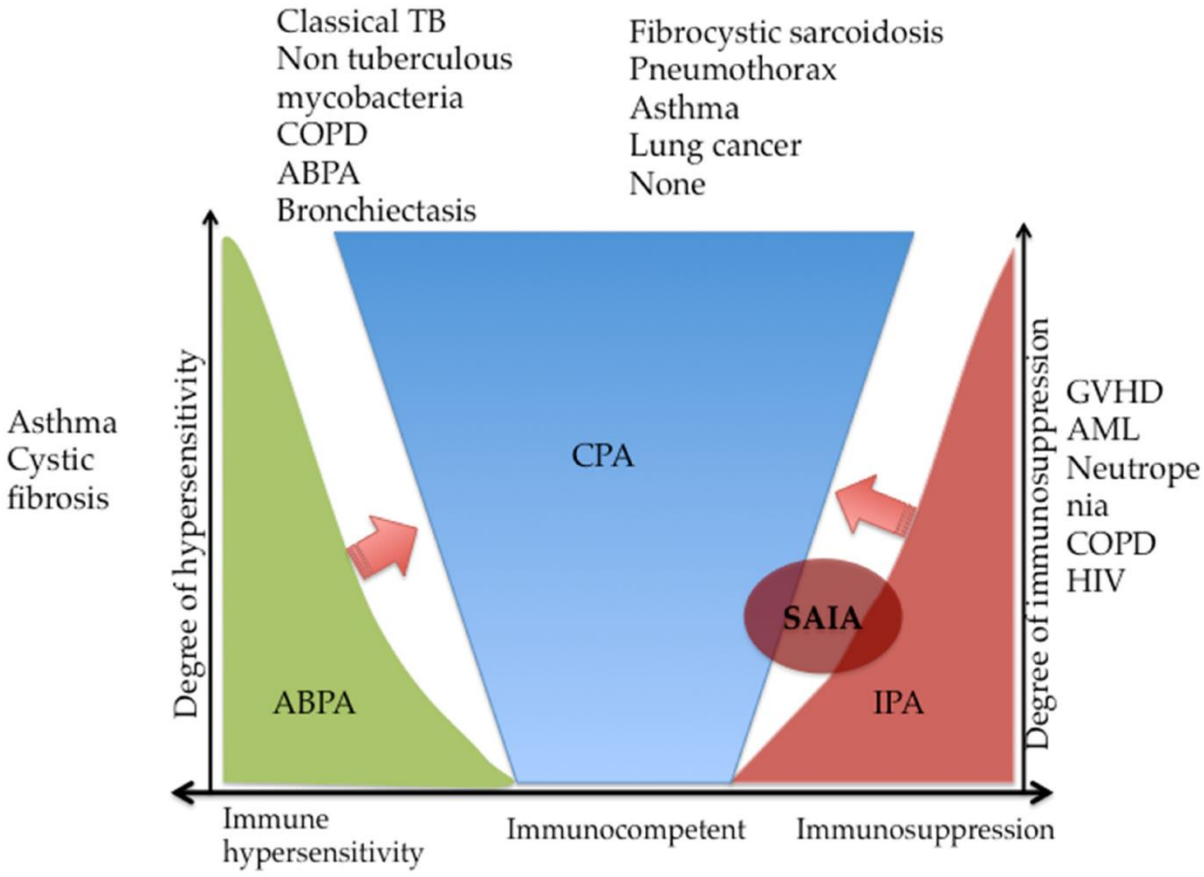


Figure 1.4: Spectrum of Aspergillosis and associated immunological status

(Adapted from Bongomin *et al*. 2020)

1.1.3 Chronic pulmonary aspergillosis (CPA)

1.1.3.1 TB as an underlying condition for CPA

Previous or concurrent underlying chronic respiratory disease is common among CPA patients (101,102). The respiratory disorders associated with CPA are PTB, sarcoidosis, COPD, pneumothorax, non-tuberculous mycobacterial lung disease (NTMLD) and lung cancer. In many countries, particularly in LMICs, PTB is the most common disorder that precedes CPA (33). In fact, several CPA studies have determined PTB as the major underlying condition, in up to 93% of CPA patients (103–105). Presence of cavities in the lung is a fundamental risk factor for the occurrence of CPA. Conditions such as cysts, abscess, infarction, fibrosis, carcinoma, irradiation and NTM infection of the lung are possible causes of lung cavities that may pave the way for the development of CPA (96,100). PTB has a huge burden in many African countries, and this signals a possible significant number of CPA patients. Despite the gradual dwindling prevalence of PTB cases, the increasing number of TB patients on treatment remains a risk factor for CPA. Moreover, data on CPA contributions from the other pulmonary diseases, such as COPD, asthma, ABPA, sarcoidosis and prior pneumothorax are limited and not widely studied in resource-limited settings. Notwithstanding, among the patients diagnosed with CPA, there are about 2-10%, who may have no recognized underlying lung disease (106).

1.1.3.2 Epidemiology of CPA

CPA is estimated to affect more than 3 million people globally having different underlying pulmonary conditions (33). Out of the total burden, PTB is reported to be the underlying condition in about 1.2 million cases. There is a varied frequency in the development of cavities post-PTB treatment, different burdens of underlying respiratory diseases, based on geographical location. However, the highest estimates of CPA developed after PTB are from resource-limited settings, in countries with high TB burden (107). Different rates of CPA have been reported among PTB patients in several countries (Figure 1.5). In a large United Kingdom survey, involving 544 patients who had developed a residual cavity one year post-TB treatment, 36% had *Aspergillus* antibodies and 22% showed aspergillomas on chest x-ray (CXR) after 3 years (108,109). This study has played a major influence in previous efforts to estimate the burden of CPA in several countries. The parameter for estimating CPA is evolving as more studies are conducted and new data furnished. For example, in the recent re-estimation of the burden of CPA in India, there was an increase in the prevalence by five-fold from 290,000 to 1.5 million using updated research findings (37,110). Until recently, CPA in Africa consisted of a few case reports and series emanating from Ghana (64), Ivory Coast (111), Ethiopia (112), Senegal (113), Nigeria (114), and Tanzania (115). In recent times,

prospective studies on CPA in relation to PTB have been implemented and reported varying prevalence rates in South African (9.9%), Uganda (4.7%, 19.8%), and recently, Nigeria (8.5%) (36,39,41,116).

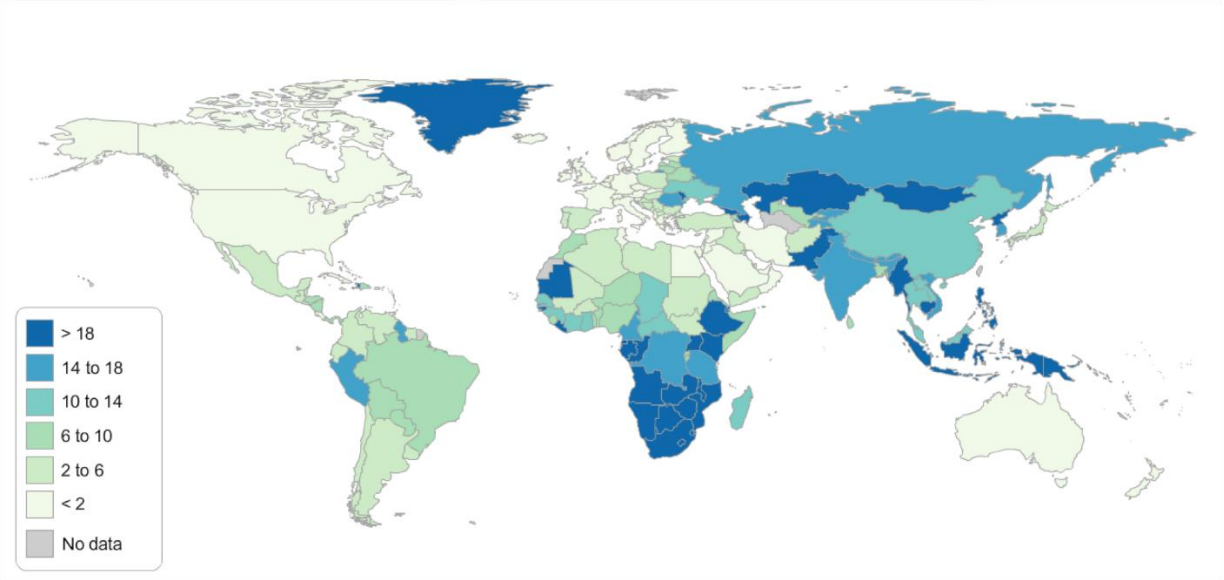


Figure 1.5: Prevalence of CPA across the globe

(Adapted from GAFFI, https://gaffi.org/why/burden-of-disease-maps/)

1.1.3.3 Clinical features of CPA

CPA manifests in different forms and classification has faced challenges in the past. The present recognized subtypes are chronic cavitary pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA), simple aspergilloma (SA) and aspergillus nodules (AN) (117). CCPA presents with one or more cavities, with or without aspergilloma(s). Aspergilloma consists of mass (*Aspergillus*) hyphae in a complex biofilm appearing as a circular entity in lung cavities and mostly occurs in the late stage of CPA. In a few cases, aspergilloma may occur isolated with few symptoms but without evidence of progression and known as SA. Demonstration of single or multiple nodules with or without cavitation usually affecting the upper lobes and non-calcifying are characteristic of AN. It is crucial to rule out malignancy in making a definite diagnosis of AN (118). Undiagnosed and untreated CCPA, may progress to extensive pulmonary fibrosis termed CFPA (119). CFPA shares similar features with CCPA plus pulmonary fibrosis, which may be progressively destructive. CNPA, also known as SAIA, is now managed as a form of IPA (106). CCPA is the commonest manifestation of CPA while AN is presumably the least common (118).

Patients with CPA generally exhibit signs and symptoms, but a few may be asymptomatic and only show radiological progression. Haemoptysis is a frequent sign in CPA and develops in

approximately 12–43% of patients (96,120,121). Haemoptysis may vary from scanty blood streaking sputum to a copious bloody sputum. Haemoptysis occurs in other respiratory diseases such as PTB but usually not severe. Persistent mild chest pain is another characteristic feature in CPA, accompanied by discomfort, which is observed in up to 37% of CPA patients (122). Weight loss and fatigue are also common, but not widespread. Cough (mainly productive) and dyspnoea are common but are not adequate to differentiate CPA from other respiratory disorders. Fever is rare in CPA and, if present, may represent a coexisting disorder. Occasionally patients are reported to experience day or night sweats but these are not discriminatory (122). The duration of disease required to define CPA has been reported to be between 1–6 months, usually 3 months distinguishing it from IPA (96,101). The average 3-month duration may be noted by changes in or worsening of clinical signs and symptoms or by progressive abnormal findings on imaging or laboratory studies.

1.1.3.4 Diagnosis of CPA

The diagnosis of CPA requires an extensive assessment of clinical features, laboratory, and radiology studies. Diagnostic guidelines for CPA have been published by the European Society for Clinical Microbiology and Infectious Diseases and European Respiratory Society (119) and Infectious Diseases Society of America (123), emphasizing the central role of advanced imaging (CT scan and MRI) (117) and serologic testing for *Aspergillus* infection (119). Unfortunately, these diagnostics are infrequently available in many resource-limited settings. Recently, the Global Action for Fungal Infections (GAFFI) developed a modified diagnostic algorithm of CPA suitable for research and clinical care in resource-limited settings to promote research so that critical data will be available to inform policy and practice, including surveillance, and to enable individualized clinical care for optimal patient management (122) (Figure 1.6).

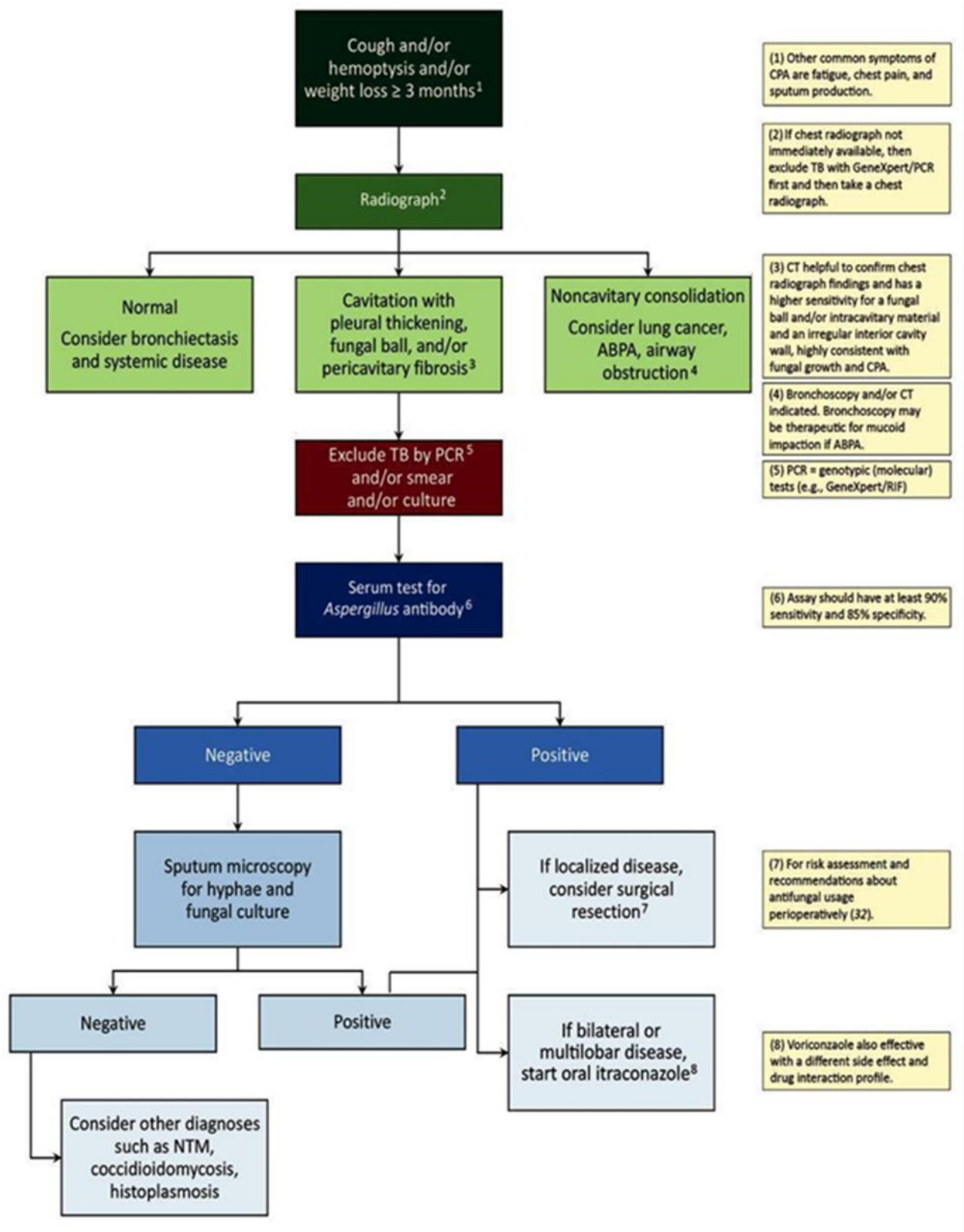


Figure 1.6: GAFFI's Diagnostic Algorithm for CPA in resource-limited settings

1.1.3.5 Radiological investigations

CXR or chest radiographs and CT scans provide important information related to cavity formation, fibrosis or pleural thickening in the lungs which are associated with CPA (122,124). The common radiologic appearances and CT scans findings compatible with CPA are provided above in the diagnostic algorithm (Figure1.6). These findings may simply be suggestive of CPA and are not specific to CPA. Many of these findings are equally observed in other pulmonary conditions including PTB and NTMLD. CXR, may however have a limited role in the final diagnosis of CPA, as some lung cavities demonstrated on CT scans are missed on CXR. Radiologists must be specifically trained to be aware of CPA and its interplay with any underlying condition present to be able to make an appropriate report to aid in the diagnosis of CPA.

1.1.3.6 Laboratory investigations

The role of the laboratory in aiding diagnosis of CPA, is to establish the presence or exposure to *Aspergillus* in relevant clinical specimens from the respiratory tract. This can involve techniques such as direct microscopy, culture, antibody detection and/or nucleic acid amplification by polymerase chain reaction (PCR). Direct microscopy of sputum from CPA patients may identify hyphae morphologically compatible with *Aspergillus* spp, usually located in the lung cavity (43). Culture can be helpful but positivity from sputum samples may be less sensitive (41%-81%) (96,104,125,126). Increasing inoculation volume, described as a high volume culture has been found to improve positivity in a recent study (127). Positive culture results are important, enabling species identification and antifungal susceptibility testing (AFST), albeit false-positive cultures do occur due to laboratory contamination or colonization. The demonstration of an immune response to *Aspergillus* by detection of serum *Aspergillus*-specific antibodies is indispensable in the laboratory confirmation of CPA (42). *Aspergillus*-specific Immunoglobulin (Ig) G antibodies are elevated between 80-92% of CPA patients with cavitary disease (122,128) and about 60% of patients with AN (106). However, elevated level of *Aspergillus*-specific IgG may also be noted with other conditions, including *Aspergillus* rhinosinusitis, ABPA, *Aspergillus* bronchitis, SAIA, recovery from IA, and community-acquired *Aspergillus* pneumonia. In view of this, corroboration with signs, symptoms and imaging finding is required to confirm diagnosis of CPA. It is noteworthy that the available assays are specific for *A. fumigatus* and may not detect antibodies of other species of *Aspergillus* that occasionally cause CPA including *A. flavus, A. terreus, and A. niger.* Also, some CPA patients have mild immunosuppression and may not demonstrate a detectable *Aspergillus*-specific IgG response. PCR detection of *Aspergillus* spp. is significantly more sensitive than routine culture and has been established to be useful in CPA (127). Recently, a new approach, pyrosequencing,

offered an additional advantage of detecting azole resistance directly from *Aspergillus* culture isolate. Unfortunately, PCR for *Aspergillus* spp. is not routinely performed in most medical centres worldwide, particularly in LMICs.

1.1.3.7 Role of Rapid Diagnostic Tests

Aspergillus-specific antibody testing has revolved in the past few decades, ranging from precipitin to automated EIA, and recently immunochromatographic test (ICT) in the form of LFA or LFD. The precipitin tests are generally complicated, less sensitive, and unstandardized (42). EIA has been the preferred and recommended technique for many years and has the advantage of quantitative measurement of antibodies. Automated EIA such as ImmunoCAP and Immunolite platforms are known to have the best performances for the diagnosis of CPA, both recording a sensitivity and specificity of 96% and 98% (Figure 1.7) (43). Unfortunately, these platforms are cost and resource intensive and are difficult to adopt in many laboratories in several resource-limited settings.

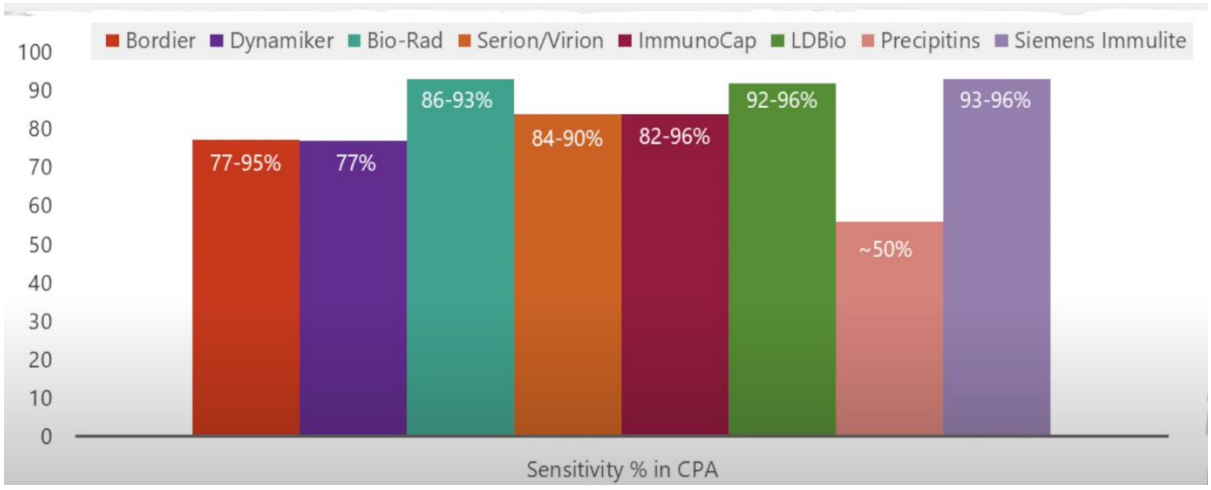


Figure 1.7: Sensitivity comparison of *Aspergillus*-specific IgG testing techniques in CPA

(Bordier, Dynamiker, Bio-Rad and Serion/Virion- Manual ELISA, ImmunoCAP and Immulite-Automated ELISA, LDBio- LFD, Precipitins/Microgen- Precipitation) (Adapted from Page *et al*. 2020)

The recent introduction of *Aspergillus*-specific antibody RDTs are anticipated to be game-changing in simplifying and encouraging the diagnosis and research on CPA in resource-limited settings where PTB burden is extremely high. Remarkedly so, the past few years have seen improvements in the clinical utility and evaluation studies from these settings. A few assays have been commercialised and evaluated, reporting high performance in supporting serological diagnosis of CPA (Table 1.4). One of these assays is the LDBio *Aspergillus*-specific antibody ICT, which was first evaluated in a concurrent prospective single-centre and retrospective multicentre study in France, reporting sensitivity of 88.9% and specificity of 96.3% (129). In another evaluation study on the

stored sera of 154 CPA patients and control patients in the United Kingdom and compared with the recommended ImmunoCAP *Aspergillus*-specific IgG titres, revealed a higher performance of the LFA, with sensitivity and specificity of 91.6% and 98% (128). In another study in Indonesia, evaluating the performance of the LDBio *Aspergillus*-specific antibody LFD in the diagnosis of CPA among symptomatic HIV negative and GeneXpert negative patients following the completion of anti-TB regimen reported a sensitivity of 80% and specificity of 70% (35). In a study analysing the efficacy of the LDBio *Aspergillus*-specific antibody LFD for CPA diagnosis in India, it was noted that, the assay had improved performance in patients with a previous history of PTB compared to those without prior PTB (130). However, in a recent study in Uganda screening for CPA in PTB patients with persisting symptoms after 2 months of anti-TB regimen, a lower sensitivity of 31.3% was reported, probably due to *A. niger* isolates frequently isolated. These assays have also been employed in real life clinical settings in Uganda (131). Overall, the meta-analysis of evaluation studies on LDBio *Aspergillus*-specific antibody LFD shows an overall sensitivity of 90% and specificity of 91% (132). There is also a newly introduced QuickIgG *Aspergillus* IgG Ab LFA based on immunofluorescence from Dynamiker Biotechnology Limited, with in-house evaluation reports promising performance (133). Era-Biology has also released its *Aspergillus* IgG detection K-set which also uses a gold colloidal ICT technology. However, the clinical utility and evaluations studies are limited and not extensively investigated for these last two assays.

Table 1.4: Overview of *Aspergillus*-specific IgG rapid diagnostic tests for diagnosis of CPA

Assay	Country	Patient group	Case Type	Sample	Sensitivity	Specificity	Ref
LDBio	France	-	Proven	Serum, plasma	88.9%	96.3%	(129)
LDBio	United Kingdom	PTB	Proven	Serum	91.6%	98%	(128)
LDBio	Indonesia	PTB	Proven	Serum	80%	70%	(35)
LDBio	Uganda	PTB	Proven, probable	Serum	31.3%		(41)
LDBio	India	PTB	Proven	Serum	86.7%	90%	(132)
LDBio	India	-	Proven Prior TB No prior TB	Serum	 73.3% 67.6%	 83.9% 81%	(130)

LDBio	Indonesia	PTB	Proven	Serum	85%	72.1%	(134)
Quic IgG*	China	-	-	Serum	87.3%	91.3%	(133)
FungiXpert*	-	-	-	Serum	-	-	(135)

*Internal evaluation - Not provided

1.1.3.8 Management of CPA

The form of CPA is critical to selecting the best management option. The ESCMID/ERS recommends that progressive and symptomatic CCPA should be treated with 4-6 months of oral triazole as a standard of care (119). Itraconazole is the first line treatment, followed by voriconazole or posaconazole in lieu of treatment failure or intolerance (119,136–138). Intravenous liposomal amphotericin B or echinocandin alone or with combination with oral triazole therapy are other alternatives suggested for CPA treatment. Patients usually take weeks to months to respond, up to 9 months. The optimal duration of antifungal therapy is not clearly established due to the occurrence of relapse. AFST is recommended when resistance is suspected but in lieu of the inconsistencies between *in vitro* AFST and clinical response. Adjunctive interferon-γ therapy has reported some success but there is presently limited evidence to enable a strong recommendation (96,119,139). Patients with SA are mostly successfully managed with surgical resection in contrast to other subtypes of CPA. The surgical techniques may include bronchoscopic removal, lobectomy, video-assisted thoracic surgery, and thoracoplasty (121,140–142).

1.1.4 Invasive Aspergillosis (IA)

1.1.4.1 Epidemiology of IA

There are an estimated 200,000 cases of IA reported annually in the world, with varying prevalence across continents (Figure 1.8) (3). A report from a multicentre registry in Europe estimated 5% prevalence in patients with acute leukaemia and mortality rates of 50 to 100% (143). There is very limited data on IA from Africa. A recent systematic review of IA in Africa revealed prevalence can be up to 27% in some settings and fatality rate is over 60% (144). The few epidemiological studies were noted to come from Northern Africa, particularly, Tunisia. This is probably due to the considerable access to *Aspergillus* antigen testing in the North African sub-region (51). In two of such detailed studies in Tunisia, one study among 105 neutropenic haematology patients, 15.2% were diagnosed with probable aspergillosis and the other study demonstrated 7.5% of proven IA and 8.2% cases of probable IA among haematological patients with at least one neutropenia episode (using the European Organisation for Research and Treatment of Cancer/National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria) (45,50).

The study also revealed that *A. niger* was possibly responsible for 35% infections, contrasting the dominance of *A. fumigatus* reported in developed countries. There have also been a number of case reports from other African countries (145–148), including one from Ghana (67).

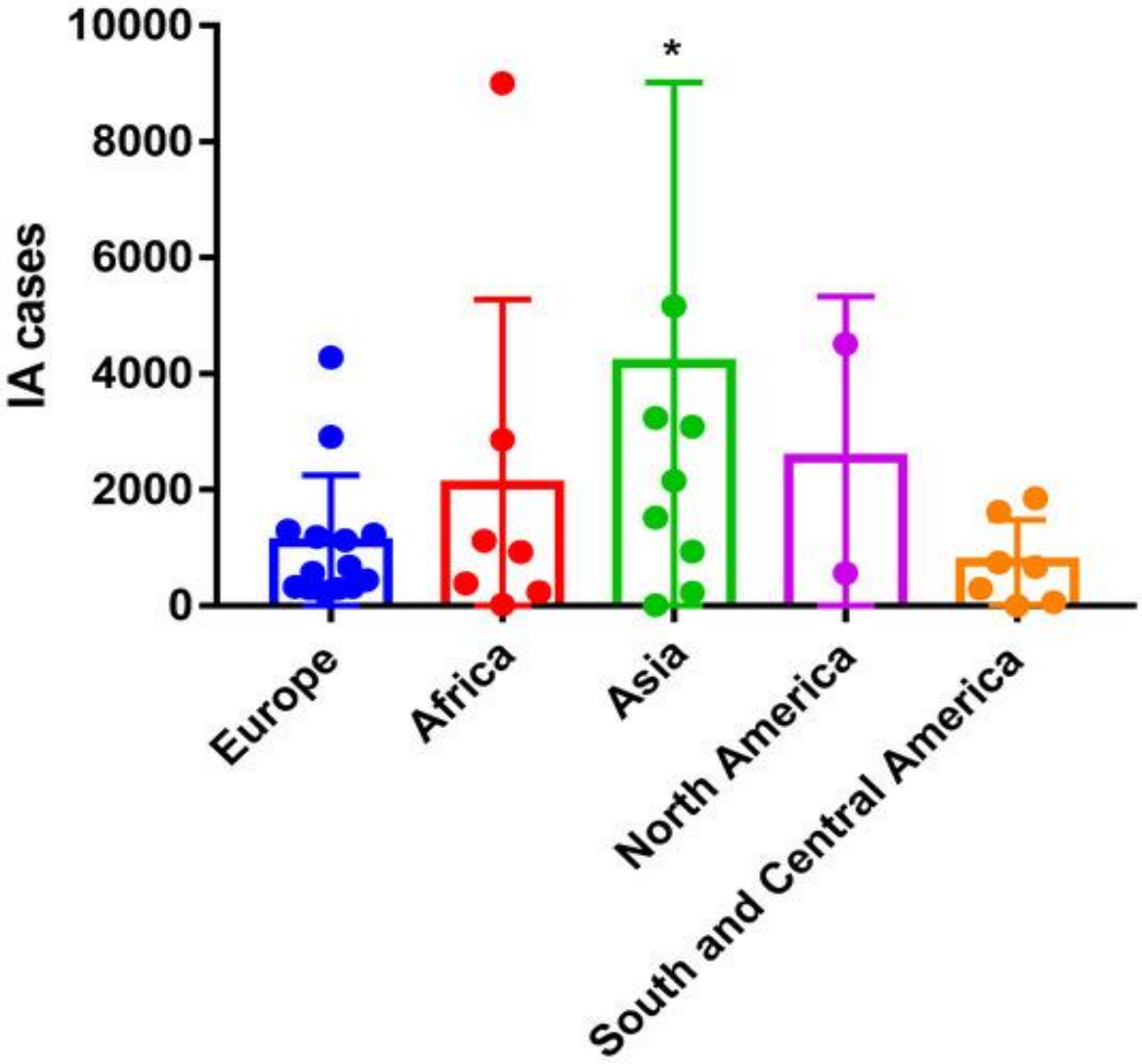


Figure 1.8: Prevalence of invasive aspergillosis in a continental level

(* p < 0.05) (Adapted from Bongomin *et al*. 2017)

1.1.4.2 Clinical features of IA

The clinical spectrum of IA is defined based on the site or organ affected after inhalation of *Aspergillus* conidia into the respiratory tract, which is the main portal of entry. Denning (149) mentions other recognized portals of entry, include damaged skin or other surgical wounds, cornea, and ear. The lung is the predominant single organ affected (usually >70%). Several studies have reported the CNS, kidney, liver, gut, heart, spleen, lymph nodes, eye, adrenal glands and sinuses as additional sites affected (2,56–58,149–154). Disseminated IA defined as involving two

or more non-contiguous visceral organs is not uncommon. Local extension around tissue planes, multifocal disease in the same organ or organ system or multiple primary infections are possible manifestations and could easily be confused with disseminated IA (149).

1.1.4.3 Haematological malignancy as an underlying condition for IA

Underlying conditions that predispose an individual to IA include haematological malignancy, immunosuppressive therapy, transplant (stem cell and solid organ), HIV, prolonged corticosteroid use, graft-versus-host disease (GVHD), ICU admission and chronic granulomatous disease (CGD). Specifically, acute leukaemia is common in 5-10% of IA patients and about 60,000 and more than 105,000 confirmed cases among COPD and transplant patients reported respectively (155,156). IA has not been strongly linked with HIV, because neutropenia, which is a classical host factor for the occurrence of IA, is not common in HIV (157). In a recent study, attempting to quantify IA deaths in HIV, there were no studies or reports from Africa (158). So, in fact, EORTC/MSG definitions (Figure 1.9) seldom identifies IA with HIV except in AHD, coupled with other host factors (159). Marijuana inhalation and alcohol consumption have been postulated while others have no observed host factors (157,159). As previously mentioned, the principal risk factor for IA is protracted neutropenia and are unable to clear exposure to significant levels of *Aspergillus* conidia (160). It is noteworthy mentioning that IA has also been diagnosed in immunocompetent hosts after a massive exposure to *Aspergillus* conidia (100).

1.1.4.4 Diagnosis of IA

The pre-mortem diagnosis of IA is often very problematic with only an estimated 50% likelihood of diagnosis before death (57). The signs and symptoms of IA, usually cough, dyspnoea and fever are non-specific and a combination of host factors, radiology findings and laboratory results (histopathology to demonstrate tissue invasion of hyphae and mycology to detect or grow *Aspergillus* from sterile clinical specimen) is particularly important for a proven diagnosis as defined by practice guidelines and diagnostic algorithms (123,161).

1.1.4.5 Radiological investigations

CXR and CT scans are non-conclusive but important components of the diagnosis of IA, particularly those affecting the respiratory tract, CNS, and sinuses. Laboratory approaches for the diagnosis of IA constitute direct microscopy, histopathology, culture, GM, beta-D glucan (BDG) and PCR. Concerns of ease of obtaining sample, sensitivity, specificity, prophylactic antifungal treatment, cost, and turn-around-time (TAT) of the different techniques require individualized diagnostic approaches. Nevertheless, a combination of tests is usually necessary and highly recommended.

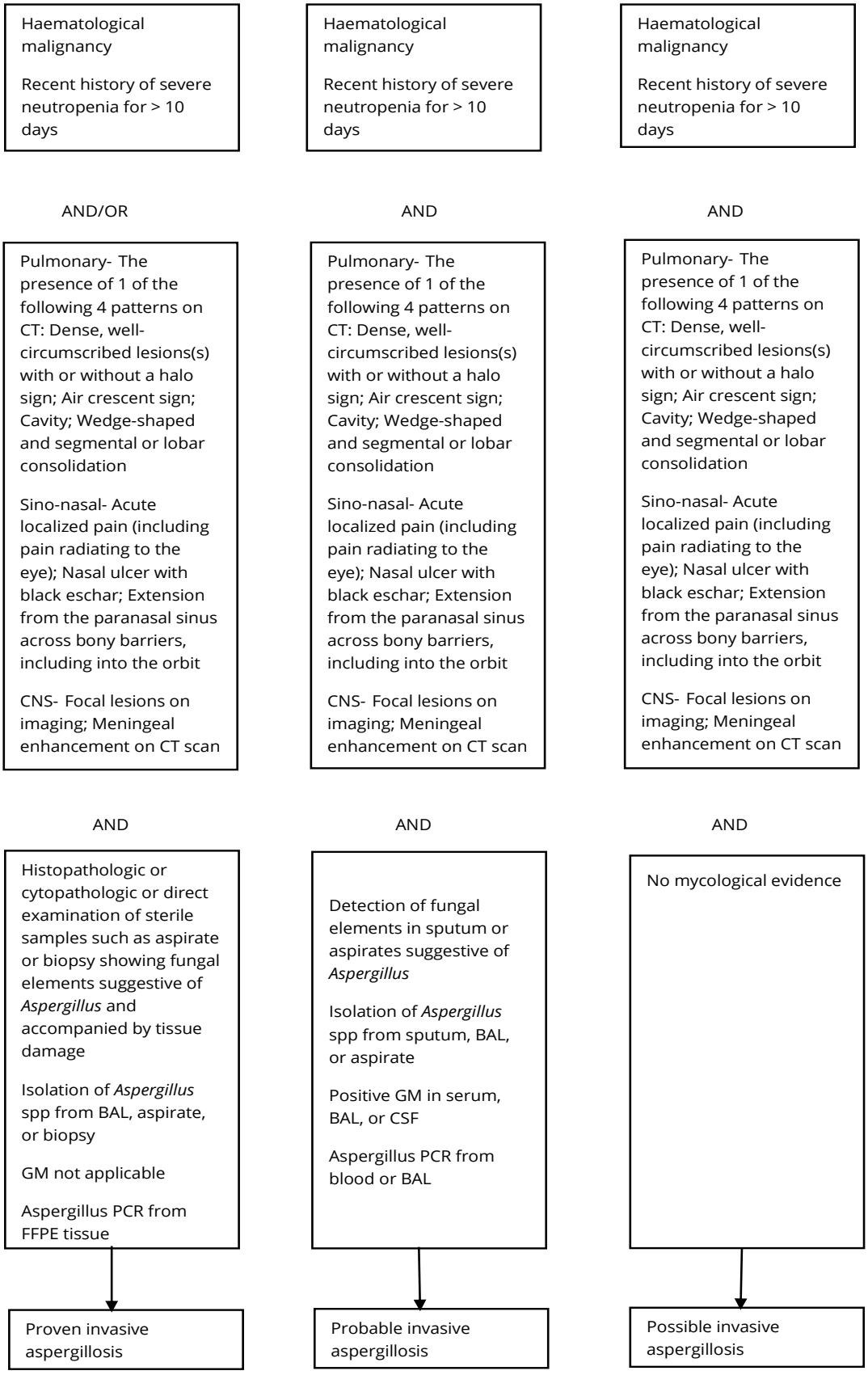


Figure 1.9: EORTC/MSGERC Diagnostic Algorithm for IA

(Adapted from Donnelly *et al*. 2020)

1.1.4.6 Laboratory investigations for IA

Direct examination of suitable clinical specimens microscopically is the simplest method of diagnosing IA. Unfortunately, microscopy lacks specificity due to the similarities between *Aspergillus* spp hyphae morphology with other filamentous fungi such as *Fusarium* spp and *Scedosporium* spp (162). Evidence of filamentous fungus and accompanying tissue invasion on histologic slides from biopsy or autopsy materials suggests a proven diagnosis (123,161). Supplementing routine haematoxylin and eosin (H&E) staining with special stains such as periodic acid Schiff (PAS) and Grocott-Gomori methenamine silver (GMS) increases sensitivity and is always recommended when IA is suspected (161). Advanced methods of identifying *Aspergillus* spp by immunohistochemical labelling has been previously described by Antinori *et al*. (56).

A positive fungal culture from a clinical sample (particularly when fungal hyphae or elements are seen at direct microscopy or histology) confirms a proven diagnosis of IA. Using the EORTC/MSGERC criteria (Figure 1.9), in the presence of relevant host factors and recognised radiologic abnormalities, a positive culture of *Aspergillus* spp from tissue and fluid specimens may represent a probable diagnosis of IA or a proven diagnosis if the sample is from a sterile site (123,161). However, culture is not sensitive and a positive culture without corresponding clinical and imaging features usually represents colonization (especially in respiratory samples) or laboratory contamination. For probable cases of IA, EORTC/MSG recommends serum and/or bronchoalveolar lavage (BAL) *Aspergillus* GM (123). GM is a polysaccharide constituent of the *Aspergillus* cell wall and established to be released during hyphal growth. In recent times, it has become the most significant tool for diagnosis and management of IA. Indeed, introduction of *Aspergillus* GM testing has significantly improved the detection rates of IA, particularly, among haematological malignancy patients (163). Periodic screening of *Aspergillus* GM among patients at high-risk patients including acute leukaemia with prolonged severe neutropenia during intensive chemotherapy. Current guidelines thus recommend serial GM testing to improve early diagnosis and follow-up of IA patients allowing for evaluation of the disease course and response to antifungal drugs. Unfortunately, GM is not specific to *Aspergillus* and can also be found in other fungi including *Penicillium*, *Fusarium*, *Alternaria*, and *Histoplasma* (164–168). Furthermore, false positives have been revealed in patients on antibiotics such as piperacillin and tazobactam and electrolyte solution (169,170). In the majority of studies among at-risk patient groups, the specificity of the GM assay is at least 85% (171). The commonest form of detecting the *Aspergillus* GM has been through EIA and used in several clinical settings globally. Over the years, EIA has been the recommended assay for detecting GM in clinical samples to diagnose IA, with several commercialised and in-house tests available. The most popular assays include Platelia *Aspergillus*

GM, Dynamiker *Aspergillus* GM, OLM *Asper*LFD and Era Biology *Aspergillus* GM test. These assays have varying sensitivity and specificity reported in clinical studies at different cut-off points of the GM index. The meta-analysis of evaluation studies report sensitivity and specificity in the ranges of 30-100% and >75% respectively (172). The pooled sensitivity and specificity for proven or probable IA was 61% and 93% respectively. In a more recent meta-analysis of 30 evaluation and clinical efficacy studies focussing on BAL samples only, the *Aspergillus* GM EIA reported an overall sensitivity of 87% and specificity of 89% (173). The diagnostic role of GM testing in serum has a pooled sensitivity of 66% and a pooled specificity of 90% (171). Additionally, new *Aspergillus* antigens monoclonal antibody (Mab) JF5 and galactofuranose-specific monoclonal antibody (mAb476) have been discovered and developed into testing kits in the form of EIAs and LFAs (174,175). The best practices, however, presently encourage combining GM testing and *Aspergillus* PCR which improves sensitivity and specificity in serodiagnosis of IA (176). Although BDG has emerged as a potential marker in the diagnosis of IA, it is less specific compared to GM with positivity levels common in cases of candidiasis, pneumocystosis and fusariosis. The BDG assay is therefore mainly recognised as a general marker rather than a specific biomarker for IA (162). There are different cut-offs for manufacturers of BDG assays. The majority of the current BDG assay platforms are time-consuming, labour-intensive, prone to contamination, require considerable technical skill, are expensive, and their clinical use may be challenging in resource-limited settings.

1.1.4.7 Role of Rapid Diagnostic Tests

After years of sustained use and well-established significance of the *Aspergillus* GM EIA in screening for IA among high-risk groups and aiding diagnosis of IA, LFA forms have been subsequently released by different manufacturers (IMMY Diagnostics and OLM Diagnostics. Some of these are CE-marked, commercialised, and others were recently introduced. Their use has been demonstrated in both serum and BAL samples for screening and diagnosing IA in both neutropenic and non-neutropenic patients, among patients with haematological malignancies and respiratory diseases. Multiple studies have evaluated these assays and reported promising performance in comparison to the standard EIA assays. Overall sensitivity and specificity ranges from 59%-97% and 66%-98% respectively (177–181) as provided in Table 1.5. An upgrade of one of the kits, is reading test results with a digital reader, which is particularly helpful for weakly positive results (178,179).

Table 1.5: Overview of *Aspergillus* GM rapid diagnostic tests for diagnosis of IA

Assay	Category	Specimen	Sensitivity	Specificity	Ref
IMMY sona Asp GM	Proven	Serum	97%	98%	(178)
IMMY sona Asp GM	Proven	BAL	91%	92%	(179)
IMMY sona Asp GM	Proven and Probable	BAL	87%	92%	(179)
IMMY sona Asp GM	Proven and Probable	BAL	77%	66%	(180)
OLM AspLFD	Proven	BAL	82%	96%	(179)
OLM AspLFD	Proven and Probable	BAL	72%	87%	(179)
OLM AspLFD	Proven and Probable	BAL	59%	78%	(180)

1.1.4.8 Management of IA

Improved outcome in the treatment of IA largely depends on early diagnosis and prompt initiation of systemic antifungal medications. Triazoles are the preferred class of antifungal agents for prevention and treatment of IA in most patients. Treatment guidelines recommend voriconazole for the primary treatment of IA (123,161). Alternatives in the absence or contraindication of voriconazole are amphotericin B deoxycholate and its lipid derivatives. There is no definite dosing of voriconazole in IA. However, the aim for dosing by the majority of experienced clinicians is to achieve a trough concentration of >1–1.5 μg/mL for efficacy but <5–6 μg/mL to minimize toxicity (161). Therapeutic drug monitoring (TDM) is suggested for patients receiving triazole-based therapy for IA, prolonged azole prophylaxis, or other therapies, for which drug interactions with azoles are anticipated, to reduce drug discontinuation due to adverse effects, identify sub-optimal exposures and minimize severe side effects. Routine AFST is not recommended during initial treatment unless azole resistance is suspected or there is treatment failure (161). Surgery, mainly debridement, is an important treatment option for localised infections in some uncommon extrapulmonary cases of IA. Surgical interventions are generally combined with systemic antifungal therapy. The therapeutic role of surgery is to decrease the extent of tissue damage and facilitate antifungal penetration. Examples of clinical manifestations of IA that may require surgical interventions include CNS aspergillosis, *Aspergillus* sinusitis, aspergillosis of the eye, *Aspergillus* osteomyelitis, *Aspergillus* endocarditis and pericarditis, and *Aspergillus* aneurysm.

1.2 CRYPTOCOCCOSIS

1.2.1 *Cryptococcus* and Cryptococcosis

Cryptococcus was first described by Prof. Otto Busse, a German pathologist, in 1894 (182). He described the fungus as a round-to-oval "corpuscles" and initially named *Saccharomyces*. Later in 1901, it was removed from the *Saccharomyces* genus to the *Cryptococcus* as it lacked common features of the former genus such as ability to produce ascospore and ferment carbon (183). The genus *Cryptococcus* presently consists of over 70 species with enormous biodiversity between the species (183,184). Serotype grouping separates the *C. neoformans* and *C. gattii* into Serotypes A, B, C, and D based on capsular polysaccharides. Many studies have reported enormous genotypic and phenotypic differences between *C. neoformans* and *C. gattii* including biochemical, pathophysiology and clinical characteristics (185–187). *Cryptococcus* has a wide range of niches including trees, soil, excreta, insects, arctic climates, and pH extremes (188–192). The pH and glucose concentration of pigeon excreta are key contributors for the environmental survival of *C. neoformans* (187). Moreover, *C. neoformans* have been isolated from the faeces of birds such as ducks, eagles, owls and a parrot (193–196). Indeed, birds are suggested as carriers and dispersal agents of *C. neoformans* and their migration is contributory to the widespread nature of the disease in humans, although disease in birds is rare due to their high body temperatures (197). On the other hand, *C. gatti* is commonly associated with woody environments or vegetation, is frequently isolated from trunk hollows, trees, and flowering plants, and thus, spreads more locally in endemic areas (198).

1.2.2 Clinical spectrum of Cryptococcosis

Cryptococcosis refers infections caused by *Cryptococcus* spp. *C. neoformans* and *C. gattii* infection affects a broad range of body sites but has a major predilection for establishing disease in the CNS and lungs, that is CM and pulmonary cryptococcosis. Uncommon body sites affected by disease include skin, eyes, and bone. Nonetheless, dissemination is possible essentially in severely immunosuppressed patients and may affect any of the body organs. The respiratory tract is the most important portal of entry for *Cryptococcus* and disease manifestations range from asymptomatic colonization of the airways or a simple lung nodule to fatal pneumonia with the presence of an acute respiratory distress syndrome (199). Cutaneous infections are the third most common clinical manifestations of cryptococcosis and patients present with non-specific skin lesions (200). Cutaneous cryptococcosis is seldom primary and mostly signals a disseminated disease (201). Bone involvement of cryptococcosis typically presents as osteolytic lesions in different types of bones, but usually affects the vertebrae (202).

1.2.3 Cryptococcal meningitis (CM)

1.2.3.1 Epidemiology of CM

In 2020, 179,000 HIV patients with CD4 cell count of less than 200 cells/μL were estimated to be positive for CrAg globally, resulting in 152,000 cases of CM (19). Out of these, SSA alone accounts for at least 70% of both CM cases and deaths respectively. Despite the decline of the prevalence of cryptococcal antigenemia (CA) and CM across the world from 2014 to 2020, deaths from CM increased from about 15% to 19% (19). A review of meningitis in HIV patients in SSA revealed higher rates of CM (19-68%) than TB meningitis (1-36%) (203). In Ghana, a retrospective study on CA and two prospective studies on CM both reported a prevalence of around 2% (68,71,72). However, a retrospective investigation of the aetiological agents of cerebrospinal meningitis isolated *C. neoformans* in 11.7% of confirmed cases (73). While another study, showed that the portion of HIV related deaths among hospitalized patients caused by CM was estimated to be 3.5% (204).

1.2.3.2 Clinical features of CM

Clinical manifestations of CM include a myriad of signs and symptoms, such as headache, fever, cranial neuropathies, altered mental activity, lethargy, memory loss, and signs of meningeal irritation (205). Symptoms usually develop over a period of one to three weeks. However, on some occasions, patients present more acutely or lack typical features, such as headache. In HIV-infected patients with CM, the burden of the fungus is usually high in the CSF and has a rapid onset of signs and symptoms and higher intracranial pressures relative to immunocompetent patients (201).

1.2.3.3 HIV as a risk factor for CM

A defect in the immune system resulting in the immune defence being compromised is the key predisposing factor for cryptococcosis. Immunocompromised conditions such as HIV infection, organ transplantation, diabetes mellitus, steroid therapy, and rheumatologic or immunologic disorder are several vulnerable conditions for cryptococcosis (200). However, HIV is the principal underlying condition for CM and the highest number of cases occurred in the 1980s during the HIV pandemic (17). CA is common in persons with AHD and is inversely proportional to CD4 count. The introduction of HAART has significantly reduced the incidence of CM in developed countries but in SSA, CM among HIV-infected adults remain frequent (21). Most CrAg seroprevalence studies focus on ART-naïve patients, but recent investigations report significant prevalence rates in ART-experienced patients (206–210). These rates are comparable to that reported in ART-naïve patients. In Cape Town, South Africa, 1 in 5 HIV-infected patients were reported to develop CM while receiving ART (after a median duration of 41 days) with 29% mortality (211). Mortality

associated with CM in Africa remains high, ranging from 20% to 50% even in settings with available first-line drugs (212).

1.2.3.4 Diagnosis of CM

Laboratory approaches for establishing a cryptococcal disease include direct microscopy, culture, histopathology, serology, and rarely molecular methods. Early diagnosis of cryptococcal infection is very critical in improving the outcomes of patients and ensuring survival. Historically, CM has been diagnosed with India ink microscopy on CSF, a rapid and inexpensive method, to demonstrate the presence of budding encapsulated yeasts. The India ink offers a background field, which is not taken up by the *Cryptococcus* capsule, forming a halo, visualized with a light microscope. Notwithstanding the simplicity and wide availability in resource-limited settings, this technique has a moderate sensitivity, up to 86% based on considerable experience of the microscopist (213). Isolating *Cryptococcus* spp from clinical samples is considered the gold standard. Culture has a slightly higher sensitivity than India ink but has a disadvantage of long TAT up to few weeks to definite results, requiring a large quantity of sample, laboratory infrastructure and technical expertise (214). Serological diagnosis of CM is achieved through the detection and/or quantification of CrAg in body fluids such as serum, BAL, CSF, and urine. CrAg is measurable in serum a median of 3 weeks before the onset of symptoms of CM, allowing for serum CrAg screening to identify patients with asymptomatic cryptococcal infection for pre-emptive antifungal treatment prior to the development of fulminant CM (215). In the past few decades, serological diagnosis has over the time played a significant role in making a definitive diagnosis of CM becoming more popular than India ink and culture. There are three main testing platforms available namely, latex agglutination test (LAT), EIA and LFA. Although the sensitivity and specificity of LAT and EIA has a great, usually more than 90% across different manufacturers these methods require steady electricity, refrigeration, cold-chain system, laboratory tools and technical expertise and may have a constrained use in resource-limited laboratories (214,216,217). Furthermore, these assays have hours from test request to delivery of reports. Comparatively, the LFA is simple, easy-to-use, and cheap with a potential of broad accessibility in resource-limited settings. The sensitivity and specificity of kits from various manufacturers are reported more than 99% (213,216). LAT, EIA and LFA are mostly used as qualitative tests and they are also used for semi-quantitative measurements of titres. Molecular detection of *Cryptococcus* spp includes pan-fungal PCR and DNA sequencing, and multiplex PCR which are generally applied in research settings where detection of *Cryptococcus* to species is important to investigate taxonomy, biological characteristics, and virulence. Conventional culture methods by canavannine glycine

bromothymol blue (CGB) test can differentiate the two species, but multiplex PCR has been shown to be more specific than CGB test (218).

1.2.3.5 Role of Rapid Diagnostic Tests

The introduction of CrAg LFAs has transformed epidemiology, diagnosis, and research of CM, particularly in Africa. The assay employs mechanisms capable of detecting all A to D cryptococcal serotypes. Furthermore, the assays allow for CrAg titre quantification relevant for predicting risk of death and risk for immune reconstitution inflammatory syndrome (IRIS) (219,220). The factors for false positive include rheumatoid factor or other fungal or bacterial infections and low fungal burden may result in false negative results (215). Multiple studies show excellent sensitivity and specificity of LFAs in serum, plasma, whole blood, finger-prick (capillary blood) in HIV patients but not in urine and saliva samples (20,221–227). Presently, there are five manufacturers of CrAg LFAs (Table 1.6). IMMY CrAg was the first introduced, FDA-approved, and CE-marked and has been broadly evaluated worldwide in different settings and in different clinical specimens. The Biosynex CryptoPS LFA (Biosynex, Paris, France) which is CE-marked and StrongStep CrAg LFA (Liming Bio, Jiangsu, China) have had its clinical utility and efficacy considerably validated (228,229). The Dynamiker CrAg LFA and FungiXpert Cryptococcal Capsular Polysaccharide K-Set are recent introductions and have limited evaluation studies.

Table 1.6: Overview of *Cryptococcal* antigen rapid diagnostic tests for diagnosis of CM

Assay	Case	Sample	Sensitivity	Specificity	Ref
IMMY CrAg	Suspected and confirmed CM	CSF	100%	100%	(220)
IMMY CrAg	Confirmed CM	Serum, CSF Urine	100% 94.4%	100% -	(230)
IMMY CrAg	Suspected CM	Plasma, CSF	99.3%	99.1%	(216)
IMMY CrAg	Suspected CI	CSF, serum	100%	99.6%	(231)
IMMY CrAg SQ,	CA screening	Plasma	98.8	97.9	(232)
CryptoPS	Suspected and confirmed CM	Plasma	95.2	92.4	(232)
Dynamiker LFA	Suspected and confirmed CM	Plasma	100	77.6	(232)
Biosynex CryptoPS	Suspected and confirmed CM	Serum Plasma CSF	74% 92% 100%	100% 100% 100%	(228)
StrongStep	Suspected and confirmed CM	CSF Plasma	100% 98%	98% 90%	(229)

Dynamiker	Symptomatic CM	CSF	100%	91%	(233)
		Serum	98%	66%	
		Plasma	100%	61%	
	Asymptomatic CA	Serum	96%	86%	
Dynamiker	Asymptomatic CA	Serum	100	89.9%	(234)

CI- Cryptococcal infection

1.2.3.6 Management of CM

The holistic management of CM involves prompt detection of infection, use of appropriate and adequate antifungal therapy, reducing increased intracranial pressure and controlling associated complications. Until recently, the WHO guidelines recommended prompt lumbar puncture with measurement of CSF opening pressure and rapid CrAg testing on strong suspicion of first episode of CM (235). And for confirmed CM cases, a short-course (one-week) induction regimen with amphotericin B deoxycholate and flucytosine followed by 1 week of fluconazole, was the preferred option among adults living with HIV or alternatively, two weeks of fluconazole and flucytosine followed by fluconazole. Consolidation (8 weeks) and maintenance therapy (at least 1 year) may subsequently follow with fluconazole. However, following a phase 3 randomized, controlled, noninferiority trial conducted in five African countries, the new WHO guidelines recommend single, high-dose liposomal amphotericin B combined with flucytosine and fluconazole (13). For patients with persistent symptoms of raised intracranial pressure, repeat daily therapeutic lumbar puncture (with measurement of CSF opening pressure where available) and CSF drainage, if required, are recommended until the symptoms resolve or the opening pressure normalizes.

The timing of ART initiation during the management of CM is critical to prevent the risk of IRIS. IRIS is a paradoxical inflammatory reaction that occurs during immunologic recovery with ART without complete or effective treatment for opportunistic infections. IRIS caused by CM is known as cryptococcal immune reconstitution inflammatory syndrome (C-IRIS) and is a common problem in resource-limited settings (236). C-IRIS can be potentially life threatening and associated with increased mortality (237). Early initiation of ART (<2weeks) after diagnosing CM has been found to increase overall mortality and thus 4-6 weeks delay is recommended (235). 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 (238).

1.3 HISTOPLASMOSIS[1]

1.3.1 *Histoplasma* and Histoplasmosis

Histoplasma capsulatum was first described in 1906 by American pathologist Samuel T. Darling, while working in Ancon Hospital, Canal Zone, Isthmus of Panama (239). The discovery was made whilst performing three unusual autopsies that consistently showed granulomas with small round or oval microorganisms in alveolar epithelial cells in the granuloma or freely in the bone marrow or spleen. He proposed the microorganism causing this new disease was a protozoan and named the organism *H. capsulatum* because it invaded the cytoplasm of histiocyte-like cells and was enveloped by a capsule. Darling summarized his findings as "a protozoan that was causing histoplasmosis, a general infection and producing pseudo-tubercules in the lungs and focal necrosis in the liver, spleen and lymph nodes" (240). Several case reports of the new microorganism were made in South America and later in other parts of the world (241). Years later, it was found that Darling erred and that *H. capsulatum* was neither a protozoan nor had capsules. In 1912, Brazilian pathologist, Henrique da Rocha-Lima, re-examined tissues from Darling's Panama patients and compared the microorganisms to *Leishmania* spp. and *Cryptoccocus farciminosum* (the cause of epizootic lymphangitis in horses) (242). Rocha-Lima observed similarities with the latter and suggested that *H. capsulatum* was a fungus rather than a protozoan. Finally, De Monbreun in 1934 confirmed *H. capsulatum* was a fungus when he cultured the organism from the bloodstream and spleen of a patient in Tennessee, USA (243).

In 1942, the first case of histoplasmosis was reported from Africa by Irish mycologist James T. Duncan (244). He noticed the organism had large yeast forms different from *H. capsulatum* and suggested a new classification. Raymond Vanbreuseghem studied similar isolates from Albert Dubois and described it as a new species in 1952 (245). It was named *Histoplasma duboisii* in honour of Albert Dubois. Subsequently, comparative studies of *H. duboisii* and *Histoplasma farciminosum* (isolated from equines in Africa and the Middle East and causes clinical disease in horses) with *H. capsulatum* established there was no significant difference and thus *H. duboisii* and *H. farciminosum* should be treated as varieties of *H. capsulatum* and not distinct species (246,247).

[1] Portions of this section on 'Histoplasmosis' has been published as Paper 1: **Ocansey BK**, Kosmidis C, Agyei M, Dorkenoo AM, Ayanlowo OO, Oladele RO, Darre T, Denning DW. **Histoplasmosis in Africa: Current perspectives, knowledge gaps, and research priorities**. PLoS Neglected Tropical Diseases. 2022 Feb 24;16(2): e0010111. (Appendix 1)

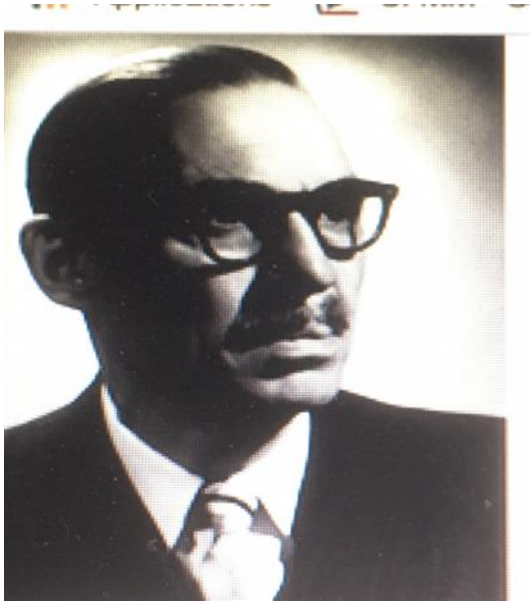


Figure 1.10: Albert Dubois
(Courtesy of Bertrand Dupont)

The traditional classification has since remained with three varieties, that is, hcc, hcd and *H. capsulatum* var. *farciminosum* (hcf). This classification depends on phenotypic characteristics such as clinical manifestation, morphology, and geographical distribution. *H. capsulatum* is a thermally dimorphic fungus; exists in nature as a saprophytic mould but in human and animal tissue, it forms small round budding parasitic yeast cells due to varying temperatures. Hcc and hcd are the pathogenic varieties in humans. Hcc and hcd cannot be differentiated in their mould forms but differ in the parasitic forms with hcd being much larger and have thicker walls than those of hcc.

Conversely, genetic studies raise concern about the current classification, and signal the likely existence of many species (248–253). These studies reveal that *H. capsulatum* consist of multiple genetically distinct groups or phylogenetic clades that qualify to be phylogenetic species. An initial study of 46 isolates (made up of hcc, hcd and hcf) and DNA sequences of four protein-coding genes revealed six clades. They are (i) North American 1 (hcc), (ii) North American 2 (hcc), (iii) Central American (hcc), (iv) South American A (hcc), (v) South American B (hcc), and (vi) Africa (hcd) (248). The study concluded that *H. capsulatum* might encompass six different species and not three varieties. Increasing the isolates to 137 and broadening the geographical distribution, a similar study identified at least eight clades. They were: (i) North American class 1; (ii) North American class 2; (iii) Latin American group A; (iv) Latin American group B; (v) Australian; (vi) Netherlands (probably derived from Indonesia); (vii) Eurasian and (viii) African clades. All but the Netherland clade were suggested for recognition as phylogenetic species (249). The African clade contained all hcd isolates but plus some hcc and hcf isolates. These findings rendered the historical

classification phylogenetically insignificant. Recently, a more robust multi-locus sequencing typing (MLST) study evaluating 234 isolates of *H. capsulatum* led to the identification of at least 11 species-level clades. The former Latin American A and Latin American B species were each divided into two different genetic clusters. Two new phylogenetic species, RJ (Southeast of Brazil) and BAC-1 (Mexico), and four different monophyletic and cryptic clades from Brazil (BR1-4) were also identified. Performing genome-wide population genetics and phylogenetic analyses on 30 *H. capsulatum* isolates from four endemic areas, Sepulvedia *et al*. (252) showed that the *Histoplasma* genus is composed of at least four species that are genetically isolated and rarely interbreed. The authors also called for a taxonomic rearrangement of the genus. It is important to note that these genetically isolated groups had varied virulence, clinical manifestation, morphology, drug susceptibility and natural history. The exact number of species belonging to the genus *Histoplasma* thus remains unclear.

The natural reservoir of hcc are bat or bird droppings and occasionally in plant crowns, bark, or rocks, which contain nutrients for fungal growth. They are commonly found in enclosed spaces such as mines, caverns, uninhabited dwellings or in open spaces such as parks and gardens. There is limited information on the ecological niche of hcd. However, cases of AH among individuals involved with collection of bat guano and living in bat infested homes suggest hcd is likely to have a similar ecological niche as hcc (254). Moreover, Gugnani *et al.* reported a natural reservoir of the hcd in soil admixed with bat guano in a bat cave at a rural town in Eastern Nigeria (255). The fungus was also isolated from the intestinal content of a bat from the cave. In the presence of their guano and humidity, a suitable microenvironment is created for harbouring by the *Histoplasma* mycelium (256).

1.3.2 Epidemiology of Histoplasmosis in Humans

Histoplasmosis refers to infections caused by *H. capsulatum* and it is the most geographically distributed of all endemic fungal infections (248,249). CH caused by hcc is common in North America particularly the central parts of the USA around the Mississippi and Ohio Rivers. CH is also found throughout South America, Africa, Asia, and Europe, which have all reported small case series or reports and significant positivity of skin-test surveys. There have been documented reports of microfoci of CH in Italy but almost no skin-test sensitivity positivity in Europe (257).

AH caused by hcd is endemic in Africa particularly in Central and Western Africa (Figure 1.11). A recent review reports that about 250 cases have been reported in indigenes from 28 countries in Africa between 1952 and 2017 (24). Cumulatively, 87% of the cases were from Western and Central Africa. There were a moderate number of cases from Eastern Africa but very few cases were

reported from Southern Africa. The study also found that both hcc and hcd coexist in Ghana, with 12 cases of histoplasmosis reported in the last six decades (1952–2017) (29,258–266).

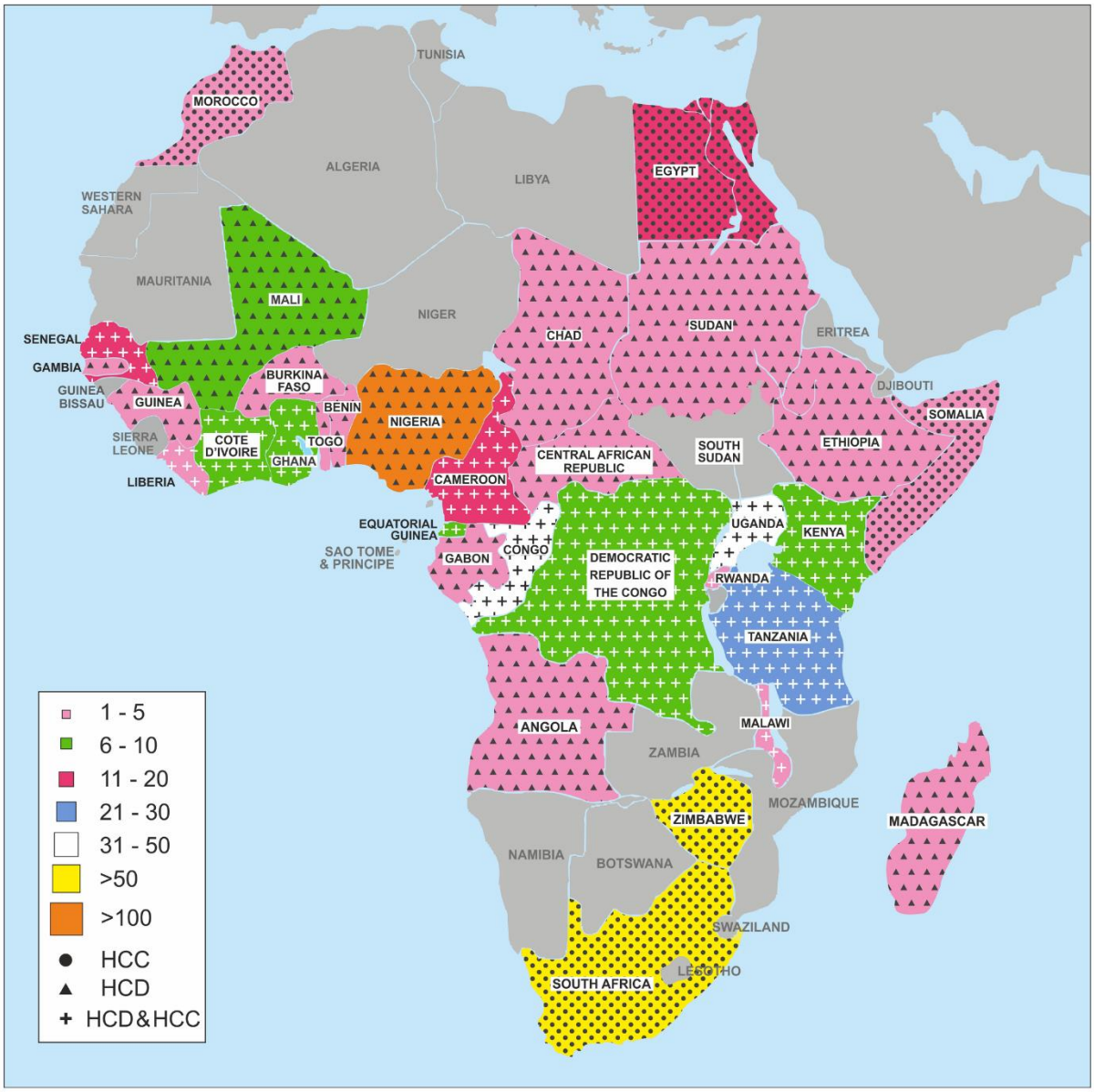


Figure 1.11: Distribution of reported cases of histoplasmosis across Africa (1952–2017)

(Adapted from Oladele *et al*. 2018)

Although AH cases have been reported in the North America, Europe, Asia and South America, travel history to Africa is common in these cases. Recently, one case of autochthonous AH was reported from India (267). Several *Histoplasma* skin sensitivity surveys have been undertaken in some parts of Africa (255,268–278). Rates of positivity ranges from 0-35%. Very few of these studies have used hcd-specific antigens. A skin sensitivity study around a bat cave vicinity using histoplasmin prepared from hcd yielded a higher prevalence of 35% (255). However, the state of

asymptomatic infections with hcd and possible progression to symptomatic disease is not clearly understood.

1.3.3 HIV as an underlying condition for Histoplasmosis

The main attributable risk factor for CH is the spread of HIV and 'disseminated' CH was classified as an AHD infection in 1987 (279). In endemic regions, CH occurs in about 2-25 % in HIV-infected patients (280). Prior to the HAART period, subclinical or symptomatic CH was reported in 12/100 person-years among HIV-infected patients in endemic settings (281). However, with the introduction of HAART, there has been significant reduction in the incidence of histoplasmosis in HIV-infected patients in the USA (265).

In contrast, AH seems to have minimal or no association with HIV, although data is scarce. A few cases of AH have been reported in HIV patients (16,28,30,59,282,283). All these cases manifested severe disease. Nevertheless, severe disease occasionally occurs in apparently immunocompetent patients (254,265,284,285). This observation is inconsistent and surprising considering the high association of CH with HIV. A review of cases in AH in Spain revealed about 77% had HIV as an underlying condition (29). All patients had migrated from Africa while a review in Togo reported only 18% of cases had occurred in HIV infected people (59). In a study in Cameroon, among 56 HIV-infected patients recruited, hcc was detected in 13% of patients (286).

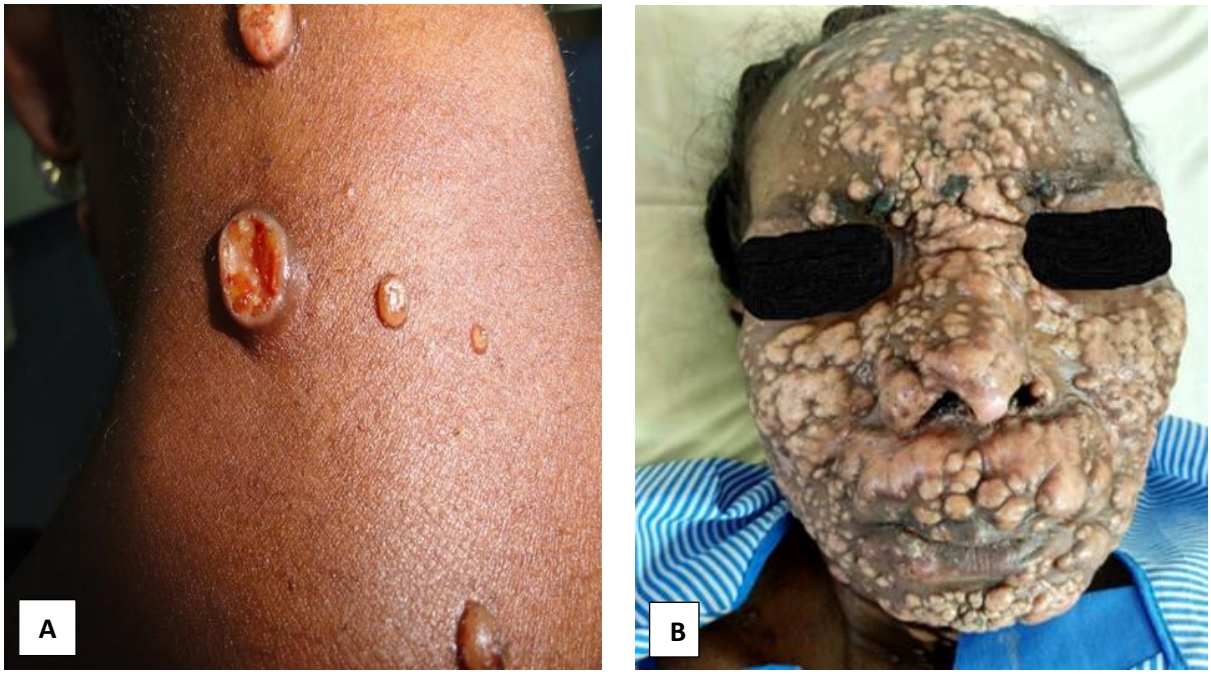
1.3.4 Clinical Features of Human Histoplasmosis

1.3.4.1 Classical Histoplasmosis (CH)

Clinical manifestations of CH vary depending on the immune status of an individual. Most cases of CH in immunocompetent individuals are asymptomatic and self-limiting after low exposure (287). Symptomatic infection mostly develops in immunocompromised patients with acquired or congenital cellular immune deficiency as well as apparently healthy individuals with high exposure (288). Pulmonary histoplasmosis is the dominant form of disease and may be acute or chronic. Acute pulmonary histoplasmosis (APH) usually presents as a non-specific flu-like illness. In the presence of an underlying lung damage such as TB and COPD, chronic pulmonary histoplasmosis (CPH), which is a slowly progressive illness, develops and often results in fibrosis and cavitation. CPH is commonly reported to mimic multidrug-resistant PTB (289,290). Disseminated histoplasmosis (DH) is frequent in individuals with T-cell mediated immunological defects. Presentation of disseminated disease may range from acute illness to indolent and chronic illness that can affect a wide range of sites including skin, liver, spleen, adrenal gland, mouth, throat, CNS, heart, gastrointestinal tract (GIT) and genitourinary tract (GUT).

1.3.4.2 African Histoplasmosis (AH)

Skin, subcutaneous tissue, lymph nodes, and bone lesions generally characterize AH. There are two main clinical presentations : i) localized: lesions are scanty and there is no systemic involvement (Photograph 1.1 A); ii) disseminated: multiple lesions through the body; usually there is systemic involvement with anaemia, loss of weight, fever and other constitutional disturbances (Photograph 1.1 B) (291). In a review of 17 cases reported by Loulergue *et al*. (28), skin lesions, weight loss, anaemia, lymph nodes, fever were the predominant clinical findings. Lesions may evolve and develop continuously, with new ones appearing as old ones heal. Thus, lesions of different sizes and at different stages of development may be present during disease. This is more prominent in disseminated cases where there are extensive lesions. The cutaneous lesions appearing during AH may have different forms and may be papular, nodular, ulcerative, eczematoid or psoriasiform lesions. Adetokunbo categorized these lesions into three main types, namely, superficial cutaneous granuloma, subcutaneous granuloma with abscess and osteomyelitis with secondary involvement of the skin (291). Involvement of bone is common in AH especially in disseminated disease and is estimated to affect more than 50% of patients (292). Bone lesions are observed in the skull, ribs, vertebrae, femur, humerus, tibia, and wrist. Lesions uncommonly develop in internal organs. Nevertheless, few cases involving internal organs including visceral organs have been reported, as a component of disseminated disease. Lesions have also been observed in liver, spleen, lungs and the GIT and may often be misdiagnosed as cancer (293–295).



Photograph 1.1: Cutaneous manifestations of AH in immunocompetent and immunocompromised patients A) Non-HIV patient with variable sizes of fleshy nodules with umbilication and a well-demarcated scalloping ulcer B) Advanced HIV Disease patient with variable sizes of fleshy hypopigmented papules and nodules with coalescing lesions especially on the nose, upper lips, and cheeks with secondary ulcers.

(*A*dapted from Ocansey *et al.* 2022)

1.3.5. Diagnosis of Histoplasmosis

Like other SFIs, diagnosis of histoplasmosis involves a combination of clinical, radiology and laboratory examinations due to non-specific signs and symptoms. Imaging studies are essentially relevant when lungs and bones are involved and can be used in monitoring therapy or identifying relapse. Although imaging findings are not pathognomonic, they should raise suspicion for mycological investigations. The definitive diagnosis is made by laboratory techniques, that is, direct microscopy, culture, histopathology, immunological assays, and molecular techniques.

1.3.5.1 Imaging investigations

For CH, imaging is very useful in lung and disseminated infections. CXR will often reveal small, scattered bilateral nodular infiltrate in APH and progressively enlarging cavities and fibrosis in CPH. CT scans and magnetic resonance imaging (MRI) commonly show single or multiple rings enhancing lesions in CNS histoplasmosis, which occurs mostly in adults (287-290). Imaging studies in AH are rather important in cases with bone involvement. The use of x-ray, CT and MRI are previously reported (285,292,296). A combination of one or more modalities may be necessary to

confirm the extent of disease. In a case report by Katchy *et al.* (296), involvement of bone was detected early when the x-ray report showed a reduction in the cortico-medullary differentiation of the distal metaphysis of the femur with soft tissue swelling, a preserved fat plane, spiculated periosteal reaction, cortical erosion and cauterization. The CT scan report confirmed the x-ray findings. Esteves *et al*. (285) also reported a case of disseminated AH in a 7-year-old girl from Guinea-Bissau where extensive imaging studies were done. Frontal x-rays of the right limb, left hand, feet, hip, and tibias were performed which showed multiple calcified and non-calcified soft-tissue lesions scattered through the body, more prominent in the lower limbs and pelvic region.

1.3.5.2 Laboratory investigations

Direct microscopy involves the preparation of a smear from the exudate or purulent discharge that may be stained or unstained based on available resources. Stains used in identification are Giemsa and GMS. The use of KOH on smears has also been successful in identifying *Histoplasma* yeast cells (28). Yeast cells are easily identified in suppurative materials from skin lesions, abscess, discharging sinuses, bone lesions and biopsy samples as the organisms are usually numerous in the infected tissue. Histologically, CH presents as well-organized granulomas with caseous centres and surrounding palisades of epithelial cells but in AH appears quite different, showing aggregates of multinucleate giant cells containing yeast cells. Other types of inflammatory cells may be present, especially lymphocytes. Supplementing H&E with PAS and GMS is recommended for better demonstration and appreciation of both inflammation reaction and yeast cells. Introduction of *Histoplasma* antigen testing is promoting diagnosis of DH. The detection of circulating antigen has been performed with several EIA methods with sensitivity in DH greater in immunocompromised patients and in patients with severe illness, than in immunocompetent patients and patients with mild disease (297). Performance of various assays is relatively higher in urine compared to blood in disseminated disease (298). The polyclonal *Histoplasma* antigen EIA (from MiraVista Diagnostics) allows the quantitative detection of *Histoplasma* GM circulating antigen with a sensitivity of 95– 100% in urine and 92–100 % in serum (299). Recently, IMMY introduced their new clarus *Histoplasma* GM EIA that has demonstrated comparable performance with previous EIA assays, with a total agreement of 91.3%-98% (300–302). New EIA assays continue to be developed with high concordance performance shown in comparison with standard recommended assays (303,304). Molecular methods are more often needed to confirm a suggested diagnosis or epidemiological studies and more explored in the research settings. The molecular methods utilised are either *Histoplasma* PCR or combined pan-fungal PCR and DNA sequencing.

1.3.5.3 Role of Rapid Diagnostic Tests

Development of LFAs for aiding the serological diagnosis in histoplasmosis is a recent observation. Presently, the EIAs remain the standard and recommended platforms as more validation, evaluation, efficacy, and clinical utility studies particularly on a large scale are being conducted (305–307). The table below illustrates the clinical performance of commercially available LFAs in comparison with EIA (Table 1.7).

Table 1.7: Overview of *Histoplasma* antigen rapid diagnostics tests for diagnosis of histoplasmosis

Assay	Case type	Sample	Sensitivity	Specificity	Ref
MVD LFA	histoplasmosis, no histoplasmosis	Urine	96%	96%	(308)
MVD LFA	histoplasmosis, no histoplasmosis	Serum Visual reading Automated reader	 96% 92%	 90% 94%	(309)
MVD LFA	suspected PDH	Urine	90.4%	92.3%	(300)
MVD LFA	histoplasmosis, no histoplasmosis	Urine	78.8%	99.3%	(310)
OIDx LFA	case series-proven and probable	Urine	100%	-	(311)

(MVD-MiraVista Diagnostics, IMMY-Immuno-Mycologics Diagnostics, OIDx-Optimum Imaging Diagnostics)

1.3.6 Management of Histoplasmosis

The management of CH is more studied and various options of antifungal investigated and recommendations established. The therapeutic approach depends on the presence of an underlying condition, tissue involved and the form of disease. It is important to identify and adequately manage any underlying existing condition. Treatment options are induction: intravenous (IV) amphotericin B for severe and disseminated disease or oral itraconazole for mild and moderate disease and non-disseminated disease, followed by maintenance therapy with itraconazole (287,312). In AH, some solitary and few isolated cases lesions heal spontaneously or simply removed by minor surgical procedure (292). However, antifungal treatment may be needed after surgical removal of lesions. In contrast to CH, there are currently no established guidelines or recommendations for antifungal treatment of AH. Successful treatment has been reported for several antifungal agents, but most clinicians apply the recommendations from the CH guidelines.

1.4 RATIONALE FOR RESEARCH STUDIES

Globally, the diagnosis and epidemiology of SFIs have seen a substantial improvement in the past two decades, particularly, in developed countries due to improved awareness, access to diagnostics and therapeutics, and expanded education, training, and research. This has consequently increased attention given to SFIs by stakeholders at national levels and adding to the sustained efforts of GAFFI in creating awareness on fungal infections globally. Improved diagnosis and continuous update of epidemiological data is strongly linked to low misdiagnosis, morbidity and mortality (3,14). Unfortunately, these advancements are yet to be realized in several LMICs, mostly in Africa. The key attributors to the status quo include inadequate awareness of SFIs among healthcare professionals, insufficient access to laboratory diagnostics and scanty epidemiological data. Meanwhile, in these settings, the population, and patient groups at risk for SFIs is immensely abundant, and the existing socio-economical and geographical conditions are favourable for SFIs. In Ghana for instance, the epidemiological data on SFIs mainly comprises case reports and case series that are completely silent on species-level aetiological agents and clinical and epidemiological studies are extremely inadequate (Table 1.3) (13,81). Fortunately, the past decade has seen growing interest and awareness on the African continent, due to the strategic efforts of local clinical and academic enthusiasts, international organizations such as GAFFI, ISHAM, ECMM, MSGERC, CDC Mycotic Disease Branch and diagnostic and therapeutic companies. The next steps are improving access to diagnosis and unravelling local epidemiological data across Africa.

Presently, routine access to fungal tests, particularly those on the WHO EDL is poor and entirely unavailable in several African settings including Ghana (81). This is probably because technical expertise in conventional methods (direct microscopy, culture, and histopathology) is extremely insufficient, and the contemporary assays (biomarker and antigen-antibody tests) are complex to broadly implement in their current forms, mainly EIA and kinetic assay (both manual and automated platforms). Although conventional methods remain the gold-standard for several SFIs, contemporary assays have become pivotal in screening and early diagnosis. EIA and kinetic assays are resource and labour-intensive. In recent times, ICT forms of contemporary assays (RDTs designed as LFA or LFD) are becoming popular. In fact, some of these assays meet the WHO's ASSURED criteria, can be used as a point of care tests at the bedside or doctor's office and included on the WHO EDL. Historically, RDTs are extensively used for aiding the diagnosis of bacterial, viral, and parasitic infections and have positively impacted diagnosis in resource-limited settings. Researchers and experts suggest fungal RDTs could revolutionize the diagnosis of life-threatening

SFIs and generate epidemiological data in resource-limited settings when broadly implemented (32,313). RDTs, thus, offer the opportunity for resource-limited settings such as Ghana to implement these assays in at-risk groups to diagnosis, as well as update epidemiological data. From our previous study, the major patient groups at risk for SFIs were HIV, TB, and haematological malignancy, and they represent a major national public health problem in Ghana (13). Thus, the choice of the at-risk patient groups studied in the research was influenced by the findings from this survey.

Furthermore, the current literature reveals a limited spectrum of SFIs that affect Ghanaians and their pattern and aetiological agents are unknown (Table 1.3). This data may be crucial to appreciate any changing epidemiological pattern such as emergence or re-emergence of infections. Considering the scarcity of adequate prospective studies, surveillance programmes or registries, analysing laboratory data provides a suitable alternative. Findings from a survey evaluating the laboratory capacity for diagnosing fungal infections in Ghana indicates SFIs are almost always going to be diagnosed by histopathology because direct microscopy and fungal culture are not routinely performed, and serological and molecular assays are not readily accessible. Review of histopathology reports has been previously used to evaluate the epidemiology of SFIs in some African countries such as Nigeria (60,314,315), Togo (59,61), and recently, Uganda (62). Therefore, retrospectively reviewing histopathology reports provide relevant demographic, clinical and laboratory characteristics. Additionally, evaluation of presumed clinical diagnosis allows for the assessment of the index of suspicion of clinicians requesting for histopathological investigations (63).

1.5 AIMS OF RESEARCH

1.5.1 Main aim

The primary aim of this research was to explore the use of RDTs at the centre of SFI diagnosis among relevant at-risk patient groups and to retrospectively evaluate the trend, spectrum, and aetiology of fungal infections in Ghana.

1.5.2 Specific objectives

I. To evaluate the frequency of CM and histoplasmosis among HIV-infected patients using CrAg LFA and *Histoplasma* EIA or LFA respectively

II. To compare the performance of *Histoplasma* EIA and *Histoplasma* LFA in detecting histoplasmosis

III. To determine the prevalence of CPA in presumed PTB patients using *Aspergillus*-specific IgG and IgM LFA

IV. To investigate the incidence of CPA among confirmed PTB patients receiving anti-TB treatment using *Aspergillus*-specific IgG and IgM LFA

V. To screen for IA among patients with haematological malignancy using *Aspergillus* GM LFA

VI. To retrospectively profile the trend, spectrum, and aetiology of histopathologically diagnosed fungal infections

1.5.3 Knowledge gaps, research questions and objectives

Drawing from the review of the literature particularly from Ghana and the established rationale of the thesis, specific knowledge gaps are identified, and strategic research questions and objectives are formulated to achieve the research aims (Table 1.8).

Table 1. 8: Knowledge gaps, research questions and objectives

Chapter	Knowledge gaps	Research questions	Research objectives
3	• CM burden in different categories of people living with HIV in Ghana is unknown • There is no epidemiological study on histoplasmosis from Ghana aside sporadic case reports • The utility of *Histoplasma* antigen testing for the diagnosis of histoplasmosis in Ghana is unknown • Performance of the OIDx *Histoplasma* LFA not externally evaluated	• How frequent is CM among different categories of people living with HIV in Ghana? • How common is histoplasmosis in people living with HIV in Ghana? • Can the OIDx *Histoplasma* LFA be a suitable alternative to the recommended IMMY *Histoplasma* EIA in detecting histoplasmosis in an African setting?	• To determine the frequency of CM and histoplasmosis among HIV-infected patients in Ghana • To compare the performance of OIDx *Histoplasma* LFA and the reference IMMY *Histoplasma* EIA in detecting histoplasmosis
4	• There is no data on the frequency of CPA among patients presumed to have new TB and TB relapse • No *Aspergillus*-specific IgG and IgM assay has not been utilized in Ghana • No study has reported clinical isolates of aspergillosis in Ghana	• How common is CPA among patients presenting with TB-like symptoms? • How to utilize the Aspergillus-specific IgG and IgM LFA to aid CPA diagnosis in Ghana? • What are the common aetiological agents of aspergillosis in Ghana?	• To determine the prevalence of CPA in patients with presumed new TB and TB relapse • To evaluate the use *Aspergillus*-specific IgG and IgM assay in Ghana • To identify the common aetiological agents of aspergillosis in Ghana
5	• No follow-up study of CPA has been done focusing on only bacteriologically confirmed PTB patients	• What is the incidence of CPA following the end of anti-TB treatment and 6 months post-treatment in bacteriologically confirmed PTB patients?	• To determine the incidence of CPA among confirmed PTB patients receiving anti-TB treatment at two timepoints, at the end of treatment and 6-month post-treatment

6	• No epidemiological and clinical data on IA in patients with haematological malignancy in Ghana • The use of *Aspergillus* GM LFA in the diagnosis of IA in West Africa unknown • Clinical experience of antifungal prophylaxis practice among patients with haematological malignancy in African settings is scarce	• How frequent IA occur among patients with haematological malignancies in Ghana? • Can the sona *Aspergillus* GM LFA reliably be diagnostic for IA? • How common is antifungal prophylaxis practiced in patients with haematological malignancies in Ghana?	• To generate epidemiological and clinical data on IA among patients with haematological malignancy in Ghana • To evaluate the clinical efficacy of *Aspergillus* GM LFA in the diagnosis of IA among haematological malignancy patients in Ghana • To evaluate the practice of antifungal prophylaxis in patients with haematological malignancies in Ghana
7	• The trend, spectrum and aetiology of fungal infections diagnosed in Ghana is unknown	• What is the fungal infections incidence pattern in Ghana? • What types of fungal infections are diagnosed in Ghana? • Are some rare moulds or yeast or endemic fungal infections diagnosed in Ghana?	• To evaluate the trend and spectrum of fungal infections in Ghana • To confirm aetiological agents of histopathologically diagnosed fungal infections with histomolecular analysis • Compare histopathology suggested aetiological agents and molecular identified agents

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CHAPTER 2: METHODOLOGY

2.1 RESEARCH FRAMEWORK AND METHODOLOGICAL APPROACH

The research project was generally designed to prospectively initiate and evaluate the use of simple RDTs for the diagnosis of SFIs in clinical settings in Ghana. RDTs that have been extensively evaluated and validated for major SFIs among common at-risk groups were selected and used in combination with other laboratory methods (such as direct microscopy, histopathology, culture) and medical imaging as provided in standard or recommended international guidelines. Additionally, to appreciate what SFIs have been diagnosed in the recent past, a retrospective study to evaluate histopathologically diagnosed fungal infections, including SFIs was implemented. Auxiliary molecular analysis on FFPE tissue blocks also allowed confirmation of the presence of fungal elements in tissues and provides relevant preliminary data for identifying aetiological agents. Both prospective and retrospective studies add to epidemiological data on SFIs in Ghana including prevalence or incidence rates, clinical characteristics, disease spectrum and causative organisms.

The studies were planned, designed and protocols developed by the author and the supervisory team at the University of Manchester, United Kingdom. Feasibility and implementation analysis of the study protocols was done by the author in collaboration with Heads of Departments/Units, where the studies were undertaken. Ethical clearance was first obtained from the institutional review boards of study sites in Ghana and afterwards, from the University of Manchester Research Ethics Committee. All studies were conducted by the author in Ghana during the duration of the PhD.

The main study site was the Korle-Bu Teaching Hospital (KBTH), which is the largest, premier tertiary in Ghana and serves as the national referral hospital. It has several specialized departments/units run by the most experienced consultants and specialists. KBTH is in Accra, the capital city of Ghana which is a cosmopolitan city habited by people from various parts of the country, with different ethnicities, languages, cultures, and customs. Four departments/units namely Fevers Unit (HIV Clinic), Chest Diseases Unit (TB Clinic), Haematology Department and Pathology Department of the KBTH was involved in the study. Additionally, there were three satellite sites, namely the Juaboso District Hospital (HIV Clinic), Ghana Standard Authority (Cellular Pathology Unit) and 37 Military Teaching Hospital (Pathology Division).

Patient identification and recruitment for studies 1 (based at HIV Clinic) and 2 (based at TB Clinic) started simultaneously and was led by the author with support from trained nurses, medical laboratory scientists and health information officers as research assistants. With regards to collection of clinical samples, the student was mainly involved in collecting samples at the TB Clinic. At the HIV Clinic, samples were collected by medical laboratory scientists or medical doctors during clinic days or ward rounds respectively. The student is then notified to come and pick the samples. However, some outpatient department (OPD) patients are referred for sample collection at the TB Clinic by the student.

All non-molecular laboratory works such as direct microscopy, culture and antigen-antibody tests were performed by the student at the TB Laboratory, Chest Clinic or the Research Laboratory, Department of Medical Microbiology. None of the study centres were performing these antigen-antibody tests at the time of the study. All antigen-antibody tests were run within 48 hours of sample collection or receipt, or occasionally only refrigerated without freezing but usually analysed the same day. All test results were shared with the clinical team. For the enzyme immunoassays (EIA), absorbance readings were done at the National Public Health Reference Laboratory, Korle-Bu. For molecular laboratory works, FFPE preparation and deparaffinization, DNA extraction and DNA amplification were done by the student and the technical team at the Clinical Virology Laboratory, Department of Medical Microbiology, UGMS. The remaining molecular works that is DNA sequencing were outsourced to Inqaba Biotec South Africa. Chest radiographs and CT scans were done at the Chest Clinic X-Ray Unit or Main Radiology Department, KBTH but when their service is unavailable outsourced to Supreme Specialist Imaging, Accra, Ghana.

Below are the respective protocols of all the five studies implemented in the thesis and the two studies that were subsequently terminated and changed due to COVID-19 associated challenges as previously expounded (see section on COVID-19 Impact Statement).

2.2 STUDY 1 PROTOCOL

Screening for Invasive Fungal Infections among Ghanaian HIV patients using non-culture-based methods

2.2.1 Study Design

This study had a prospective cross-sectional design

2.2.2 Study Sites

The study was conducted at two sites, that is HIV Clinic and Infectious Disease wards of the KBTH, Greater Accra region and HIV Clinic, Juaboso District Hospital, Western region.

2.2.3 Study population

Participants in this study included HIV-infected patients both newly diagnosed and ART experienced (currently on ART or lost-to-follow-up on ART) patients returning to care with complaints. Patients were eligible irrespective of presenting symptoms, disease stage, CD4 cells count or ART status. Additionally, healthy ART experienced patients with well-controlled viral load (< 20 copies/ml or target not detected), no new complaints, and so, likely at low risk for CM or DH returning to clinic for ARV restock were recruited into the control group to serve as control for the screening tests.

2.2.3.1 Inclusion criteria

i. Aged 18 years and above
ii. Participant or relative has given informed consent
iii. Confirmed HIV diagnosis

2.2.3.2 Exclusion criteria

i. Intake of antifungal drugs for at least 2 weeks in the last 3 months
ii. Previous history of cryptococcosis or histoplasmosis

2.2.4 Sample size

Recruitment targets:

- 150 newly diagnosed HIV patients and ART experienced patients returning to care
- 75 healthy ART experienced patients

Patients were recruited from September 2020 to November 2021.

2.2.5 Ethical Issues

Ethical clearance for the study was obtained from the Scientific and Technical Committee and Institution Review Board of the Korle-Bu Teaching Hospital (STC/IRB/00058/2020) (Appendix 2), University Research Ethics Committee of the University of Manchester, UK (UREC Ref: 2020-9372-16067) (Appendix 3) and administrative authorization from the Juaboso Government Hospital before commencing the recruitment. Recruitment into the study was preceded by prior explanation to the patients using the Study 1 Participant Information Sheet (Appendix 4). The

information sheet summarised the purpose of the study, procedure, and the expected contributions from patients. Patients had the opportunity to ask questions or raise concerns which were answered and resolved respectively. Study 1 Informed consent (Appendix 5) was obtained by the student or research assistants. Information sheet and consent form was translated and validated in the common local dialect Twi (Appendices 6 and 7). For patients in critical condition and unable to give personal consent, consent was provided by their relatives, kinsmen or care giver. Patient had the opportunity to withdraw consent at any time without giving any reason, since participation in the research was entirely voluntary.

2.2.6 Procedures

i. Patient's sociodemographic and occupational history data were collected by interviews and clinical data was anonymously retrieved from medical records onto the Study 1 questionnaire (Appendix 8) by the student or research assistants. Clinical characteristics data will include drug history (including ART and antifungals) and laboratory results (especially CD4 cells count and viral load).

ii. A 3 ml venous blood and urine (5-10ml) was collected from each patient into a serum separator tube (SST) (Becton Dickinson Company, Franklin Lakes, New Jersey, USA) and urine container (Life Medical Supplies, Korle Bu, Accra, Ghana) respectively when collecting samples for routine laboratory investigations. Blood samples were collected by the Fevers Unit's phlebotomists, who are licensed medical laboratory scientists with many years of experience and routinely collects blood samples at the Unit. Whole blood was allowed to clot, spun and serum separated. Serum and urine samples are sent to the Medical Microbiology Research Laboratory or TB Laboratory for analysis.

iii. CrAg LFA testing was done on sera using the CrAg LFA kit (Immuno-Mycologics Diagnostics, Norman, USA). When CrAg was positive, the same kit was used to perform CrAg semi-quantitative test to determine the titre following the manufacturer's titration formula. Urine samples were analysed with both IMMY clarus *Histoplasma* EIA (Immuno-Mycologics Diagnostics, Norman, Oklahoma, USA) and OIDx *Histoplasma* LFA (Optimum Imaging Diagnostics, Scarborough, Maine, U.S) *Histoplasma* GM antigen. The optical density (OD) of IMMY clarus *Histoplasma* EIA was recorded from a microplate reader and test line intensity of OIDx *Histoplasma* LFA was determined visually. All tests were performed according to manufacturer's instructions. When delays in testing was anticipated, samples were stored at 2-8°C for up to 72 hours.

iv. Patients with a positive urine IMMY *Histoplasma* EIA and in those unable to provide urine samples their serum was tested with both *Histoplasma* antigen tests.

v. Additional samples such as CSF, biopsies, blood, or sputum were received for further confirmatory tests from patients with either a positive serum CrAg (with titre >1:160) or positive urine *Histoplasma* EIA test as part of routine clinical care.

vi. When serum CrAg was positive at a titre ≥1:160 or 1: 80 with strong correlating neurological manifestations, the clinical team analysed eligibility for lumbar puncture (LP). The LP was performed as soon as feasible (mostly within 72 hours) and CSF tested by CrAg LFA, India ink and fungal culture.

vii. When urine *Histoplasma* GM EIA was positive, sputum, biopsy or blood was collected, based on patients' clinical manifestation of possible disease. Direct examination with Giemsa, histological examination with periodic-acidic Schiff (PAS) and fungal culture on Sabouraud dextrose agar, were done on received samples.

viii. The results of all investigations were passed on to the clinical team.

ix. Diagnosis, and directed management, of cryptococcosis and histoplasmosis was made by the clinical team incorporating clinical assessment, screening, and confirmatory test results. Treatment and three-month outcome details of patients who were diagnosed with cryptococcosis and histoplasmosis was obtained from medical records.

x. In the control group, after adequately informing patients and obtaining consent, blood and urine samples were collected and tested for CrAg and *Histoplasma* antigen respectively.

2.2.7 Data handling

Study data was managed according to the University of Manchester guided and supported data management plan. All information obtained from the participants was solely used for the purpose of this study. Confidentiality of the information provided by the participants was ensured and safeguarded. All paper documents were stored in a locked cabinet under the care of the student and digitised as soon as practicable. Access was restricted to main researchers that is student, academic supervisors, and lead collaborators in Ghana. All computer files were protected by a password. Copies of the files was created for backup storage in minimal two different computers and a specific internet drive. Every data entry process was evaluated to ensure that data is safe. The confidentiality of patient identity was maintained via coding process. When data is complete, the patient identifiers was replaced with a code number. The study was subject to the audit and

monitoring regime of the University of Manchester. The participants' privacy and anonymity will also be secured during the storage and publication of the research data.

2.2.8 Statistical analysis

All statistical analysis was performed with Statistical Products and Services Solutions (SPSS), version 25 (IBM Corp, Armonk, New York, USA) using a 5% significance level. Prevalence of cryptococcosis and histoplasmosis was determined as a diagnostic positivity percentage of the total number of recruited participants. Descriptive statistics including parameters such as frequencies, percentages, means, standard deviations, medians and IQR were used to summarize the patients' sociodemographic and clinical features for positive and negative screening cases, confirmatory results, and performance characteristics for IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA. Kappa index (κ) was used to measure the agreement between the IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA, with the former as the reference standard with the following interpretation for strength of agreement: poor (<0.00), slight (0 to 0.20), fair (0.21 to 0.40), moderate (0.0.41 to 60%), substantial (61 to 80%), and near perfect (81 to 100%). Also, point biserial correlation (r_{pbis}) was used to determine associations between the optical density (OD) of IMMY *Histoplasma* EIA and the test line intensity of OIDx *Histoplasma* LFA.

2.3 STUDY 2 PROTOCOL

Screening for Chronic Pulmonary Aspergillosis among suspected TB Patients in Ghana

2.3.1 Study Design

The study was a cross-sectional survey

2.3.2 Study Site

The study site was the Chest Clinic, Chest Diseases Unit, Department of Medicine, Korle-Bu Teaching Hospital, Accra. The Clinic acts as the national TB referral centre and hosts a specialized TB laboratory that receives samples from different parts of Ghana.

2.3.3 Study population

The study targeted at patients receiving care at the Chest Clinic of the KBTH, Greater Accra for suspected PTB or those referred from within the Greater Accra and other regions of the country to the TB laboratory for GeneXpert MTB (Xpert® MTB/RIF, Cepheid, California, USA) testing irrespective of their symptoms. Also, blood donors determined to have no symptoms and signs of

a respiratory condition or history of PTB, or any other chronic respiratory condition via an interview, were also recruited as control group participants from the KBTH Blood Bank, Southern Blood Area, National Blood Service, Ghana. The control group was included mainly to assess the specificity of the *Aspergillus*-specific antibody test.

2.3.3.1 Inclusion criteria

i. Aged 18 years and above
ii. Participant/relative has given informed consent
iii. Referred for GeneXpert MTB testing

2.3.3.2 Exclusion criteria

i. Asymptomatic individuals doing PCR TB testing for non-clinical purposes such as screening for travelling or school admission

2.3.4 Sample size

Recruitment targets:

- 180 patients suspected and being investigated for PTB
- 90 healthy blood donors

Patients were recruited from September 2020 to May 2021.

2.4.5 Ethical Issues

In Ghana, ethical approval was obtained from the Scientific and Technical Committee and Institutional Review Board of the Korle-Bu Teaching Hospital (STC/IRB/00058/2020) and the National Blood Services Ghana (NBSGRD/201410/02) (Appendix 2). In the UK, the University Research and Ethics Committee of the University of Manchester (Ref: 2020-9368-16168) (Appendix 3) also approved this study. Written informed consent was obtained from all participants. Patients with suspected PTB were consecutively approached for recruitment into the study. Prior to recruitment the Study 2 PIS (Appendix 4) was explained to the patient and any questions or concerns resolved. Interested patients then signed the Study 2 consent forms (Appendix 5). The PIS and the consent form was translated and validated into the common local dialect, Twi (Appendices 6 and 7) for some patients. For patients in critical condition and unable to give personal consent, permission was sought from relatives, kinsmen or care giver.

2.3.6 Procedures

i. Patients' demographics, clinical and socioeconomic details were collected via interviews using the Study 2 questionnaire (Appendix 8) by the student or research assistants. attending physician and recorded onto a structured questionnaire. Details collected included age, gender, smoking status, occupation history, documented respiratory disease (such as asthma, COPD), present symptoms and duration of symptoms.

ii. A 4ml sputum and 4 ml venous blood sample was collected from each participant into a sterile universal container and SST respectively. Sputum and blood samples were sent to the Medical Microbiology Research Laboratory or TB Laboratory for analysis.

iii. Direct microscopy for fungal elements (using KOH) and fungal culture was carried out on sputum samples. To enhance the growth of *Aspergillus* spp, a modified version of high-volume culture was adopted. Briefly this was done by inoculating an aliquot (1-2 ml) of undiluted sputum on Sabouraud dextrose agar and incubated at 37 °C for up to 8 days. When delayed testing is anticipated, samples were stored at -20°C at the Medical Microbiology Research Laboratory. All procedures were carried out following standard operating procedures as well as health and safety regulations.

iv. Whole blood was allowed to clot, spun and serum separated. Sera were tested for *Aspergillus*-specific antibodies with LDBio *Aspergillus* IgG & IgM LFA (LDBio Diagnostics, Lyon, France) and HIV antibody testing with HIV ½ RDT (Healgen Scientific LLC, Texas, USA) and confirmed with OraQuick HIV ½ RDT (OraSure Technologies, Pennsylvania, USA). Serum samples were run immediately or occasionally stored at 4^{o}C and run within 24 hours. Testing was done according to the manufacturer's instruction. Serum aliquots was stored in a -80^{o}C freezer at the Medical Microbiology Research Laboratory.

v. Chest radiograph was done for patients by radiographers at the Clinic and images reviewed and reported by a consultant radiologist. Chest radiograph was done for all patients except for patients who had obtained one within the previous month. The following predetermined abnormalities which often suggest a diagnosis of CPA including cavitation (single or multiple with sizes noted), fungal ball, pleural thickening and pericavitary fibrosis or infiltration was particularly noted for look out by the radiologist.

vi. Chest CT scan was done for patients with a positive *Aspergillus* antibody test or cavitation on chest radiograph including MTB positive cases. Among the MTB positive cases with cavitation, CT scan was done to unravel any concealed imaging features of CPA to identify possible PTB-CPA coinfection.

vii. Xpert MTB/RIF results were retrieved from laboratory records. TB diagnosis was confirmed when *Mycobacterium tuberculosis* was detected in a patient's sputum by Xpert MTB/RIF assay.

Patients with suspected or confirmed PTB were classified as new PTB and relapsed PTB as follows:

- new PTB-patients with no prior history of PTB
- relapsed PTB-patients who had been treated successfully for PTB in the past

viii. A case of CPA was defined following the guidelines for CPA diagnosis in resource-constrained settings, developed by the GAFFI international expert panel (2016). The panel defined a case of CPA as follows:

a. weight loss, persistent cough, and/or haemoptysis for >3 months

b. chest images showing progressive cavitary infiltrates and/or a fungal ball and/or pericavitary fibrosis or infiltrates or pleural thickening

c. a positive *Aspergillus* IgG assay or other evidence of *Aspergillus* infection.

ix. Patients who met criteria (a) and (b) above, but not (c), or met criteria (a) and (c), but not (b) were categorized as probable CPA.

x. In the control group, after adequately informing patients and obtaining consent, 4 ml venous blood samples were collected and tested for *Aspergillus*-specific antibody. Samples were collected by the Blood Bank nurses during donation.

2.3.7 Data handling

Study data was managed according to the University of Manchester guided and supported data management plan. All information obtained from the participants was used for the purpose of this study. Confidentiality of the information provided by the participants was ensured and safeguarded. All paper document was stored in a locked cabinet under the care of the PI and digitised as soon as practicable. Access was restricted to main researchers that is PI, academic supervisors, and lead collaborators in Ghana. All computer files were protected by a password. Copies of the files was created for backup storage in minimal two different computers and a specific internet drive. Every data entry process was evaluated to ensure that data is safe. The confidentiality of patient identity was maintained via coding process. When data is complete, the patient identifiers was replaced with a code number. The study was subject to the audit and monitoring regime of the UoM. The participants' privacy and anonymity will also be secured during the storage and publication of the research data.

2.3.8 Statistical analysis

All statistical analysis was performed with SPSS version 25 (IBM, Armonk, New York, USA) using a 5% significance level either with Chi Square or Fisher's exact tests. Summary statistics of variables (sociodemographic and clinical characteristics) was analysed using frequencies, means, standard deviations, medians, and interquartile ranges (IQR). Differences between groups was assessed using chi-square test, Fisher's exact test or Student's t-test were appropriate. CPA prevalence was calculated by the Jeffrey's method using a confidence interval of 95%. Fisher's exact tests were employed to compare proportions between groups. Logistic regression was carried out to assess the effect of individual symptoms and socioeconomic details on the likelihood of acquiring CPA.

2.4 STUDY 3 PROTOCOL

Screening for Chronic Pulmonary Aspergillosis in Confirmed Tuberculosis Patients Receiving Treatment: A Prospective Follow-Up Study

2.4.1 Study Design

A prospective longitudinal study design was implemented in this study. The study was an extension of the Study 2 above where follow-ups were made at two different time points.

2.4.2 Study Site

The study site was continued at the Chest Clinic, Chest Diseases Unit, KBTH, Accra. Patients receiving care at different health facilities were engaged to visit the Chest clinic at stipulated study schedules for review or follow-up.

2.4.3 Study Population

Patients recruited in Study 2, who had a positive GeneXpert MTB test report and subsequently placed on standard anti-TB treatment.

2.4.3.1 Inclusion criteria

i. Aged 18 years and above.
ii. Patient has a positive GeneXpert MTB test report.
iii. Patient/relative has given informed consent.

2.4.3.2 Exclusion criteria

i. Patients with a previous history of PTB
ii. Patients with Rifampicin resistance

2.4.4 Sample size

Recruitment targets: There were 41 patients eligible for follow-up and 20% lost to follow-up was assumed at both time points.

- 33 patients at first follow-up
- 26 patients at second follow-up

Patients were recruited from June 2021 to November 2021 at first time point and November 2021 to April 2022 at second time point.

2.4.5 Ethical Issues

The ethical issues previously described in Study 2 above equally applied to this study.

2.4.6 Procedures

i. Participants was checked on monthly via telephone calls after a prior permission has been obtained. They were encouraged to be available for follow-up. Participants was resurveyed at 6 and 12 months after treatment of PTB.
ii. Patients' demographics and baseline CPA screening findings including laboratory and chest radiograph reports were extracted from the primary research data, Study 2.
iii. Patients were then followed-up and further screened for CPA at two timepoints, within one month after completing treatment (T_1, 6-7 months from diagnosis) and 6–7-month post-treatment (T_2, 12-13 months from diagnosis).
iv. CPA screening involved assessment of symptoms, *Aspergillus*-specific antibody testing, sputum *Aspergillus* culture, chest radiograph and/or computed tomography (CT) scan.
v. Serum samples were obtained from all patients for *Aspergillus*-specific antibody testing with the LDBio *Aspergillus* IgG & IgM LFA (LDBio Diagnostics, Lyon, France) following the manufacturer's instructions.
vi. Sputum *Aspergillus* culture was done for all patients using a modified version of the high-volume culture method by inoculating an aliquot (1–2 ml) of undiluted sputum on Sabouraud dextrose agar and incubated at 37°C for up to 8 days.
vii. Chest CT scan was done for patients with positive *Aspergillus* serology or cavitation on baseline chest radiograph with new or persistent respiratory symptoms. All imaging

investigations were evaluated by a consultant radiologist (HG) blinded to clinical and laboratory findings. Xpert MTB/RIF and/or acid-fast bacilli (AFB) smear results were retrieved from laboratory records.

viii. CPA was defined based on the GAFFI diagnostic criteria (2018).

ix. Additionally, the quality of life (QoL) of patients were evaluated at both timepoints using the St. George's Respiratory Questionnaire (SGRQ), which scores patients from 1 (excellent health) to 100 (very ill).

2.4.7 Data handling

Study data was managed according to the University of Manchester guided and supported data management plan. All information obtained from the patients was used for the purpose of this study only. Confidentiality of the information provided by the patients was ensured and safeguarded. All paper document was stored in a locked cabinet under the care of the student and digitised as soon as practicable. Access was restricted to the student and academic supervisors. All computer files were protected by a password. Copies of the files was created for backup storage in minimal two different computers or storage devices and the University of Manchester student internet drive. Every data entry process was evaluated to ensure that data is safe. The confidentiality of patient identity was maintained via coding process. When data is complete, the patient identifiers was replaced with a code number. The study was subject to the audit and monitoring regime of the University of Manchester. The participants' privacy and anonymity will also be secured during the storage and publication of the research data.

2.4.8 Statistical analysis

Data was analysed with SPSS version 25 (IBM, New York, USA) at 5% significance level, using either Chi Square or Fisher's exact tests. Summary statistics were presented using frequencies and percentages for categorical variables. Fisher's exact tests were employed to compare proportions of the various characteristics of patients recruited at both timepoints.

2.5 STUDY 4 PROTOCOL

Invasive Aspergillosis among Haematological Malignancy Patients in Ghana

2.5.1 Study Design

This was a cross-sectional study.

2.5.2 Study Site

The study was conducted at the Department of Haematology, KBTH and UGMS. This centre is the national referral facility for the management of patients with haematology malignancies.

2.5.3 Study population

Patients with haematological malignancy including newly diagnosed, known cases receiving treatment or relapsed patients were prospectively recruited.

2.5.3.1 Inclusion criteria

i. Aged 18 years and above.
ii. Participant/relative has given informed consent.
iii. Newly diagnosed (last 4 months), active haematological malignancy receiving treatment or relapse

2.5.3.2 Exclusion criteria

i. Patients with aplastic anaemia were excluded as it represents a heterogenous group and not listed as a host factor among the haematological malignancy group in the EORTC/MSGERC definitions.

2.5.4 Sample size

Recruitment target:

- 65 patients with haematological malignancy

Recruitment was done during different phases of diagnosis and management as follows; a) within a week of new diagnosis, b) within a week of relapse, c) prior to starting chemotherapy d) during chemotherapy and e) end of chemotherapy.

2.5.5 Ethical issues

The study was approved by the Scientific and Technical Committee and Institutional Review Board of the KBTH (STC/IRB/00058/2020) (Appendix 2) and the UREC of the University of Manchester (Ref: 2022-13962-25109) (Appendix 3). Written informed consent was obtained from all participants. Recruitment into the study was preceded by prior explanation to the patients using the Study 4 PIS (Appendix 4) and patients' questions and concerns resolved. Consents were subsequently obtained with Study 4 consent forms (Appendix 5) by the student or research assistant. PIS and consent form was translated and validated to the local Ghanaian dialect, Twi (Appendices 6 and

7). For patients in critical condition and unable to give personal consent, permission was sought from relatives, kinsmen or care giver.

2.5.6 Procedures

i. Clinical and demographic data of patients were collected from medical records and documented onto the Study 4 questionnaire (Appendix 8).

ii. 3 ml of venous blood and sputum was obtained from each patient into a SST and universal container, respectively.

iii. Another venous blood sample was scheduled for collection within the next 7 days of collecting the first blood sample when feasible.

iv. If nasal aspirate, cerebrospinal fluid (CSF) or bronchoalveolar lavage fluid (BALF) are collected during routine clinical care aliquots were obtained for research laboratory analysis where possible. However, none of these specimens were collected.

v. Blood and sputum samples were labelled with unique patient and specimen information and transported to the sent to TB Laboratory immediately. Samples was refrigerated at 2-8°C or frozen at -20°C when delay is anticipated.

vi. Portions of sputum was directly examined using potassium hydroxide (KOH) (HiMedia Laboratories, Mumbai, Maharashtra, India) and lactophenol cotton blue (LPCB) (HiMedia Laboratories, Mumbai, Maharashtra, India)

vii. Remaining undiluted sputum was cultured on SDA, incubated at 37°C for up to 8 days and fungal growth identified by conventional methods using macroscopic and microscopic examination.

viii. Serum was analysed for *Aspergillus* GM antigen using the IMMY sōna® *Aspergillus* GM LFA (Immuno-Mycologics Diagnostics, Norman, Oklahoma, USA) following the manufacturer's instructions.

ix. Patients had a chest CT scan at the Radiology Department, KBTH or the Supreme Specialist Scan Limited. If patients had nasal or neurologic symptoms, CT scan of sinus and brain were conducted respectively.

x. All CT scans were reviewed by a radiologist blinded to clinical and laboratory data to assess abnormalities associated with IA and other invasive fungal infection.

xi. Cases of IA were classified as proven, probable, and possible based on the newly updated 2020 EORTC/MSGERC definitions.

2.5.7 Data handling

Study data was managed according to the University of Manchester guided and supported data management plan. All information obtained from the participants was used for the purpose of this study. Confidentiality of the information provided by the participants was ensured and safeguarded. All paper document was stored in a locked cabinet under the care of the PI and digitised as soon as practicable. Access was restricted to main researchers that is PI, academic supervisors, and lead collaborators in Ghana. All computer files were protected by a password and copies of the files was created for backup storage in minimal of two different computers and a specific internet drive. Every data entry process was evaluated to ensure that data is safe, and the confidentiality of patient identity was maintained via coding process. When data is complete, the patient identifiers was replaced with a code number. The study was subject to the audit and monitoring regime of the UoM. The participants' privacy and anonymity will also be secured during the storage and publication of the research data.

2.5.8 Statistical analysis

The data was analysed with SPSS version 25 (IBM, Armonk, New York, USA) with a $P < 0.05$ considered statistically significant using Chi-square. Summary statistics were presented using frequency as numbers and percentages for all categorical variables. Mean and standard deviation was calculated for non-normally distributed continuous variables.

2.6 STUDY 5 PROTOCOL

An Overview of Fungal Infections in Ghana: A 10-Year Retrospective Study of Histopathologically Diagnosed Cases

2.6.1 Study Design

This was a retrospective review of laboratory data and secondary analysis of archived samples.

2.6.2 Study Sites

This study was undertaken at the main pathology laboratory references within the Greater Accra which receives tissue samples from all over the country. These are the Histopathology Laboratory, Department of Pathology, KBTH; Histopathology Laboratory, J.M Wadhwani Department of

Anatomical Pathology, 37 Military Hospital and Histopathology Laboratory, Cellular Pathology Division, Ghana Standard Authority.

2.6.3 Study population

All histopathology reports describing the presence of fungal elements in deep tissues irrespective of the underlying condition or clinical presentation.

2.6.3.1 Inclusion criteria

i. Archived tissue blocks available for re-examination.

2.6.3.2 Exclusion criteria

i. Descriptions consistent with superficial or mucocutaneous fungal infections

2.6.4 Sample size

All positive reports with accompanying tissues block meeting the inclusion criteria were collected.

2.6.5 Ethical Issues

University Research Ethics Committee and Institutional Review Board approval was obtained from UoM and KBTH respectively before commencing the research. Information sheet and consent form was translated in a local dialect participant is comfortable with.

2.6.6 Procedure

i. The histopathology laboratory reports from 2012 to 2021 of the three Pathology Laboratories were reviewed to identify reports that mentioned the presence of fungal elements and distinctive features signalling the presence of a fungal infection. The reports were manually reviewed at the KBTH and 37 MH while at the GSA, reports were electronically searched. The following keywords were looked for or used: fungi, fungal element (s), fungal bodies, hyphae, yeast, pseudohyphae and spores. Secondarily, distinctive morphological appearance of fungal elements such as spherules, sclerotic/muriform/medlar/copper penny bodies and grains were looked for. All positive cases were included irrespective of underlying condition or site of sample collection.
ii. The sociodemographic and clinical details including age, gender, site of tissue collection, underlying diseases, clinical suspicion for fungal infection and microbiological results were extracted from laboratory records.
iii. Additionally, type of histological stain(s) used, and type of fungal element or feature seen, and fungal infection suggested by pathologists were all extracted from laboratory reports.

iv. The corresponding archived tissue blocks of positive cases were then retrieved for research analysis.
v. Tissue blocks were re-cut with the microtome (Leica Biosystems, Deer Park, Illinois, USA), re-stained with fungal stain, Periodic acid Schiff and re-examined by the student and a consultant pathologist for confirmation of the presence of fungal elements.
vi. Tissue blocks were deparaffinized, tissue digested, and DNA extracted Quick-DNA FFPE miniprep kit (Zymo Research Corporation, Irvine, California, USA) following the manufacturer's instructions.
vii. Pan-fungal PCR was carried out on DNA products for secondary confirmation of the presence of fungal elements using an in-house hemi-nested ITS gene PCR protocol. PCR assay was performed on a Maxygene II Thermal Cycler (Axygen Scientific, Union City, California, Spain).
viii. PCR products were purified and outsourced to a private genomics products and service provider, Inqaba Biotech for sequencing in South Africa.

2.6.7 Data handling

Study data was managed according to the University of Manchester guided and supported data management plan. All information obtained from the cases was only used for the purpose of this study. Confidentiality of the information extracted about the cases was ensured and safeguarded. All paper document was stored in a locked cabinet under the care of the PI and digitised as soon as practicable. Access was restricted to main researchers that is PI, academic supervisors, and lead collaborators in Ghana. All computer files were protected by a password. Copies of the files was created for backup storage in minimal two different computers and a specific internet drive. Every data entry process was evaluated to ensure that data is safe. The confidentiality of patient identity was maintained via coding process. When data is complete, the patient identifiers was replaced with a code number. The study was subject to the audit and monitoring regime of the UoM. Also, patient's privacy and anonymity will also be secured during the storage and publication of the research data.

2.6.8 Statistical analysis

All statistical analysis was performed with SPSS version 25 using a 5% significance level. Summary statistics of variables (sociodemographic, clinical and histopathology characteristics) was analysed using frequencies, percentages, means and standard deviations.

2.7 PROTOCOLS FOR TERMINATED STUDIES

2.7.1 Invasive Fungal Infections in Ghanaian HIV Patients: An Autopsy Study

2.7.1.1 Study Design

The study will be a cross-sectional study

2.7.1.2 Study Site

The study will be conducted at the Mortuary Unit, Department of Pathology, KBTH and University of Ghana Medical School. The Unit is the premier mortuary facility for the management of deceased patients at the KBTH and surrounding health facilities.

2.7.1.3 Study population

The study will be undertaken among deceased persons with HIV infection referred for autopsy or deceased patients diagnosed with HIV at autopsy.

2.7.1.3.1 Inclusion criteria

i. Persons aged 18 years and above who died with HIV and are to undergo autopsy
ii. Subject's relative has given informed consent

2.7.1.3.2 Exclusion criteria

i. Poorly preserved cadavers
ii. Organ donors and a medico-legal autopsy

2.7.1.4 Sample size

Recruitment target:

- 100 deceased persons with HIV infection

2.7.1.5 Ethical issues

The study was already approved by the Scientific and Technical Committee and Institutional Review Board of the KBTH (STC/IRB/00058/2020) (Appendix 2) and the UREC of the University of Manchester (Ref: 2020-9529-16112) (Appendix 3). Subjects will be enrolled from deceased HIV patients referred to the Mortuary Unit (Department of Pathology) of the Korle-Bu Teaching Hospital, Accra, Ghana. Prior to registration and booking for autopsy, Participant Information Sheet (Appendix 4) will be handed to the deceased's relative/kinsmen or caregiver, clearly explained to them and their questions answered. Those willing to allow involvement of the

deceased will be asked to give consent and obtained with consent forms (Appendix 5) by author. After obtaining consent, their deceased individual will be recruited as a subject. PIS and consent form was translated and validated to the local Ghanaian dialect, Twi (Appendices 6 and 7).

2.7.1.6 Procedures

i. Clinical (medical, drug, laboratory, and radiology) and demographic data will be collected from patient's medical records and documented onto research file after consent has been obtained from relatives.
ii. Information to be collected include patient's demographics, clinical history/features, including clinically ascertained cause of death, laboratory results, radiology findings (when available chest X-rays, CT, MRI and ultrasound scans that had previously been done for subjects will be accessed) and treatment regimen
iii. Research procedures and the routine autopsy procedure will run concurrently and so samples for research will be collected during routine autopsy
iv. Samples to be collected include blood, urine, cerebrospinal fluid, lung washings and tissue (from lung, heart, kidney, spleen, brain, and liver). Pus and effusion will be collected if present. Not all subjects will have extra sampling for research purposes; most of these samples will be obtained as part of the routine autopsy. In the case when a sample is not being obtained for routine autopsy, then a sample will be obtained for research purposes.
v. Peripheral or cardiac (preferably the right chamber) blood samples (5 mL) will be collected into a SST. 10 mL of CSF will be collected into a 50 ml falcon tube. Tissue (3 cm^3) samples will be collected from the lung, brain, liver, kidney, heart, and spleen into a 50 mL falcon tube containing 10 mL of phosphate buffered saline (PBS) and phosphate buffered formalin (PBF). In addition, grossly injured organs, cavities, and sinuses will be sampled. Multiple samples will be taken where there is more than one lesion or gross abnormality. Both lungs will be washed with physiological saline and 20 mL lung washings sampled into a 50 mL falcon tube. 10-20 mL of urine will be collected from the exposed bladder with syringe and needle into a 50mL falcon tubes. 5-10 ml of pus and effusions will be collected into a falcon tube when present.
vi. All samples will be labelled with unique patient and specimen information and transported to the TB laboratory immediately.

vii. Whole blood samples will be allowed 30 minutes to clot and then spun to obtain serum. Samples may be refrigerated at 2-8°C or frozen at -20°C when delay is anticipated.

viii. Direct microscopy: Lung washings, tissue, pus and effusion will be examined directly with KOH, Giemsa and GMS stains.

ix. Culture: Fungal culture will be carried on lung washings, CSF, and tissues in PBS on SDA, incubated at 37°C for up to 4 weeks and fungal growth identified by conventional methods using macroscopic and microscopic examination.

x. Antigen-antibody testing; Serum, urine, CSF, and lung washing will be screened for *Aspergillus* antigen using sōna® *Aspergillus* GM LFA (Immuno-Mycologics Diagnostics, Norman, Oklahoma, USA), *Histoplasma* antigen using clarus® *Histoplasma* GM EIA (Immuno-Mycologics Diagnostics, Norman, Oklahoma, USA) and *Cryptococcal* antigen using CrAg LFA (Immuno-Mycologics Diagnostics, Norman, Oklahoma, USA).

xi. Histopathology: Histological examination will be conducted on tissues in PBF and stained by H&E, GMS and PAS strictly following SOPs. Photomicrographs will be taken for slides showing the presence of fungal elements.

xii. PCR: PCR will be carried out on lung tissue/lung washing for *Pneumocystis jirovecii* Polymerase chain reaction (PCR) will be carried out on lung tissue/lung washing for *Pneumocystis jirovecii.*

xiii. Serum samples will be stored at -80 degrees Celsius for a maximum of 5 years (before being discarded) and other samples discarded according to existing guidelines.

xiv. Research investigations findings will be made available to the doctor who requested the autopsy following the existing results dispatching policy at the Department of Pathology.

xv. Autopsy procedures and examination of histology slides will be done by a pathologist. However, all mycology laboratory work will be carried out by the principal investigator.

2.7.1.7 Data handling

Study data was managed according to the University of Manchester guided and supported data management plan. All information obtained from the participants will be used for the purpose of this study. Confidentiality of the information provided by the participants will be ensured and

safeguarded. All paper documents will be stored in a locked cabinet under the care of the PI and digitised as soon as practicable. Access was restricted to main researchers that is PI, academic supervisors, and lead collaborators in Ghana. All computer files will be protected by a password and copies of the files will be created for backup storage in minimal of two different computers and a specific internet drive. Every data entry process will be evaluated to ensure that data is safe, and the confidentiality of patient identity will be maintained via coding process. When data is complete, the patient identifiers will be replaced with a code number. The study will be subject to the audit and monitoring regime of the University of Manchester. The participants' privacy and anonymity will also be secured during the storage and publication of the research data.

2.7.1.8 Statistical analysis

Data generated will be analysed with SPSS version 25 (IBM, Armonk, New York, USA) with a $P < 0.05$ considered statistically significant using Chi-square. Summary statistics will be presented using frequency as numbers and percentages for all categorical variables. Mean and standard deviation was calculated for non-normally distributed continuous variables.

2.7.2 Establishing A Ghana/West Africa Registry for African Histoplasmosis

2.7.2.1 Study Design

The study will be a prospective longitudinal study

2.7.2.2 Study Site

The main study will be based at the Dermatology Clinic, KBTH. Satellite sites where samples will be expected from where the Komfo Anokye Teaching Hospital, Kumasi, Ghana; Tokoin University Hospital Centre, Lomé, Togo; and Lagos University Teaching Hospital, Lagos, Nigeria. Advertisements, including social media, will be made to clinicians and scientists about the establishment of the African histoplasmosis registry for them to report or submit diagnosed cases or liaise with Dermatology Clinic to facilitate diagnosis of suspected cases. For health facilities other than the research sites in Ghana, the initial diagnosis will be made by an attending doctor and then reach out to the research dermatologists (Dr Mark-Young Seadey-Accra and Dr Martin Agyei-Kumasi) for confirmation of diagnosis.

2.7.2.3 Study population

Patients diagnosed with African histoplasmosis will be recruited for this study

2.7.2.3.1Inclusion criteria

i. Persons aged 16 years and above (majority of African histoplasmosis cases have been reported in teens and young adults)
ii. Participant/relative has given informed consent

2.7.2.3.2 Exclusion criteria

i. Patients there are no clinical samples available or cannot be provided

2.7.2.4 Sample size

Recruitment target:

- 40 patients diagnosed with African histoplasmosis

The aim will be to collect as many cases of African histoplasmosis as possible but a minimum of 40 patients will be required for data analysis.

2.7.2.5 Ethical issues

The study was approved by the Scientific and Technical Committee and Institutional Review Board of the KBTH (STC/IRB/00058/2020) (Appendix 2) and the UREC of the University of Manchester (Ref: 2020-9593-16127) (Appendix 3). Informed consent will be sought from those to be enrolled in the study by the principal investigator or external collaborators. After the diagnosis of African histoplasmosis has been made, the Participant Information Sheet (Appendix 4) will be handed to patients, clearly explained to them and their questions answered. Patients willing to partake will be asked to give a written consent (Appendix 5). Consenting patients will be recruited as participants. PIS and consent form has already translated to the local Ghanaian dialect, Twi (Appendices 6 and 7). For patients in critical condition and unable to give personal consent, permission will be sought from relatives, kinsmen or care giver.

2.7.2.6 Procedures

Investigations for suspected cases

i. Skin biopsy, a blood sample (3 mL) and a urine sample will be collected from participants if not already obtained during routine diagnostic workup. Skin biopsy will be collected into 50 mL falcon tubes containing PBS and PBF. When clinically present, pus will be sampled. One pus sample and biopsy in PBS will be stored at -20°C. When suppurative lesions are present, pus will be collected by attending physician into two sterile 5 ml falcon tubes. One pus sample and biopsy in PBS will be stored at -20°C. Additionally, when indicated in

disseminated cases, biopsy of affected organ(s) will be taken for histology and mycological examination.

ii. For bone involvement and suspected disseminated cases, the attending physician may conduct appropriate imaging studies. KOH preparation, Giemsa and GMS staining will be undertaken on pus sample and examined following SOPs. Histological examination will be carried on biopsy in PBF following SOPs. Photomicrographs will be taken for all positive cases. Skin lesions will be photographed. Imaging findings contributory to diagnosis will be documented.

iii. Positive cases will be assessed for HIV infection (if not already done) and any other immunodeficiency (that is, a CD4 count will be done). Participant will be assessed for HIV infection (if not already done) and any other immunodeficiency (i.e. a CD4 count will be done).

Investigations for diagnosed cases

i. Informed consent will be sort from AH diagnosed patients to be enrolled into study by research dermatologist/principal investigator.

ii. Case report forms (CRF) will be completed for enrolled patients. The CRF will anonymously capture case information about demographics, clinical history, signs and symptoms, risk factors and exposures and diagnosis approach

iii. Photographs of lesions and imaging findings will be taken.

iv. 5 mL EDTA blood and urine will be collected from participants.

v. As part of the study, patients will be provided with free itraconazole for a maximum of four months. As part of the study, If the doctor decides itraconazole (standard or recommended antifungal for histoplasmosis) is indicated for the treatment of African histoplasmosis, we will supply itraconazole for free for 4 months. This is because itraconazole is generally quite expensive, unavailable, and inaccessible in Ghana. The monitoring of the treatment and possible side effects will be the responsibility of the treating doctor, but we will monitor the outcomes.

vi. Pus and biopsy samples are cultured to isolate *Histoplasma capsulatum* var. *duboisii* following SOPs. Pus and biopsy samples will be cultured to isolate *Histoplasma capsulatum* var. *duboisii*

vii. *Histoplasma* antigen and antibody testing in urine and blood respectively. *Histoplasma* antigen and antibody testing will be done in urine and blood respectively

viii. var. *duboisii* isolates will be stored in glycerol at -80°C for later taxonomic studies. *Histoplasma* isolates will be stored and archived in glycerol at -80°C for later taxonomic studies

ix. DNA will be extracted and collected from blood sample for long-term storage and subsequent genetic analysis of susceptibility to African histoplasmosis. DNA will be extracted from blood sample for long-term storage, archiving and subsequent genetic analysis of susceptibility to African histoplasmosis.

Follow-up investigation

i. Participants will be checked on monthly via telephone calls. They will be encouraged to be available for follow-up where they will be reimbursed their transport fares. Monthly follow-up on patients for clinical examination findings during reviews. Collection of data on management and outcome on a case management form (CMF). Participants will complete the WHOQOL-BREF questionnaire for an assessment of their quality of life.

ii. Imaging studies will also be repeated for previously abnormal findings. Photographs will be taken again for lesions and imaging findings.

iii. Once treatment is completed patients will be followed up for relapse for 6 months. Photographs will be taken again for skin lesions. Once treatment is completed, patients will be followed up monthly for relapse for 6 months.

2.7.2.7 Data handling

Study data was managed according to the University of Manchester guided and supported data management plan. All information obtained from the participants will be used for the purpose of this study. Confidentiality of the information provided by the participants will be ensured and safeguarded. All paper documents will be stored in a locked cabinet under the care of the PI and digitised as soon as practicable. Access was restricted to main researchers that is PI, academic supervisors, and lead collaborators in Ghana. All computer files will be protected by a password and copies of the files will be created for backup storage in minimal of two different computers and a specific internet drive. Every data entry process will be evaluated to ensure that data is safe, and the confidentiality of patient identity will be maintained via coding process. When data is complete, the patient identifiers will be replaced with a code number. The study will be subject to

the audit and monitoring regime of the UoM. The participants' privacy and anonymity will also be secured during the storage and publication of the research data.

2.7.2.8 Statistical analysis

The data will be analysed with SPSS version 25 (IBM, Armonk, New York, USA) with a $P < 0.05$ considered statistically significant. Summary statistics were presented using frequency as numbers and percentages for all categorical variables. Mean and standard deviation was calculated for non-normally distributed continuous variables. Differences between variables in groups will be analysed by chi-square test, Fisher's exact test or Student's t-test. Predictors of treatment outcome will be identified by univariate analysis using logistic regression for different variables.

2.8 ETHICS APPROVAL AND MODIFICATIONS

The studies conducted in the thesis were subjected to ethical review by the University of Manchester and three local institutions, that is, KBTH, National Blood Service and 37 Military (Teaching) Hospital (37 MH). For KBTH and 37 MH, all five studies were advised to be put together into a single project proposal or protocol during application. However, for University of Manchester, it was required for individual studies put through separate applications except Study 2 (cross-sectional CPA in PTB) and Study 3 (longitudinal CPA in PTB) which was put together because it involved same patients. Cumulatively, the primary ethical application, review and approval process took about seven months and two months for the modifications and details are illustrated in Table 2. 1.

Table 2.1: Timeframes for obtaining approvals for studies

Institution	**Date of application**	**Date of approval**
Primary		
Korle-Bu Teaching Hospital		
-Scientific and Technical	19th March 2020	21st May 2020
-Ethical	21st May 2020	11th June 2020
University of Manchester		
-Study 1	16th April 2020	21st July 2020
-Study 2 and 3	16th April 2020	28th July 2020
National Blood Service	17th December 2020	4th February 2021

Secondary/Modifications		
Korle-Bu Teaching Hospital	27th August 2021	31st August 2021
37 Military (Teaching) Hospital	1st November 2021	1st February 2022
University of Manchester		
-New Study 4	27th April 2022	23rd August 2022

2.9 ATTACHMENT WITH UNIVERSITY OF GHANA MEDICAL SCHOOL

In Ghana, the PhD student was attached to the Department of Medical Microbiology, University of Ghana Medical School, Korle-Bu Campus, which also hosted the project. Prof Japheth Opintan, a faculty member at the Department, facilitated laboratory works and offered laboratory supervision. Also, clinical samples and isolates are archived for long term storage at the Department. The author was also partially accommodated at the TB Laboratory, Chest Clinic, KBTH.

2.10 CONTRIBUTIONS OF KEY EXTERNAL COLLABORATORS

The project involved an extensive collaboration and support from a team of multidisciplinary clinicians and academics, including pulmonologists, physician specialists, dermatologists, pathologists, infectious diseases, clinical/medical microbiologists, radiologists, haematologists, and oncologists. The detailed contribution of collaborators from Ghana are documented in Table 2.2.

Table 2.2: Key Collaborators in Ghana and their contributions

Name	**Institution (s) – Position (s)**	**Contribution (s)**
Dr Isabella Asamoah	Department of Medicine and Therapeutics, KBTH and UGMS - Infectious Diseases Consultant	-Reviewed Study 1protocol -Facilitated recruitment in Study 1 -Reviewed Study 1 manuscript (i.e., Chapter 3)
Dr Hafisatou Gbadamosi	Department of Radiology, KBTH and UGMS – Consultant Radiology	-Reported and reviewed imaging investigations (both chest x-ray and CT scan) in Studies 2, 3 and 4 -Reviewed manuscripts for Studies 2, 3 and 4 (i.e., Chapter 4, 5 and 6)

Mr Prince Ashong-Pappoe	Department of Medical Microbiology, UGMS – Research Scientist	-Facilitated molecular aspects of Study 5
Dr. Abraham Adjei	Chest Clinic, KBTH – Physician Specialist	-Facilitated recruitment in Study 2 -Reported and reviewed initial chest x-ray of Study 2 -Reviewed manuscript for Study 2 (Chapter 4)
Prof Japheth Opintan	Department of Medical Microbiology, UGMS – Medical Microbiologist	-Reviewed all study protocols -Facilitated the laboratory works of all studies -Reviewed the manuscripts for all published studies
Dr Peter Puplampu	Department of Medicine and Therapeutics, KBTH and UGMS - Infectious Diseases Consultant	-Reviewed Study 1protocol -Facilitated recruitment in Study 1 -Reviewed Study 1 manuscript (i.e., Chapter 3)
Dr Jane Afriyie-Mensah	Department of Medicine and Therapeutics, KBTH and UGMS – Consultant Pulmonologist	-Reviewed Studies 2 and 3 protocols -Facilitated recruitment in Studies 2 and 3 -Reviewed Studies 2 and 3 manuscripts (i.e., Chapter 4 and 5)
Dr Isaac Erskine	Department of Pathology, KBTH and UGMS – Specialist Pathologist	-Reviewed Study 5 protocol -Supported the extraction of cases or samples in Study 5 -Reported and reviewed histopathology slides
Dr Solomon Quayson	Department of Pathology, KBTH and UGMS – Consultant Pathologist	-Facilitated the implementation of Study 5 -Reviewed Study 5 protocol -Supported the extraction of cases or samples in Study 5
Prof Yvonne Dei-Adomako	Department of Haematology, KBTH and UGMS – Consultant Haematologist	-Reviewed Study 4 protocol -Facilitated recruitment in Study 4

		-Reviewed Study 4 manuscript (i.e., Chapter 6)

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CHAPTER 3: STUDY 1– SCREENING FOR INVASIVE FUNGAL INFECTIONS AMONG HIV PATIENTS USING NON-CULTURE-BASED ASSAYS

ABSTRACT

HIV is the commonest risk factor for SFIs, particularly in sub-Saharan African where the burden remains high. SFIs frequently associated with HIV are cryptococcal meningitis (CM), disseminated histoplasmosis (DH), *Pneumocystis jirovecii* pneumonia, oropharyngeal and oesophageal candidiasis are classified as AIDS-defining disease. Among these infections, CM and DH have the highest morbidity and mortality and are rarely the target of antifungal prophylaxis. CM diagnosis has greatly improvement since the introduction of the cryptococcal antigen (CrAg) lateral flow assay which is positive in almost all cases and more simplified treatment regimen recently reported. Also, the evolution of simple *Histoplasma* antigen (Histo Ag) assays is revealing evidence of histoplasmosis complicating HIV in Africa particularly mimicking tuberculosis a phenomenon well established in Latin America. Although, enzyme immunoassays are currently recommended, LFAs have been introduced and being evaluated but rarely studied in Africa. In Ghana, CM and DH are rarely suspected by clinicians due to limited epidemiological data and insufficient access to these simple but critical diagnostic tools. The aim of this study was to screen people with HIV (PWH) irrespective of symptoms with CrAg and Histo Ag and confirm positive cases with conventional methods. Additionally, a comparative analysis was made between the established IMMY Histo Ag EIA and newly introduced OIDx Histo Ag LFA for case detection of histoplasmosis. This was a cross-sectional study conducted among PWH in Ghana who are unwell. Sociodemographic and clinical data were collected by questionnaire through interviews and review of medical records. Serum and/or urine were obtained from patients and screened for CrAg and Histo Ag, using IMMY CrAg lateral flow assay (LFA) and IMMY *Histoplasma* enzyme immunoassay (EIA) kits, respectively, regardless of present symptoms. Samples run with IMMY *Histoplasma* EIA were simultaneously run with OIDx *Histoplasma* LFA. Results of antigen screening was shared with the attending clinical team and further testing with conventional techniques such as direct microscopy, culture, or histopathology for confirmation of disease was requested when indicated. Laboratory investigations were conducted by the research team while diagnosis incorporating clinical assessment, screening and confirmatory testing results and treatment decisions were made by the clinical team. Treatment and outcome information on CM and DH patients were retrospectively collected and evaluated. Overall, 150 patients were recruited. There were 73% (*n* = 109) females, and the age range was 18–62 years. Serum samples were obtained from all recruited

patients, but 107 patients provided urine samples. The prevalence rates of CrAg and Histo Ag were 2.7% (4/150) (95% CI, 0.1 – 5.3%) and 4.7% (5/107) (95% CI, 0.7 – 8.7%), respectively. The OIDx *Histoplasma* LFA showed a high concordance (98.4%) with the IMMY *Histoplasma* EIA. All antigen-positive cases by standard tests were diagnosed with CM and DH. Antifungal treatment was given in five patients and follow-up revealed two deaths and three recoveries. Histoplasmosis among PWH may be more common than previously anticipated and may be more frequent than cryptococcosis in Ghana. The performance of the OIDx *Histoplasma* LFA should be further explored.

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3.1 INTRODUCTION

Invasive fungal infections (IFIs) are an important cause of ill-health and deaths among people with HIV (PWH). Despite the global roll-out of highly active antiretroviral therapy (HAART), SFIs continue to affect PWH particularly in sub-Saharan Africa (SSA). This has largely been attributed to delayed HIV diagnosis, interruption of ART care, and high burden of advanced HIV disease (AHD) (1). Globally, SFIs are collectively estimated to cause about 47% of all AIDS-related deaths (2). The SFIs associated with the highest morbidity and mortality in PWH are cryptococcal meningitis (CM), disseminated histoplasmosis (DH), and *Pneumocystis jirovecii* pneumonia (PJP) (3).

Annually, over 200,000 CM cases occur globally, with 73% in SSA and responsible for 15% of AIDS-related deaths (4). Presently, the World Health Organization (WHO) recommends testing for cryptococcal antigen (CrAg) in PWH with a CD4 count less than 100 cells/μL (5). This recommendation has been evaluated to be cost-effective even at a low CrAg prevalence rates of 1.4% (6). Earlier studies on CrAg screening and CM had focused on ART-naïve patients, but recent studies among ART-experienced patients report similar rates (7–9). Among the ART-experienced population, virologic failure or viral non-suppression has been associated with a high burden of CM (10,11). In Ghana, only two major studies have been specifically conducted on CM. One was a retrospective study in PWH with CD4 count less than 100 cells/μl and the other a prospective study in PWH presenting with signs of meningitis; both reported a low prevalence of about 2% (2/92 and 1/53) (12,13). In related studies, *Cryptococcus neoformans* was the aetiological agent in 6% (5/84) and 11.7% (19/163) of cerebrospinal meningitis(14,15). Also, during the early ART era, a retrospective study reported CM as the cause of death in 3.3% (4/123) of hospitalized HIV/AIDS patients in Ghana (16).

Despite histoplasmosis being endemic in Africa, it seems underdiagnosed in many parts of the continent with scarce epidemiological data (17). In Cameroon, among patients with a clinical suspicion of histoplasmosis, *Histoplasma capsulatum* was detected in 7% (18). In Latin America, another endemic area, histoplasmosis is estimated to be a predominant HIV co-infection(19). In view of this, the Pan-American Health Organisation (PAHO) in collaboration with the WHO released guidelines for the diagnosis and management of DH among PWH (20). The burden of DH in Latin America is believed to be similar in SSA (21,22). In Ghana, of 12 individual histoplasmosis cases reported in the last six decades, 11 occurred in PWH (17).

Several antigen detection assays exist for CM and DH, and they have been exploited in screening programmes or studies in some countries in the Americas and Africa (23–29). These assays have been evaluated in previous studies (22,23,26,28,29,30–32). In Guatemala, an implemented

screening for CM, DH and TB reduced HIV deaths by 7% (24). For CM, there are many rapid diagnostic tests (RDTs) with an established high analytical performance, clinical relevance and more recently semi-quantitative forms have been introduced and are being evaluated (31,33). Although these tests have revolutionized CM diagnosis, increasing availability and accessibility to these tests have been slow and remain absent in many SSA countries including Ghana (34,35). Unlike CM, most available assays for DH are based on EIA, a technique which may not be readily available in many resource-limited laboratories due to required equipment and personnel training. RDTs have been recently introduced by manufacturers including MiraVista Diagnostics and Optimum Imaging Diagnostics (OIDx) for detecting Histo Ag in urine and/or serum, but only the former has been widely evaluated (36–38). However, internal evaluation studies of OIDx *Histoplasma* LFA reported a sensitivity of 95.1% and specificity of 96.1% (39). These *Histoplasma* RDTs are anticipated to consolidate the diagnostic efforts in detecting cases of DH in PWH especially in resource-limited settings. However, aside antigen detection tests, conventional techniques such as direct microscopy, histopathology and culture may be employed as confirmatory tests to prove infections and identify aetiological agents.

In Ghana, studies on CM are scarce and there are no studies on histoplasmosis in any risk group. CM and DH are thus rarely on the diagnostic radar of clinicians due to the limited epidemiological data. Generating epidemiological data is critical to informing and directing practice and policy changes. In this study, we screened for CrAg and Histo Ag, and subsequently established proven cases of cryptococcosis and histoplasmosis among PWH in Ghana and compared the performance of the OIDx *Histoplasma* LFA with IMMY *Histoplasma* EIA.

3.2 METHODS

Settings, Participants and Samples

In this prospective cross-sectional study, PWH returning to care (currently on ART or lost-to-follow-up on ART) and newly diagnosed HIV patients aged 18 years and above were recruited irrespective of presenting symptoms, disease stage, CD4 counts or ART status. Patients who had taken antifungal drugs for at least two weeks in the last three months, or with previous CM or DH were excluded. The study was conducted at the Korle-Bu Teaching Hospital and Juaboso Government Hospital, in the Greater Accra and Western North regions of Ghana respectively. Patients were recruited from September 2020 to November 2021. A well-structured questionnaire was used to anonymously collect sociodemographic by interviews and clinical data extracted from medical records. Blood and urine samples were collected from all participants. Additional samples such as CSF, biopsies, blood, or sputum were received for further confirmatory tests from patients with

either a positive serum CrAg (with titre >1:160, which is the optimal cut-off titre for predicting concurrent subclinical CM (40) or a lower titre with patient presenting with signs and symptoms suggestive of CM) or positive urine *Histoplasma* EIA test as part of routine clinical care. To serve as control for the screening tests, healthy PWH with well-controlled viral load (< 20 copies/ml or target not detected), no new complaints, and so, likely at low risk for CM or DH returning to clinic for ARV restock were recruited. Blood and urine samples were collected from these group for screening tests (Figure 3.1).

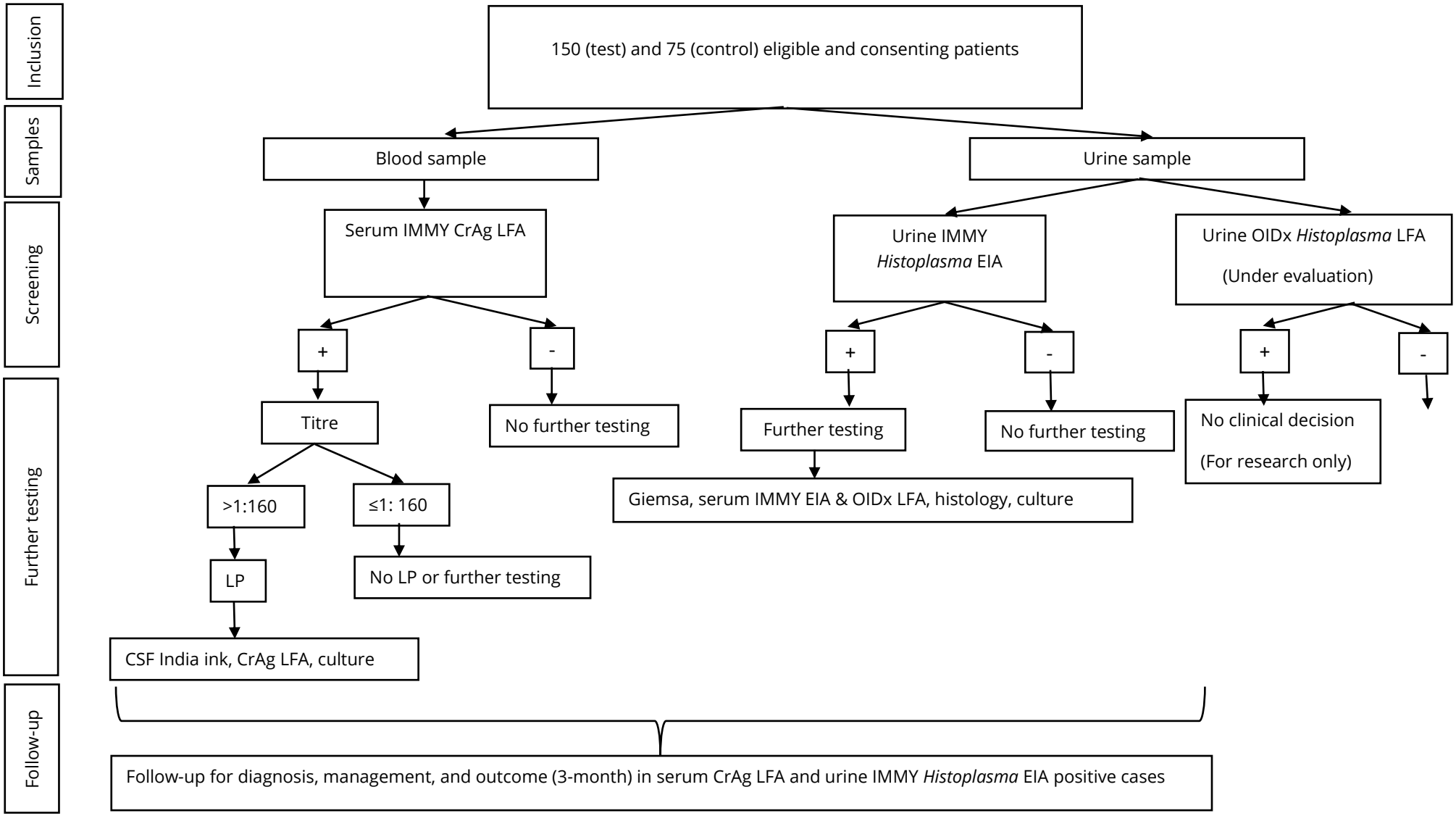

Figure 3.1: Study workflow

Screening and Confirmatory testing

CrAg screening was done on sera, using the IMMY CrAg LFA (Immuno-Mycologics Diagnostics, Oklahoma, U.S). When CrAg was positive, CrAg semi-quantitative test was done to determine the titre. Urine samples were analysed with both IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA (Optimum Imaging Diagnostics, Scarborough, Maine, USA). The optical density (OD) of IMMY *Histoplasma* EIA was recorded from a microplate reader and test line intensity of OIDx *Histoplasma* LFA was determined visually. All tests were done strictly following the manufacturer's instructions. In patients with a positive urine IMMY *Histoplasma* EIA and in those unable to provide urine samples both Histo Ag were tested on their serum. None of the study centres were performing these antigen tests at the time of the study. All antigen tests were run within 48 hours of sample receipt (after only refrigeration without freezing), and usually the same day. All screening test results were shared with the clinical team.

When serum CrAg was positive at a titre ≥1:160 or 1: 80 with strong correlating neurological manifestations, the clinical team analysed eligibility for lumbar puncture (LP). The LP was performed as soon as feasible (mostly within 72 hours) and CSF tested by CrAg LFA, India ink and fungal culture. When urine *Histoplasma* GM EIA was positive, sputum, biopsy or blood was collected, based on patients' clinical manifestation of possible disease. Direct examination with Giemsa, histological examination with periodic-acidic Schiff (PAS) and fungal culture on Sabouraud dextrose agar, were done on received samples. Outpatients were called back for admission appropriately. The results of all investigations were passed on to the clinical team.

Diagnosis, Management and Outcome

Diagnosis, and directed management, of cryptococcosis and histoplasmosis was made by the clinical team incorporating clinical assessment, screening and confirmatory test results (5,20,41). Treatment and three-month outcome details of patients who were diagnosed with cryptococcosis and histoplasmosis was obtained from medical records.

Data analysis

Data analysis was done using Statistical Products and Services Solutions (SPSS), version 25 (IBM Corp, Armonk, NY, USA). Descriptive statistics were used to summarize the patients' sociodemographic and clinical features for positive and negative screening cases, confirmatory results, and performance characteristics for IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA. Kappa index (κ) was used to measure the agreement between the IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA, with the former as the reference standard with the following interpretation for

strength of agreement: poor (<0.00), slight (0 to 0.20), fair (0.21 to 0.40), moderate (0.0.41 to 60%), substantial (61 to 80%), and near perfect (81 to 100%). Also, point biserial correlation (r_{pbis}) was used to determine associations between the optical density (OD) of IMMY *Histoplasma* EIA and the test line intensity of OIDx *Histoplasma* LFA. The alpha level was set at 0.05.

Patient Consent Statement

Ethical clearance for the study was obtained from the Institution Review Board of the Korle-Bu Teaching Hospital (STC/IRB/00058/2020), University Research Ethics Committee of the University of Manchester, UK (UREC Ref: 2020-9372-16067) and administrative authorization from the Juaboso Government Hospital. Prior to recruitment, a Participant Information Sheet was shared with patients, any questions or concerns respectively answered and resolved and a written informed consent subsequently obtained.

3.3 RESULTS

Patients' background details

Overall, 150 patients with 54 (36%) inpatients were recruited into the study. There were 109 (72.7%) females, and the mean age was 42.9 years (range, 18-62). Recent (within the last three months) CD4 count, and viral loads were available for 73 (48.7%) and 139 (92.7%). The median and interquartile range for CD4 count and viral load were1049.1 cells/µl (258.4-1480.6) and 18367.9 copies/ml (4524.1-44633.9), respectively. Of the recruited patients, 78 (52.0%) were presently on ART, 41 (27.3%) were lost to follow-up on ART and 31 (20.7%) were ART naïve or newly diagnosed. The main exposure risks identified were farming or agricultural activities (20.7%, *n* = 31), and livestock rearing or animal contact (10.0%, *n* = 15) (Table 3.1).

Table 3.1: Demographics, clinical details, and exposure factors in positive antigen and negative antigen groups

Participants' features	**Total (150)**	**Positive CrAg+ HistoAg (9)**	**Negative CrAg +HistoAg (141)**
	Number (%)	**Number (%)**	**Number (%)**
Sex			
Male	41 (27.3%)	2 (22.2%)	39 (27.7%)
Female	109 (72.7%)	7 (77.8%)	102 (72.3%)

Age (years)			
Median	42.9	43.0	42.6
ART status			
Newly diagnosed	31 (20.7%)	2 (22.2%)	29 (30.9%)
Defaulter	41 (27.3%)	3 (33.3%)	38 (30.0%)
On ART	78 (52.0%)	4 (44.5%)	74 (52.5%)
Patient group			
Outpatient	96 (64%)	3 (33.3%)	93 (66.0%)
Inpatient	54 (36%)	6 (66.7%)	48 (34.0%)
CD4 count (cells/µl)			
Number	73 (48.7%)	4 (44.4%)	69 (48.9%)
Median	1409.1	204.5	1362.0
Viral load (copies/ml)			
Number	139	8	131
Median	18367.9	105345.3	16890.3
Exposure factors			
Farming	31 (20.7%)	7 (77.8%)	24 (17.0%)
Animal contact	15 (10.0%)	3 (33.3%)	12 (8.5%)

Prevalence of serum CrAg and urine Histo Ag and confirmatory tests

Out of the 150 patients, 43 did not provide urine samples. CrAg LFA was positive in 2.7% (4/150) (95% CI, 0.1 – 5.3%) patients while IMMY *Histoplasma* EIA was positive in 4.7% (5/107) (95% CI, 0.7 – 8.7%). All four serum CrAg positive samples had titres above 1: 160, but CSF sample was received from only three and positivity rates were 100% (3/3), 66.7% (2/3) and 33.3% (1/3) for CrAg LFA, India ink and culture, respectively. Four whole blood and three skin biopsies (patients showing skin lesions) were received from the urine IMMY *Histoplasma* EIA positive patients for confirmatory

testing. Recorded positivity was 50% (2/4) for whole blood Giemsa staining, 100% (3/3) for histology on skin biopsy but there was no *Histoplasma* growth in any sample. Out of the five serum samples from positive urine IMMY *Histoplasma* EIA cases, only three tested positive. IMMY *Histoplasma* EIA performed on the serum of 43 patients without urine samples were all negative (Table 3.2). No sample was positive for both CrAg and Histo Ag. Additionally, serum CrAg LFA and urine IMMY *Histoplasma* EIA was negative among all patients in the control group.

Table 3.2: Screening results in total participants and confirmatory findings in cryptococcosis and histoplasmosis cases

Screening tests		**Total**	**Cryptococcosis**	**Histoplasmosis**
Serum CrAg				
	Number	150	4	5
	Pos (%)	4 (2.7)	4 (100)	0 (0)
Urine Histo GM EIA				
	Number	107	4	5
	Pos (%)	5 (4.7)	0 (0)	5 (100)
Confirmatory tests				
CSF				
	Number	3	3	–
	CrAg Pos (%)	3 (100)	3 (100)	–
	India ink Pos (%)	2 (66.7)	2 (66.7)	–
	Culture Pos (%)	1 (33.3)	1 (33.3)	–
Whole blood				
	Number	4	–	4
	Giemsa Pos (%)	2 (50)	–	2 (50)
Skin biopsy				

	Number	3	–	3
	Histology Pos (%)	3 (100)	–	3 (100)
	Culture Pos (%)	0 (0)	–	0 (0)

Comparison of IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA

IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA were performed on 230 samples comprising 107 urine samples from the test group, 43 serum samples from patients unable to provide urine samples, serum samples from five patients whose urine were positive for IMMY *Histoplasma* EIA and 75 urine samples from the control group (Table 3.3). Among the 107 urine samples, there were six positives with OIDx *Histoplasma* LFA including all five that were positive for IMMY *Histoplasma* EIA. The OIDx *Histoplasma* LFA also recorded three positives among the 43 serum samples and two positives in control urine samples. Additionally, for the three IMMY *Histoplasma* EIA positive sera from patients with *Histoplasma* antigenuria, the OIDx *Histoplasma* LFA were also positive. Thus, there were six positives for only OIDx *Histoplasma* LFA (where IMMY *Histoplasma* EIA was negative). Evaluation of agreement between the OIDx *Histoplasma* LFA and IMMY *Histoplasma* EIA, showed an overall high concordance (98%).

Table 3.3: Comparison of EIA and LFA in urine and serum samples and their performance characteristics

Specimen		**IMMY Histo EIA**	**OIDx Histo LFA**	**% Agreement**
Urine (*n* = 107, test group)				
	Pos (%)	5 (4.7%)	6 (5.6) [a]	99
Serum (*n* = 43, no urine samples)				
	Pos (%)	0 (0)	3 (7.0)	93
Serum (*n* = 5, urine *Histoplasma* GM EIA positive)				
	Pos (%)	3 (60)	3 (60) [a]	100
Urine (*n* =75, control group)				
	Pos (%)	0 (0)	2 (2.7)	97

Urine (*n* =182, test and control groups)				
Pos (%)		5 (4.7%)	2 (2.7)	98
Parameter (Based on urine samples)				
	Sensitivity	100.0%	100.0%	
	Specificity	100.0%	98.3%	
	PPV	100.0%	62.5%	
	NPV	100.0%	100.0%	
	Accuracy	100.0%	98.4%	
Parameter (Based on serum samples)				
	Sensitivity	60.0%	60.0%	
	Specificity	100.0%	93.0%	
	PPV	100.0%	50.0%	
	NPV	95.6%	95.2%	
	Accuracy	95.8%	89.6%	

a = EIA positives were also LFA positive; Kappa = 0.90; $p < 0.0001$

The Kappa index indicated a near perfect agreement between the two test kits, and this was statistically significant (Kappa value = 0.90; $p < 0.0001$) (Table 3.3). The point biserial correlation analysis involving the IMMY *Histoplasma* EIA optical densities and the OIDx *Histoplasma* LFA test line intensities of all the positive cases (for both the test and control groups) revealed a medium, but statistically insignificant association between the two variables ($r_{pbis} = 0.38$, $p = 0.19$).

CM was diagnosed in all patients with positive serum CrAg LFA while five out of the six patients with positive urine Histo Ag (both IMMY *Histoplasma* EIA and OIDx *Histoplasma* LFA) were diagnosed with DH (Table 3.4). Among the cases with only a positive OIDx *Histoplasma* LFA both in test and control groups, there was no evidence of histoplasmosis infection. Thus, the prevalence of CM was determined to be 2.7% (4/150), and that of DH was 4.7% (n=5/107). The patients diagnosed with an SFI had a lower CD4 count (median SFI, 204.5 vs. median non-SFI, 1362.0) and a higher viral load (median SFI, 105,345.3; vs. median non-SFI, 16,890.3) than those not diagnosed

with SFIs. However, there was no significant difference (p = 0.26) between CD4 and viral load for CM and DH patients.

Diagnosis, Treatment and Outcome

Table 3.4: Demographics, clinical details, risk exposure, laboratory findings, diagnosis, treatment, and outcomes of patients diagnosed with cryptococcosis and histoplasmosis

Age	Sex	Patient Group	ART Status	Risk	sCrAg	uHisto	sHisto	cCrAg	cDM	cCul	bDM	tDM	tPath	tCul	Dx	Rx	Outcome
43	F	In	LA	–	+	–	–	+	+	–	ND	ND	ND	ND	CM	FLC, FLC+AMB	Recovered
30	F	Out	A	Fa, AC	–	+	+	ND	ND	ND	–	–	+	–	DH	LTFU	–
45	F	In	LA	Fa	+	–	–	+	+	–	ND	ND	ND	ND	CM	FLC, FLC+AMB	Recovered
43	F	In	A	Fa	–	+	+	ND	ND	ND	+	+	+	–	DH	ITR	Died
67	M	Out	A	–	+	ND	–	ND	ND	ND	ND	ND	ND	ND	CM	FLC	Recovered
50	M	In	A	Fa, AC	–	+	–	ND	ND	ND	ND	–	–	–	CH	LTFU	–
28	F	Out	N	Fa, AC		+	+	ND	ND	ND	ND	+	+	–	DH	RT	–
19	F	In	N	Fa	+	–	–	+	+	+	ND	ND	ND	ND	CM	NT	Died
43	M	In	LA	Fa	–	+	+	ND	ND	ND	+	–	–	–	DH	ITR	Recovered

Abbreviations: [A] on ART; [LA] lost to follow-up on ART; [F] female; [M] male; [In] Inpatient; [Out] Outpatient; [Fa] farming; [AC] animal contact; [ND] not done; [CM] cryptococcal meningitis; [DH] disseminated histoplasmosis; [FLC] fluconazole; [AMB] amphotericin B (conventional); [ITR] itraconazole; [LTFU] lost to follow-up; [RT] refused treatment; [NT] not treated; [R] recovered

Two patients diagnosed with DH were lost to follow-up. One patient with CM died within a week of diagnosis prior to antifungal treatment. One DH patient, who was newly diagnosed with HIV refused any form of treatment. Out of the five patients that received antifungal therapy, the CM patients were treated with amphotericin B deoxycholate 1mg/kg/day for two weeks and fluconazole 1200 mg/daily for 12 weeks as induction regimens depending on availability and patients' financial capacity while the two DH patients were placed on itraconazole 400 mg/daily for six months. A 3-month follow-up showed all CM were doing well but one DH patient had died. Overall mortality rate for patients diagnosed with SFIs was 33.3%.

3.4 DISCUSSION

These findings show the potential impact of antigen detection assays for diagnosis of CM and DH in LMIC settings where WHO-listed essential diagnostics are unavailable (34,42). Importantly, the prevalence of histoplasmosis (4.7%) (95% CI, 0.7 – 8.7%) was shown to be probably higher than that of cryptococcosis (2.7%) (95% CI, 0.1 – 5.3%). This is the first epidemiological study of histoplasmosis among any risk-group in Ghana providing important data to guide diagnostic decisions for this largely unrecognized infection. Similar studies in South Africa, Guatemala and Mexico also reported higher Histo Ag positivity than other SFIs (24,27,29). In our study, direct examination and histopathology for DH were helpful and were contributory in establishing proven CM or DH. However, culture positivity was very low. The possible explanation for this observation is early treatment for CM patients because serum CrAg titre was > 1: 2560 in all patients and not directly inoculating biopsy on media as *H. capsulatum* rarely survives in clinical specimen and must be inoculated as soon as possible to increase recovery. Considering the time, cost, equipment, and training required to run an EIA, efforts are now directed at developing LFAs (36–38). The world's first LFA for *Histoplasma* detection was developed by MiraVista Diagnostics. Evaluation studies have revealed high concordance, Kappa index ranging from 0.66 – 0.90 between the MiraVista *Histoplasma* LFA and EIAs in both urine and serum (36–38). The recently introduced OIDx *Histoplasma* LFA, only has internal evaluations to date(39). Our study shows that the OIDx *Histoplasma* LFA has excellent sensitivity (100%), specificity (98.3%) and in comparison, with the IMMY *Histoplasma* EIA shows a near perfect agreement (98.4%). However, there were six false positives. Comparing our findings with a study of the MiraVista *Histoplasma* LFA on urine samples, the OIDx *Histoplasma* LFA had a higher sensitivity (OIDx ,100.0% vs MVista, 93.2%) but lower specificity (OIDx, 98.3% vs MVista, 99.3%) in proven cases (38). Contrarily, on sera the OIDx *Histoplasma* LFA had a lower sensitivity (OIDx, 60.0% vs MVista, 96%) but higher specificity (OIDx, 93.0% vs MVista, 90%) (36). It is, however, worth noting that, our study had a different population,

very few positives compared to the MiraVista *Histoplasma* LFA studies and thus the comparison may be invalid. In another evaluation using frozen urine samples (Nigeria), much lower sensitivity of the OIDx *Histoplasma* LFA was found (unpublished data). Our findings also suggest OIDx *Histoplasma* LFA may be superior to the existing OIDx *Histoplasma* EIA in terms of sensitivity, specificity and accuracy (OIDx LFA, 100%; 98%; 98% vs OIDx EIA, 92%; 32%; 51%) (43) . Our study is the first using freshly collected samples.

Unfortunately and needing critical attention is that the current Ghana HIV guidelines only acknowledge CM as the HIV-associated SFIs of concern (44). This, in addition to inadequate awareness, has resulted in SFIs being rarely suspected or investigated. In a few circumstances where these tests may be requested, they are not available in the in-hospital laboratory, and are expensive at private facilities, as patients need to pay out-of-pocket. This is because the investigations are not captured on the National Health Insurance Scheme (NHIS) in Ghana, despite falling under the HIV/AIDS symptomatic management for opportunistic infections category (45). However, both CrAg LFA and Histo Ag EIA are on the WHO essential diagnostic list, and Ghana is yet to develop its Essential Diagnostic list, as in many other countries (42).

The prevalence of cryptococcosis in the present study is slightly higher than reported in prior studies (12,13). Cryptococcal disease studies in Ghana have consistently reported lower rates in comparison to other countries in SSA (7,9,31,46,47). One probable reason for this observation is that all the studies in Ghana were done in the two largest cities, while most of the other studies elsewhere are conducted in small towns or rural areas and mostly in AHD. In this era when CD4 testing is not popular locally, this is challenging.

Clinical suspicion of SFIs was only made prior to screening tests in three of nine patients who were eventually diagnosed. We, therefore, extrapolate that the majority of these SFIs are likely to be missed in Ghana particularly for DH. Among patients diagnosed with SFIs, the majority were on admission (66.7%) and not receiving ART (55.5%). This is a well-established phenomenon, not just for SFIs, but for other opportunistic infections (14,24,29,48). Improving early HIV diagnosis and maintaining those taking ART in care should reduce the prevalence of SFIs.

Presently, treatment of CM and DH in Ghana is faced with challenges due to inadequate availability and accessibility of antifungal agents, especially amphotericin B and flucytosine (49). These difficulties were demonstrated in our study. For the CM patients, two received fluconazole alone initially due to accessibility and financial constraints but later changed to amphotericin B when they failed to respond. This also comes with the additional challenge of local clinicians' experience

with the use and managing the side-effects of amphotericin B(5). Fortunately, a recent study reveals a single high-dose liposomal amphotericin B is as efficient as a week of daily amphotericin B deoxycholate (50). The use of fluconazole monotherapy is popular in many LMIC settings, but is sub-optimal in the management of CM (51,52). Notwithstanding, one CM patient has been on fluconazole monotherapy and is doing well with good adherence to ART. Regarding DH, both patients received itraconazole. However, one patient had severe disease and amphotericin B deoxycholate would have been the preferred therapy, if available. Four patients comprising one CM and three DH did not receive antifungal treatment. The CM patient died while efforts were being made to acquire amphotericin B which out-of-stock at the hospital. This re-emphases the importance of prompt initiation of effective antifungal agents although mortality remains high at about 44% even with treatment(53). Two patients with DH were lost-to-follow-up and the third person refused any form of treatment including free ARV possibly because patient had to be admitted longer and the cost of treatment is borne by the patient out-of-pocket.

There were some limitations to our study. The selected study sites, sampling and number of positives may not be reflective of a broader nationwide conclusion. The diagnosis of CM or DH may be missed if a false negative antigen assay which occasionally happens with low fungal burden or prozone effect. The study population shifted from only high-risk ART-experienced patients to include newly diagnosed due to the decline in clinic attendance during the peak of COVID-19. This resulted in convenient sampling, and thus a relatively low proportion of patients with AHD. Moreover, CD4 and viral load tests results were not available for many patients. CD4 count has become unpopular in the era of 'test and treat', and attention is now focused on viral loads. Unfortunately, viral load tests were not done sometimes due to reagent shortages. Furthermore, post-mortem investigation was not done on patients who died with SFIs to ascertain if it was the cause of death. Despite the above limitations, the present study generated novel epidemiological data for DH in Ghana, showing that DH is an important but largely unrecognized opportunistic fungal infection in PWH.

3.5 CONCLUSION

This study reveals that histoplasmosis and cryptococcosis may be an unrecognized but relatively common SFI in Ghana, and diagnostics like CrAg LFA and *Histoplasma* EIA could facilitate diagnosis. Notably, histoplasmosis may be at least as common as cryptococcosis. The OIDx *Histoplasma* LFA appears to have good sensitivity and acceptable specificity, but more data are needed to confirm this.

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CHAPTER 4: STUDY 2– SCREENING FOR CHRONIC PULMONARY ASPERGILLOSIS AMONG PRESUMED TUBERCULOSIS PATIENTS IN GHANA

ABSTRACT

Chronic pulmonary aspergillosis (CPA) may mimic pulmonary tuberculosis (PTB). The two diseases are clinically indistinguishable and may result in CPA misdiagnosed as PTB or vice versa. Although, PTB is largely recognised as a differential diagnosis of CPA and often ruled out prior to CPA diagnosis, the converse is uncommon. The aim of this study was to determine the proportion of CPA cases among patients being assessed for PTB. A cross-sectional survey was conducted among consecutive patients referred for GeneXpert *Mycobacterium tuberculosis* (MTB) test for the diagnosis of PTB at the Korle-Bu Teaching Hospital, Accra, Ghana. Patients' demographics, clinical and socioeconomic details were obtained using a structured questionnaire. Blood was collected for *Aspergillus* and HIV serology, and sputum samples obtained for *Aspergillus* culture. Chest radiograph was obtained, and computed tomography (CT) scan was also done for patients with positive *Aspergillus* serology or cavitation. CPA was defined using an algorithm developed by the Global Action for Fungal Infections (GAFFI) international expert panel. One hundred and fifty-four patients were included in the analysis, of whom 134 (87%) did not have a prior PTB diagnosis. There were 41 (26.6%) GeneXpert positive cases. CPA prevalence was 9.7% (95% CI, 5.0 – 14.4%) overall, but 50% (95% CI, 28 – 72%) in patients with a prior history of PTB and 3.7% (95% CI, 0.5 – 6.9%) in those without previous PTB. Although CPA is rarely considered as a differential diagnosis of PTB in Ghana, our findings show that CPA may affect half of patients being assessed for PTB relapse. Efforts to diagnose CPA should be prioritised in this patient group.

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4.1 INTRODUCTION

Pulmonary fungal infections have increased in clinical significance in recent times, and although many of them mimic pulmonary tuberculosis (PTB), chronic pulmonary aspergillosis (CPA) is one of the commonest (1). CPA is a slow, progressive, and destructive lung disease associated with both respiratory and systemic symptoms. Globally, approximately 3 million people suffer from CPA, with 1.2 million occurring as a sequel of PTB (2). In Ghana, CPA among PTB patients is estimated at 2600 cases annually (3). PTB is a common differential diagnosis of CPA, and could occur before, after, or infrequently, together with CPA (4). There are many similarities between PTB and CPA in terms of risk factors, clinical presentation, and radiological features, making the two diseases clinically indistinguishable (5). This may result in misdiagnosis of CPA as PTB, or vice versa. As PTB is more common and largely recognized globally, the index of suspicion for PTB is likely higher compared to CPA, particularly in settings with a high PTB burden. Being mostly diagnosed as a post-PTB complication, CPA may be misdiagnosed as relapsed PTB infection and managed as such (5). CPA may, also be occasionally misdiagnosed as primary TB infection (5). Some studies have reported CPA misdiagnosed as acid-fast bacilli (AFB) smear-negative or GeneXpert *Mycobacterium tuberculosis* (MTB)-negative PTB and resulting in worsening symptoms and anti-TB treatment failure (6,7). Previous and present guidelines for CPA diagnosis have recommended a necessary exclusion of PTB (4,8). However, with emerging concerns of primary CPA and CPA co-existing with PTB, it may be equally important to rule out CPA when making a diagnosis of PTB to avoid inappropriate exposure of patients to anti-TB medications.

Unfortunately, differentiating CPA from PTB in many high TB burden countries, which are mostly resource-constrained, is a major challenge. This is probably because of inadequate awareness, unavailable diagnostic laboratory support and omission of CPA as a differential diagnosis in existing local guidelines. To improve the *status quo*, the Global Action for Fungal Infections (GAFFI) convened an international expert panel in 2016 to develop a CPA guideline specific for resource-constrained settings (8). However, the lack of *Aspergillus* serology testing capacity, which is key to CPA diagnosis poses, a significant limitation to the general use of these guidelines (9–11). Until recently, the common standard and commercially available methods were precipitins and enzyme immunoassay (EIA). The drawbacks of these techniques include cost, long turnaround time, poor interlaboratory reproducibility, and variable cut-off values (9,12–14). Additionally, these tests require sophisticated equipment and adequate laboratory expertise. LDBio Diagnostics introduced a new rapid diagnostic test (RDT) in the form of a lateral flow assay (LFA) for the detection of *Aspergillus*-specific IgG and IgM antibodies based on immunochromatography

technology, that meets the World Health Organization (WHO) ASSURED ("Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end users") criteria and may be more suitable for use in resource-constrained settings. Several evaluation studies or clinical use of the LFA have reported a general high analytical performance and strong clinical relevance (15–19).

In this study, we screened for CPA among patients presumed to have PTB using the LDBio *Aspergillus*-specific IgG & IgM LFA and the CPA guideline for resource-constrained settings (8). We also evaluated the significance of CPA as a differential diagnosis of PTB and assessed the clinical relevance of the LDBio *Aspergillus*-specific IgG & IgM LFA in CPA diagnosis.

4.2 METHODS

Study design and site

The study was a cross-sectional survey conducted at the Chest Clinic, Chest Diseases Unit, Department of Medicine, Korle-Bu Teaching Hospital, Accra. The clinic acts as the national TB referral centre and hosts a specialized TB laboratory that receives samples from different parts of Ghana.

Study population

Patients seen at the Chest Clinic of the Korle-Bu Teaching Hospital for suspected PTB or those referred from other parts of the country to the TB laboratory for GeneXpert MTB (Xpert® MTB/RIF, Cepheid, California, USA) testing were recruited, irrespective of their symptoms. Also, blood donors determined to have no symptoms and signs of a respiratory condition or history of PTB, or any other chronic respiratory condition via an interview, were also recruited as control group participants from the National Blood Service, Ghana. This was mainly done to assess the specificity of the LFA test.

Ethical approval was obtained from the Institutional Review Board of the Korle-Bu Teaching Hospital (STC/IRB/00058/2020) and the National Blood Services Ghana (NBSGRD/201410/02) and University Research and Ethics Committee of the University of Manchester (Ref: 2020-9368-16168). Written informed consent was obtained from all participants.

Investigations

Patients' demographics, clinical and socioeconomic details were collected via interviews using a questionnaire. Serum samples were obtained for *Aspergillus* serology with LDBio *Aspergillus* IgG & IgM LFA (LDBio Diagnostics, Lyon, France) and HIV antibody testing with HIV ½ RDT (Healgen

Scientific LLC, Texas, USA) and confirmed with OraQuick HIV ½ RDT (OraSure Technologies, Pennsylvania, USA). Sputum was obtained for high-volume culture to enhance *Aspergillus* detection, a modified version of Vergidis *et al.* (20), by inoculating an aliquot (1-2 ml) of undiluted specimen on Sabouraud dextrose agar and incubated at 37 °C for up to 8 days. Chest radiograph was done for all patients unless patient had obtained one within the previous month. Xpert MTB/RIF results were retrieved from laboratory records. Chest computed tomography (CT) scan was done for patients with positive *Aspergillus* serology or cavitation on chest radiograph including MTB positive cases. Among the MTB positive cases with cavitation, CT scan was done to unravel any concealed imaging features of CPA to identify possible PTB-CPA coinfection. In the control group, only serum samples were obtained for *Aspergillus* serology.

Case definition

TB was diagnosed if *Mycobacterium tuberculosis* was detected in a patient's sputum by Xpert MTB/RIF assay. Patients with suspected or confirmed PTB were classified as new PTB and relapsed PTB as follows:

i. new PTB-patients with no prior history of PTB

ii. relapsed PTB-patients who had been treated successfully for PTB in the past

A case of CPA was defined following the guidelines for CPA diagnosis in resource-constrained settings, developed by the GAFFI international expert panel (2016) (8). The panel defined a case of CPA as follows:

i. weight loss, persistent cough, and/or haemoptysis for >3 months

ii. chest images showing progressive cavitary infiltrates and/or a fungal ball and/or pericavitary fibrosis or infiltrates or pleural thickening

iii. a positive *Aspergillus* IgG assay or other evidence of *Aspergillus* infection.

Patients who met criteria (i) and (ii) above, but not (iii), or met criteria (i) and (iii), but not (ii) were categorized as probable CPA, a modified version of a classification described by Setianingrum *et al.* (21).

Data analysis

Data was analysed with SPSS version 25 (IBM, New York, USA) at 5% significance level, using either Chi Square or Fisher's exact tests. Summary statistics were presented using frequencies and percentages for categorical variables, and median values for non-normally distributed continuous

variables. Fisher's exact tests were employed to compare proportions between groups. Logistic regression was carried out to assess the effect of individual symptoms and socioeconomic details on the likelihood of acquiring CPA.

4.3 RESULTS

From October 2020 to May 2021, 183 consecutive patients referred for Xpert MTB/RIF were screened, but 21 (11.5%) were either less than 18 years or unable to provide sputum and/or blood and were excluded. Of the 162 recruited, a complete data set for evaluation of CPA was available for 154 (84.2%) patients. The 154 patients comprised 92 (59.7%) males, with a median age of 41.5 years and range of 18 to 96 years (Table 4.1). There were 134 (87%) and 20 (13%) patients being assessed for new PTB and 'relapsed' PTB, respectively. The time from completion of TB treatment to recruitment in the 'relapse' group was 1to 24 years (median 4). The median duration symptoms prior to presentation among patients was nine weeks, with a range of 1 to 21 weeks. Ninety blood donors were recruited in the control group from March to April 2021.

Table 4.1: Characteristics of 154 patients referred for GeneXpert TB according to eventual CPA diagnosis

Features	Total (n=154)	CPA (n=15)	Non-CPA (n=139)	P value
Demographics				
Male	92 (59.7%)	11 (73.3%)	81 (58.3%)	
Female	62 (40.3%)	4 (26.7%)	58 (41.7%)	0.410
Age, median (range)	41.5 (18-96)	47 (28-96)	43.4 (18-78)	0.765
Clinical details				
History of previous PTB	20 (13%)	10 (66.7%)	10 (7.2%)	0.002
Persistent cough	138 (89.6%)	15 (100%)	123 (88.5%)	1.0
Haemoptysis	26 (16.9%)	7 (46.7%)	19 (13.7%)	0.023
Chest pain	74 (48.1%)	11 (73.3%)	63 (45.3%)	0.395
Dyspnea	57 (37%)	4 (26.7%)	53 (38.1%)	0.570
Fatigue	111(72.1%)	11 (73.3%)	100 (71.9%)	0.101
Weight loss	110 (71.4%)	10 (66.7%)	100 (71.9%)	0.203
Chronic condition				

HIV infection	44 (28.6%)	3 (20%)	41 (29.5%)	0.550
Asthma	6 (3.9%)	2 (13.3%)	4 (2.9%)	0.040
COPD	9 (5.8%)	3 (20%)	6 (4.3%)	0.044
Diabetes mellitus	7 (4.5%)	1 (6.7%)	6 (4.3%)	0.500
Hypertension	25 (16.2%)	3 (20%)	22 (15.8%)	0.101
Lung cancer	1 (0.6%)	0	1 (0.7%)	1.0
Socioeconomics				
Practice of traditional cooking	83 (53.9%)	7 (46.7%)	76 (54.7%)	0.433
Residence in damp house	12 (7.8%)	2 (13.3%)	10 (7.2%)	0.330
Engagement in agricultural activities	30 (19.5%)	2 (13.3%)	28 (20.1%)	0.740
History of smoking	27 (17.5%)	2 (13.3%)	25 (18%)	1.0

Laboratory results

Laboratory findings of the 154 patients are shown in Table 2. *M. tuberculosis* was detected in 41 (26.6%) patients, of whom 35 (85.4%) were classified as new PTB and six (14.6%) had relapsed PTB. *Aspergillus* serology was positive in 9.9% (*n* = 16) of participants but there was no imaging data for two (12.5%) of these and hence they were excluded from the analysis. There were 44 (28.6%) HIV-positive patients, of whom two (4.5%) had positive *Aspergillus* serology. *Aspergillus* serology was positive in one (1.1%) participant in the control group, with no respiratory signs and symptoms or previous history of PTB. Culture was positive for *Aspergillus* spp. in 32 (20.8%) cases, yielding 38 isolates. The main species were *A. fumigatus* (47.4%, *n* = 18) and A. *niger* (36.8%, *n* = 14) (Table 4.2).

Table 4.2: Laboratory results of 154 patients referred for GenXpert TB according to eventual CPA diagnosis

Variable	Total (n=154)	CPA (n=15)	Non-CPA (n=139)	P value
Aspergillus serology	14 (9.1%)	14 (93.3%)	0	<0.001
Positive *Aspergillus* culture	32 (20.8%)	10 (66.7%)	21 (15.1%)	0.024
Aspergillus spp. isolates	38 (24.7%)	13 (86.7%)	25 (18.0)	<0.001
Aspergillus spp. Distribution				
A. fumigatus	18 (47.4%)	9 (69.2%)	9 (36%)	
A. niger	14 (36.8%)	3 (23.1%)	11 (44%)	
A. flavus	5 (13.2%)	1 (7.7%)	4 (16%)	
A. terreus	1 (2.6%)	0	1 (4%)	
HIV reactive	44 (28.6%)	3 (20%)	41 (29.5%)	0.560
M. tuberculosis detected	41 (26.6%)	4 (26.7%)	37 (26.6%)	1.0
MTB load distribution				
Trace	2 (4.9%)	2 (50.0%)	0	0.009
Very low	5 (12.2%)	2 (50.0%)	3 (8.1%)	0.050
Low	3 (7.3%)	0	3 (8.1%)	1.0
Medium	9 (22%)	0	9 (24.3%)	0.060
High	22 (53.7%)	0	22 (59.5%)	0.013

Radiological findings

Of 154 patients, chest radiograph was normal in 96 (62.3%) (Table 4.3). The common abnormalities reported were infiltration (23.4%, *n* = 36), cavitation (16.9%, *n* = 26), fibrosis (12.3%, *n* = 19) and pleural thickening (15.6%, *n* = 24). Chest CT scan was done in 17 (53.1%) of the 32 patients eligible for the procedure; the remainder either died or were lost to follow-up. The major CT scan findings were cavitation (100%, *n* =17; two of these contained a fungal ball), fibrosis (88.2%, *n* =15) and pleural thickening (88.2%, *n* =15). Out of the 26 participants with cavitation, 11 (42.3%) had a positive *Aspergillus* IgG/IgM assay. Cavitation (*p* = 0.005), paracavitary fibrosis (*p* = 0.005) and pleural thickening (*p* = 0.04) were seen more often in patients with CPA (Table 4.3).

Table 4.3: Imaging (chest radiograph and/or CT scan) findings for 154 patients

Variable	Total (n=154)	CPA (n=15)	Non-CPA (n=139)	*p* value
Infiltration	36 (23.4%)	8 (53.3%)	28 (20.1%)	0.169
Cavitation	26 (16.9%)	12 (80%)	14 (10.1%)	0.005
Paracavitary fibrosis	19 (12.3%)	11 (73.3%)	8 (5.8%)	0.005
Pleural thickening	24 (15.6%)	10 (66.7%)	14 (10.1%)	0.040
Bronchiectasis	11 (7.1%)	3 (20%)	8 (5.8%)	0.077
Nodules	16 (10.4%)	2 (13.3%)	14 (10.1%)	0.066
Fungal ball	2 (1.3%)	2 (13.3%)	0	0.009
Pleural effusion	12 (7.8%)	1 (6.7%)	11 (7.9%)	1.0

CT scan contributed to CPA diagnosis in 11 patients (Figures 1 and 2).

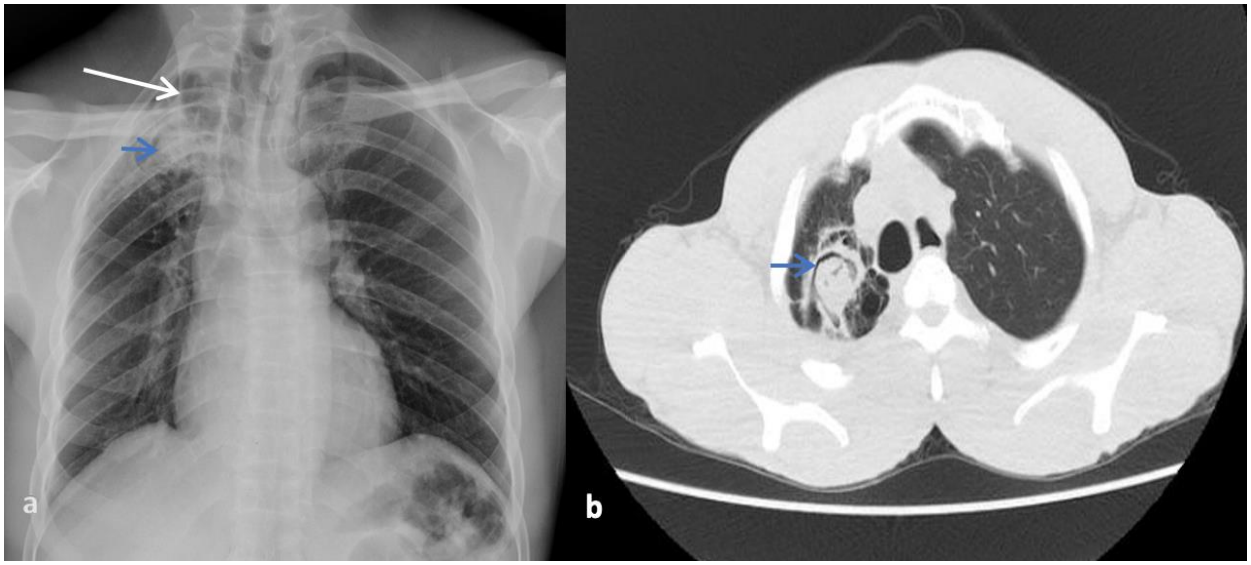

Figure 4.1: a. Plain frontal radiograph of a CPA patient, shows a right apical lung cavity (long white arrow) with a soft tissue density within it (short arrow), this is associated with pericavity fibrosis and volume loss evidenced by mediastinal shift to the right. b. Axial chest CT scan in lung window confirmed the presence of a right apical lung aspergilloma with an air crescent sign (short blue arrow) and surrounding fibrosis.

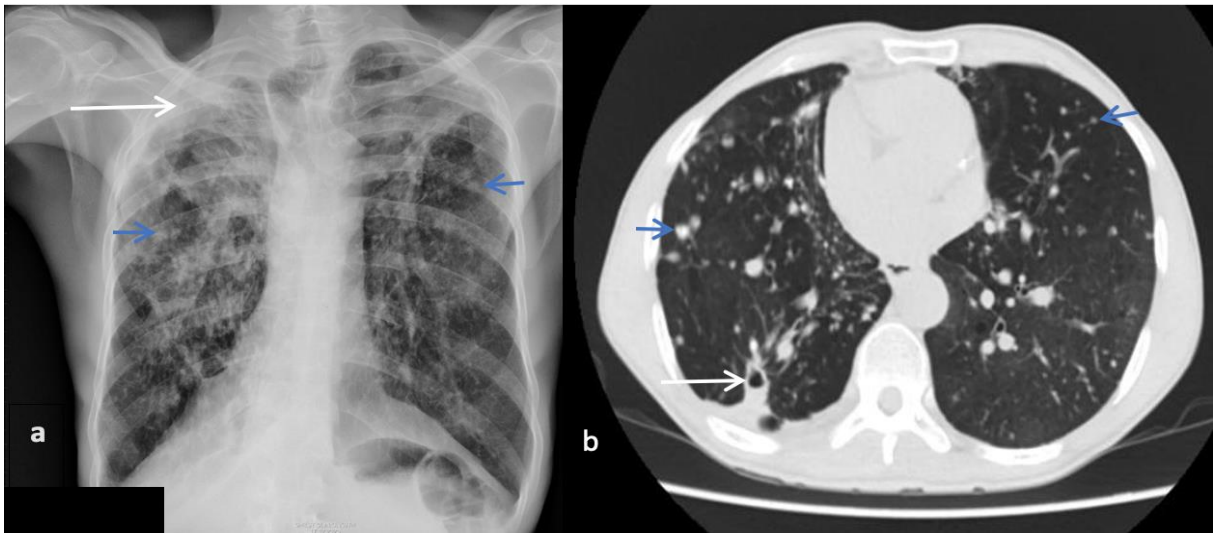

Figure 4.2: a. Plain frontal radiograph of another CPA patient, demonstrates bilateral apical lung fibrosis with associated hilar retraction and distortion. Right apical pleural thickening (long white arrow) and adjacent consolidation. Background of diffuse bilateral lung nodules (short arrows). b. Corresponding Axial Chest CT scan- lung window demonstrates a small right lower lobe superior segment cavity, which was not demonstrated on the plain radiograph and multiple bilateral lung nodules (short blue arrows)

CPA classification

Of the 154 patients, 15 (9.7%, 95% CI 5.0 - 14.4%) met the criteria for CPA, including three probable CPA cases. Ten (66.7%) CPA cases had previous PTB, representing 50% (10/20) (95% CI, 28 – 72%) of the patients being assessed for relapsed PTB. The predisposing conditions in the remaining CPA patients were chronic obstructive pulmonary disease (COPD) (*n* = 3) and asthma (*n* = 2). Four cases classified as CPA, all with previous history of PTB infection, had trace or very low levels of *M. tuberculosis* detected on GeneXpert, and were placed on TB treatment. The diagnosis of CPA was made in these patients because the imaging features were more suggestive of CPA including cavitation with irregular intraluminal lining of cavity, pleural thickening adjacent cavity, paracavitary fibrosis and one with a fungal ball. Additionally, *Aspergillus* antibody test was positive in all four patients and *Aspergillus* spp. was isolated in three patients. Three subsequently had a negative sputum Xpert MTB and/or AFB smear one month later but the fourth patient had a positive AFB smear which showed scanty organisms. The proportion of CPA based on HIV status were 10.9% (12/110) and 6.8% (3/44) for HIV- negative and HIV- positive, respectively (p = 0.560). The most common symptoms among CPA cases were fatigue (73.3%, *n* = 11), weight loss (73.3%, *n* = 11) and haemoptysis (46.7%, *n* = 7).

Haemoptysis was proportionately more common in CPA than other symptoms. No association was found between CPA diagnosis and socioeconomic details as potential risk factors.

4.4 DISCUSSION

This study is the first epidemiological study on CPA from Ghana and provides data for differential PTB diagnosis. It is common practice in high TB prevalence areas that patients previously treated for PTB who present with new symptoms are generally diagnosed with PTB relapse even when sputum AFB smear or Xpert MTB are negative. Our study showed that half of these patients had CPA. Therefore, a prior history of PTB treatment in a patient presenting with suspected relapse should raise suspicion for CPA.

The current study revealed a CPA prevalence of 9.7% among patients presenting with presumed PTB. Varying rates have also reported in Uganda (22) and Nigeria (6), Iran (23), Pakistan (24), Indonesia (15,21), Uganda (16) , and Brazil (25) largely due to difference in study designs, population, investigations, and sampling methods. Also, the present study identified 50% prevalence of CPA in patients with prior PTB. This is similar to recent reports from Vietnam (54.3%) (26) and India (57%) (27). CPA and PTB often present with clinically indistinguishable symptoms; fever is more common in PTB, and haemoptysis in CPA, but are not sufficiently distinctive features to be used as a definite diagnostic tool. A recent review established a common association between CPA and TB in Africa (28). Considering the high burden of TB in many African countries such as Ghana, CPA is likely to be misdiagnosed as PTB (5). In recent times, the new GAFFI case definition for CPA for resource-constrained healthcare settings, utilising the new *Aspergillus* IgG & IgM LFA, is improving epidemiological studies and providing more clinical experience of CPA in resource-constrained settings (7,15–19).

In the present study, three of four patients with concomitant CPA and PTB had a negative AFB smear within a month. The average interval from completion of TB treatment to recruitment for these patients was four years. Possibly, their Xpert MTB could have been false positives due to detection of residual MTB DNA or non-viable non-intact bacilli (29–31). The role of Xpert MTB in detecting reinfection or relapse accurately is unclear, often associated with high occurrence of false-positives especially with low mycobacterial burden, and short time post-treatment, as noted in our study (30,31). The likelihood of false positivity is reported to decrease with the longer time since successful treatment of PTB but the total duration is not known (30). Nevertheless, one study indicates that this can be up to four years after successful completion of appropriate treatment (31). The fourth patient had a positive AFB smear result, and probably had PTB-CPA co-infection.

The profound drug-drug interaction between rifampicin and oral antifungal azoles requires that clinicians to select between these diagnoses, and not attempt treating both PTB and CPA together. Some very ill patients with CPA require intravenous antifungal therapy while those with a single aspergilloma can be cured with surgical resection (4). However, the majority require at least 12 months of oral antifungal (itraconazole or voriconazole) treatment for chronic cavitary pulmonary aspergillosis (CCPA) to reduce symptoms, prevent progression or relapse and improve overall quality of life (4). A recent randomized controlled trial in India demonstrated that 12 months of oral itraconazole was superior to a 6-month regimen in reducing relapses of CPA at two years (32).

The commonest symptoms for CPA cases recorded in the current study were fatigue, weight loss and haemoptysis. Although patients with active PTB also had these symptoms, haemoptysis was more common in CPA, and should raise suspicion for diagnosis(5). COPD was more common in patients with CPA than PTB, although overall numbers were small. We observed no significant statistical difference of CPA in HIV positive patients (6.8% vs 10.9%), similar to other studies (6,16,22,33). Though *Aspergillus* serology was positive in less than 10% of our screened patients, it contributed to greater than 90% of CPA diagnosis. One CPA patient who was HIV-positive, had a negative *Aspergillus* IgG & IgM LFA result. This maybe because of a reduced capacity to elicit production of antibodies; the correlation between immunodeficiency and negative *Aspergillus* serology has been previously described (6,9,33). Unfortunately, the participant in the control group with a positive Aspergillus antibody test was unable to be reached for imaging investigations. In addition, we did not observe an association with various sociodemographic practices common in Ghana that could potentially lead to fungal exposure.

The commonest organism implicated in CPA is *A. fumigatus*; it is especially reported in Europe and USA., where it accounts for over 90% of all cases (34–36). However, in Africa and Asia, *A. flavus* and *A. niger* are frequently isolated, as demonstrated in our study and elsewhere (6,15,16,34). The frequent imaging findings of CPA, as stipulated in many guidelines, reported in several epidemiological studies, and considered to be more linked to CPA are cavitation, pericavitary fibrosis and pleural thickening (4,5,8,15,21,22). These three features were present in 67–80% of CPA cases in our study on chest radiograph and/or CT scan. Three patients without cavitation on chest radiograph were diagnosed with probable CPA based on parenchymal fibrosis and/or bronchiectasis coupled with positive *Aspergillus* serology and/or culture. It is possible these patients had cavitation that could have been revealed by CT scan as observed in two other patients with CPA. CT scan is an important complementary investigation to chest radiography when available especially when *Aspergillus* serology is positive. In the present study, additional cavitation

and fungal balls detected by CT scan in four patients were missed on chest radiographs. Similar observations were made by Page *et al.* (22) and Nguyen *et al.* (26).

4.5 CONCLUSION

In conclusion, the early differentiation of active PTB, post-TB lung disease and PTB plus CPA co-infection, in settings with high TB burden, may require a broader screening strategy at the investigation stage. The present study contributes to the efforts of identifying an efficient framework for routine or systematic screening for CPA in PTB. Access to readily available diagnostics, in addition to algorithms that easily identify patients with CPA, will improve patient care and outcomes.

Our study is not without limitations. Our inability to do CT scans for all eligible patients due to loss to follow-up or death was a major challenge. Secondly, prior to PTB retreatment, a repeat Xpert MTB and/or culture was not done for CPA patients from whom MTB was detected to rule out false positive results. Additionally, patients being assessed for PTB relapse were few, and the CPA rates in this group may not be representative.

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CHAPTER 5: STUDY 3– SCREENING FOR CHRONIC PULMONARY ASPERGILLOSIS IN CONFIRMED AND TREATED TUBERCULOSIS PATIENTS

ABSTRACT

Chronic pulmonary aspergillosis (CPA) often occurs in patients previously treated for pulmonary tuberculosis (PTB). Limited studies have looked at the development of CPA at different times following the completion of PTB treatment. This prospective longitudinal study aimed to determine the incidence of CPA at two time points, at the end of PTB treatment (T_1) and six months post-treatment (T_2). Patients with confirmed PTB from a previous study who were placed on anti-TB medication were followed up and screened for CPA at T_1 and T_2 by assessing symptoms, evaluating quality of life and screening for *Aspergillus* by antibody testing and culture. CPA was defined based on the Global Action for Fungal Infections (GAFFI) diagnostic algorithm. Forty-one patients were enrolled, of whom 33 (80%) and 28 (68%) were resurveyed at T_1 and T_2, respectively. The rate of new CPA was 3% (1/33) and 7.4% (2/27) at T_1 and T_2 respectively with an overall incidence of 10.7% (3/28) (95% CI, 1.1 – 20.3%) among patients followed at both T_1 and T_2. Positive *Aspergillus*-specific antibody test was an indicator for CPA in all three patients. *Aspergillus*-specific antibody screening during and after the end of anti-TB treatment may be important for early detection of CPA in high PTB burden settings.

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5.1 INTRODUCTION

Pulmonary tuberculosis (PTB) remains a major global health problem, with high burden in low and middle-income countries (LMICs). In 2020, there were 4.8 million people diagnosed with PTB globally and 59% were bacteriologically confirmed (1). About 85% of people diagnosed with PTB are generally successfully treated with a 6-month drug regimen (1). Unfortunately, patients with PTB after successful treatment, become more exposed to secondary respiratory infections that are uncommon in patients without prior PTB (2). These infections can be chronic and associated with high morbidity and mortality. Chronic pulmonary aspergillosis (CPA) is one of the common infections and the high-risk group are patients with PTB with overt lung cavities (3). The prevalence of CPA as a sequel of PTB worldwide was estimated at 1.2 million in 2011 (4). Several cross-sectional studies have been conducted on CPA as a secondary infection of PTB in different countries and reported varying rates (5–9). However, none of these studies have solely focused on bacteriologically confirmed PTB and so may have included individuals without prior PTB and may not be a true definition of post-PTB complication. In recent times, there has been an interest in evaluating the timing of the incidence of CPA from diagnosis, during treatment to post-treatment of PTB (3,10,11). Such data could help guide screening strategies for early CPA diagnosis.

In addition, CPA may rarely co-exist with PTB as a primary CPA-PTB co-infection(12–14). Genetic and environmental factors may also influence the development of CPA at different times among individuals (10,15). Presently, CPA has been identified in PTB patients from early PTB treatment and close to 40 years after completing treatment (3,10,16). This prospective longitudinal study was conducted to evaluate the incidence of CPA at two time points following the completion of PTB treatment in a cohort of new bacteriologically confirmed PTB patients using *Aspergillus*-specific antibody testing as a screening tool.

5.2 METHODS

Adult (≥ 18 years) patients whose GeneXpert MTB was positive in a previous study (17) and subsequently placed on anti-TB treatment were enrolled for this study. Patients were receiving care for suspected PTB at the Chest Clinic of the Korle-Bu Teaching Hospital, Accra, Ghana or referred from various health facilities across the country to the Chest Clinic TB laboratory for GeneXpert MTB and rifampicin (RIF) resistance testing (Xpert® MTB/RIF, Cepheid, California, USA). Patients with a previous history of PTB were excluded.

Patients' demographics and baseline CPA screening findings including laboratory and chest radiograph reports were extracted from the primary research data. Patients were then followed-up and further screened for CPA at two time points, within one month after completing treatment

(T_1, 6-7 months from diagnosis) and 6–7-month post-treatment (T_2, 12-13 months from diagnosis). CPA screening involved assessment of symptoms, *Aspergillus*-specific antibody testing, sputum *Aspergillus* culture, chest radiograph and/or computed tomography (CT) scan.

Serum samples were obtained from all patients for *Aspergillus*-specific antibody testing with the LDBio *Aspergillus* IgG & IgM LFA (LDBio Diagnostics, Lyon, France) following the manufacturer's instructions. Sputum *Aspergillus* culture was done for all patients using a modified version of the high-volume culture method by inoculating an aliquot (1–2 ml) of undiluted sputum on Sabouraud dextrose agar and incubated at 37°C for up to 8 days (17). Chest CT scan was done for patients with positive *Aspergillus* serology or cavitation on baseline chest radiograph with new or persistent respiratory symptoms. All imaging investigations were evaluated by a consultant radiologist (HG) blinded to clinical and laboratory findings. Xpert MTB/RIF and/or acid-fast bacilli (AFB) smear results were retrieved from laboratory records. Additionally, the quality of life (QoL) of patients were evaluated at both timepoints using the St. George's Respiratory Questionnaire (SGRQ), which scores patients from 1 (excellent health) to 100 (very ill) (18,19). CPA was defined based on the Global Action for Fungal Infections (GAFFI) diagnostic criteria (20).

Data was analysed with SPSS version 25 (IBM, New York, USA) at 5% significance level, using either Chi Square or Fisher's exact tests. Summary statistics were presented using frequencies and percentages for categorical variables. Fisher's exact tests were employed to compare proportions of the various characteristics of patients recruited at both timepoints.

5.3 RESULTS

There were 47 Xpert MTB positive cases, of whom 41 were diagnosed as new cases. Of the 41 new PTB patients, 33 (80%) and 28 (68%) were resurveyed at T_1 and T_2, respectively. In all, three of the 13 patients who were not resurveyed at T_1 and T_2, died and the remainder were lost to follow-up for varying reasons. Details of enrolled patients at both timepoints including those lost to follow-up are shown in Figure 5.1.

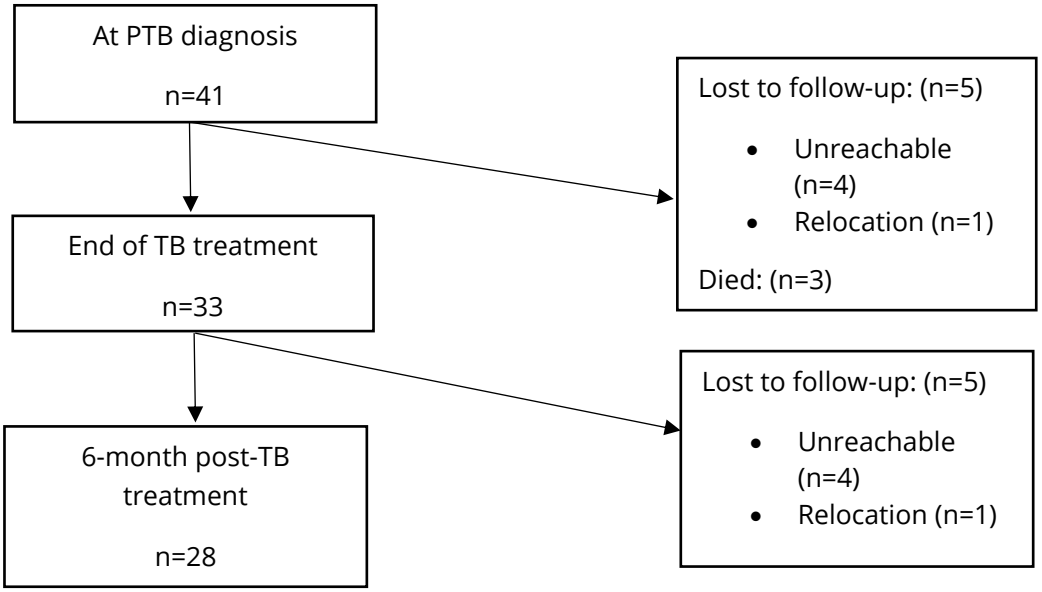

Figure 5.1: Overview of 41 patients enrolled.

There were more male patients (78%, *n*=32) and the mean age was 40.2 years (range, 18-75 years) (Table 5.1). Of 41 positive Xpert MTB cases, the majority (78%, *n*=32) had a high or medium MTB load or concentration. No RIF resistance was recorded, but there were three cases of RIF indeterminate results and so resistance could not be established. At baseline, *Aspergillus*-specific antibody was negative in all patients. Cavitation was visible on chest radiograph in 18 patients at baseline. There were 10 patients whose sputum grew *Aspergillus* spp. All 41 patients were placed on a standard first-line anti-TB regimen, comprising rifampicin, isoniazid, pyrazinamide, and ethambutol. The baseline SGRQ score was higher than 50 in 32% (13/41) patients, with an average of 43.1 (Table 5.1).

Table 5.1: Patients' characteristics at the three time points.

Features	**Baseline (n=41)**	**End of PTB Rx (n=33)**	**6-month post-PTB Rx (n=28)**	**P-value**
Demographics				
Male	32 (78%)	27 (81.8%)	23 (82.1%)	
Female	9 (22%)	6 (18.2%)	5 (17.9%)	<0.001
Age (mean/ range)	40.2 (18-75)	41.2 (18-75)	39.7 (18-75)	0.962
Symptoms				

Haemoptysis	9 (22%)	2 (6.1%)	2 (7.1%)	<0.001
Chest pain	19 (46.3%)	4 (12.1%)	6 (21.4%)	0.253
Dyspnea	16 (39%)	1 (3%)	1 (3.6%)	0.071
Fatigue	33 (80.5%)	6 (18.2%)	2 (7.1%)	<0.001
Weight loss	36 (87.8%)	6 (18.2%)	3 (10.7%)	<0.001
Laboratory				
MTB load distribution				
Trace	2 (4.9%)	-	-	
Very low	3 (7.3%)	-	-	
Low	4 (9.8%)	-	-	
Medium	9 (22%)	-	1 (3.6%)	
High	23 (56.1%)	-	-	<0.001
+ *Aspergillus* serology	0	1 (3%)	3 (10.7%)	<0.001
+ *Aspergillus* culture	10 (24.4%)	9 (27.3%)	8 (28.6%)	<0.001
Aspergillus spp. Distribution				
A. fumigatus	8 (16%)	6 (18.2%)	4 (14.3%)	
A. flavus	1 (2.4%)	0	1 (3.6%)	
A. niger	4 (9.7%)	5 (15.1%)	4 (14.3%)	
A. terreus	0	1 (3%)	0	<0.001
+ HIV serology	12 (24%)	8 (23.5%)	6 (21.4%)	0.003
+ AFB smear	-	3 (9.1%)	0	<0.001
Imaging				
Cavity	9 (22%)	6 18.2%)	4 (14.3%)	<0.001
Infiltration	14 (34.1%)	3 (9.1%)	2 (7.1%)	0.020

Fibrosis	25 (60.9%)	4 (12.1%)	6 (21.4%)	<0.001
Pleural thickening	20 (48.8%)	4 (12.1%)	4 (14.3%)	0.333
Bronchiectasis	19 (46.3%)	2 (6.1%)	3 (10.7%)	0.252
Fungal ball	0	0	1 (3.7%)	<0.001
Quality of Life				
SGRQ (mean ± SD)	43.1±9.127	9.3±6.848	13.1±18.064	0.124

Rx-Treatment, HIV-human immunodeficiency virus, SD-standard deviation

Of 33 patients resurveyed at T_1, 90.9% (30/33) had completed treatment and achieved microbiological cure (AFB smear negative) and three patients were declared to have failed treatment due to persisting symptoms and positive AFB smear. *Aspergillus*-specific antibody was positive in one patient who was presumed to have failed treatment due to persisting symptoms but AFB smear negative and was placed on second-line treatment. This patient met the criteria for CPA with suggestive imaging findings on CT scan (Figure 5.2) and sputum growing *A. fumigatus* and *A. niger* (Table 5.2). SGRQ score improved significantly at T_1 for patients without CPA or treatment failure, from an average score of 51.4 to 3.8 signifying PTB treatment success. The SGRQ for the CPA patient decreased from 45 at baseline to 29 at T1 while the three patients with treatment failure had their SGRQ reduced from an average of 51 to 26.3, respectively.

The 28 patients rescreened at T_2, included the previous CPA patient. Twenty-seven had achieved microbiological cure while one was confirmed as PTB relapse. Of the 27 patients with a previous negative *Aspergillus* antibody test, 7.4% (2/27) seroconverted. These two new patients also met the criteria for CPA, with one having a fungal ball (Figure 5.3 and 5.4) and both had *A. fumigatus* growing from sputum (Table 5.2). One of the CPA patients was HIV positive. Again, the two new patients developing CPA at T_2 were symptomatically worse with increasing SGRQ scores from 14.5 at T_1 to 36 at T_2. Generally, among patients resurveyed at both time points, the average SGRQ scores for those without CPA had improved significantly compared to those with CPA. The other reasons for poor SGRQ score were anti-TB treatment failure and PTB relapse.

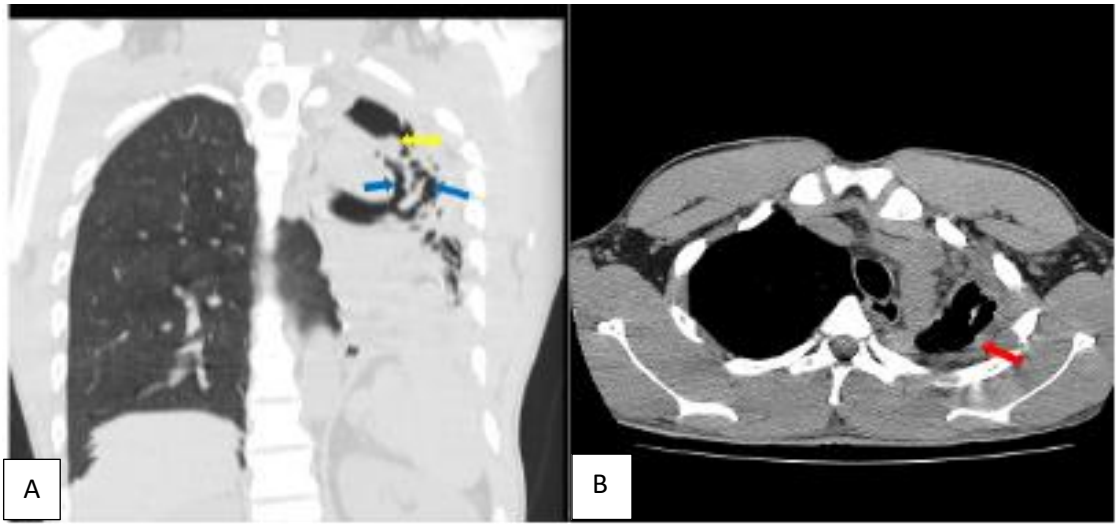

Figure 5.2: Axial non contrast CT scan, coronal reformatted lung, and axial mediastinal windows of the chest of the patient diagnosed with CPA at T1 A). Extensive left lung traction bronchiectasis (blue arrows) with ipsilateral lung volume loss, left apical lung cavity with intracavitary material (yellow arrow), B) left apical lung pericavitary pleural thickening (red arrow).

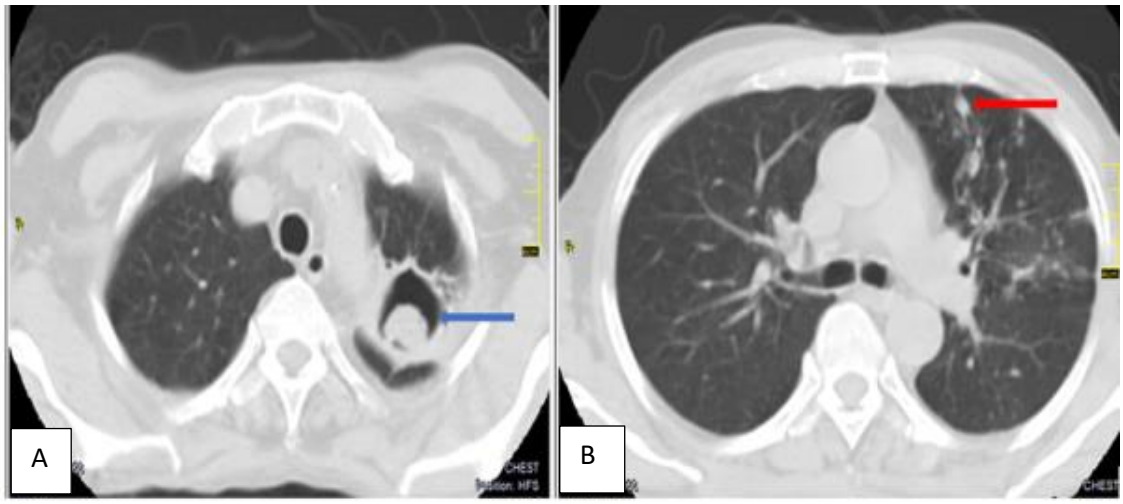

Figure 5.3: Axial contrast CT scans, axial lung window of chest of second CPA patient diagnosed at T2 showing A) a soft tissue mass within a left apical lung cavity (blue arrow) with a characteristic crescent of air around it, the monad sign, indicative of an aspergilloma. B). Nodular opacities (red arrow) and non pericavitary fibrotic changes in the left upper lobe.

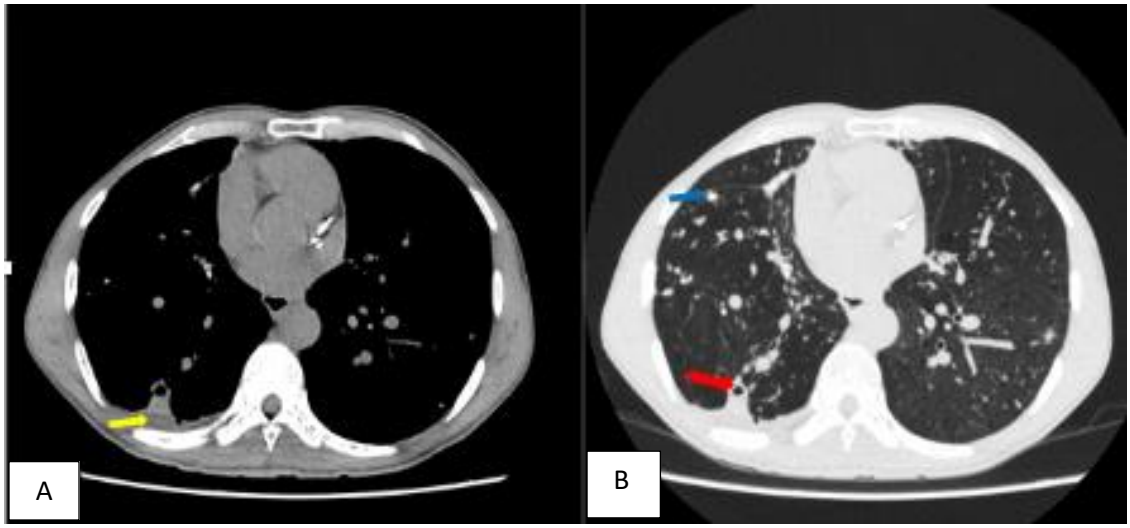

Figure 5.4: Axial non contrast CT scan, axial mediastinal and lung windows of the chest of third CPA diagnosed at T2, demonstrating A) right lung nodules (blue arrow); B) small right lower lobe cavity with pericavitary infiltration (red arrow) as well as a small pleural effusion (yellow arrow).

Table 5.2: Demographics, symptoms, laboratory, imaging and QoL details of the three CPA patients

Age	Sex	Symptoms	ASPG Ab	Culture	CT scan at T_1 /T_2	SGRQ at baseline, T_1, and T_2
30	M	Cough, haemoptysis, chest pain, weight loss	Positive	*A. fumigatus, A. niger*	Cavities, intracavitary material, pericavitary infiltration, pleural thickening adjacent cavity, bronchiectasis, pleural effusion	45, 29, 83
49	M	Cough, dyspnoea, weight loss	Positive	*A. fumigatus*	Cavities, fungal ball, pleural thickening adjacent cavity, pericavitary fibrosis, bronchiectasis	46, 14, 23
50	M	Haemoptysis, cough, fatigue	Positive	*A. fumigatus*	Cavities, intracavitary material, pleural thickening adjacent	30, 15, 49

					cavity, pericavitary fibrosis, nodules, bronchiectasis	

ASPG- *Aspergillus*, Ab-antibody, M-male

The overall incidence of CPA among the resurveyed patients over 12-months from the time of PTB diagnosis was 10.7% (3/28) (95% CI, 1.1 – 20.3%). The incidence of CPA at end of PTB treatment (T_1) was 3% which later increased to 7.4% at 6-month post treatment (T_2). This represents 42.9% (3/7) of patients with new or persisting symptoms suggestive of PTB after either 6 months or 12 months post initial PTB diagnosis. Haemoptysis was only noted in patients who had CPA when resurveyed at T_1 and T_2. The common CT scan findings among the three CPA patients were cavitation, pleural thickening, pericavitary fibrosis and bronchiectasis (Table 5.2). Two of the three CPA patients had cavitation on baseline chest radiograph, representing 11.1% (2/18) of all cavitations.

5.4. DISCUSSION

We prospectively followed up new bacteriologically-confirmed PTB patients from a previous study (17) to evaluate the incidence of CPA from the time of PTB diagnosis, to after completing PTB treatment and finally six months after treatment. CT scan was done in all CPA patients in our study. This makes the diagnosis more robust because CT scan which is very important for confirming CPA diagnosis with a maximum score point in the recently published CPA EQUAL scores (21). This study adds to previous studies that have indicated the need to regularly screen for CPA post-PTB treatment (3,5,10). It also contributes to the emerging evidence that CPA may develop during or early after completion of anti-TB medications, as previously reported (10). The current study also shows the increasing incidence rate of CPA among post-PTB patients when resurveyed over time similar to previous findings (10).

Interestingly, at baseline, no patient met the criteria for CPA, that is we found no PTB-CPA coinfection at the time of confirmed PTB diagnosis. However, it possible for CPA to develop two months after initiating anti-TB treatment as reported by two recently published studies in Indonesia (22) and in Uganda (6). First, Setianingrum *et al.* (10) in Indonesia, reported CPA incidence of 7.9% among PTB patients who were within 2 months into anti-TB treatment. It is important to note however that, their cohort included at least 42% new non-bacteriologically confirmed PTB cases, some may probably had had CPA with other respiratory disorders, including asthma and chronic pulmonary obstructive disease (COPD) as an underlying condition. Similarly, another study in Uganda reported about 20% rate of CPA incidence among PTB with persisting

symptoms after 2 months of treatment (6). In a recently published study, a possible PTB-CPA coinfection was reported but there were still doubts with regards to the existence of a true PTB infection (17). As more studies are conducted on CPA in patients presenting with PTB, the phenomenon of co-infection may be further explored.

In the current study, T_1 CPA patient resurveyed after six months, continued to have features of CPA including a positive *Aspergillus*-specific antibody test and *Aspergillus* culture accompanied by a worsening SGRQ score. Similar observations have been made in other studies (3,10). However, it has been reported that some of these patients may no longer have the features of CPA when resurveyed without any antifungal treatment, surgical procedure or radiotherapy (10). The data available on this phenomenon is scanty, notwithstanding, it is widely accepted that some cases of CPA may be self-resolving or remain static for long periods (13).

Aspergillus specific-antibody testing was critical in identifying potential cases of CPA and distinguishing them from treatment failures or PTB recurrent cases with CT scan investigations. It is worthy of note that, all PTB patients who were eventually diagnosed with CPA, first had their *Aspergillus*-specific antibody test being positive, acting as an indication for advanced imaging for features suggestive of CPA. Notwithstanding, *Aspergillus*-specific antibody in any of the current available forms, including precipitins, counter immuno-electrophoresis, immunodiffusion, complement fixation, enzyme immunoassay or immunochromatography tests have limitations and associated with false positives and false negatives (22,23). In fact, evaluation studies and clinical use of the LDBio *Aspergillus*-specific IgG and IgM assay employed in our study, has reported varying sensitivities and specificities pooled at 90% and 91% respectively among different populations and widely recommended for screening of CPA cases (6,8,24–28). Although, an elevated *Aspergillus*-specific IgG level is superior compared to other immunoglobulins in the diagnosis of CPA, it is possible for some CPA to have normal levels of *Aspergillus*-specific IgG but raised levels of *Aspergillus*-specific IgM, which may be positive in about half of CPA patients (22). Thus, an *Aspergillus*-specific IgG and IgM assay may offer additional sensitivity. Like *Aspergillus*-specific IgM, *Aspergillus*-specific IgA and IgE can also be positive when symptoms and imaging features suggestive of CPA are observed and *Aspergillus*-specific IgG levels are normal. *Aspergillus*-specific IgE may be linked to allergic aspergillosis as the underlying condition for CPA, but also may be independently elevated.

The performance of some *Aspergillus*-specific antibody assays is negatively affected by patient's immunodeficiencies, and performance is great in the absence of immunodeficiency. However, the LDBio *Aspergillus*-specific IgG and IgM assay has been previously reported to be minimally affected

by immunodeficiency and thus may perform acceptably well in both HIV seronegative and seropositive patients (29). Notwithstanding, CPA is rarely associated with HIV and rather more common in patients without apparent or with subtle immunodeficiency. Our study suggests the LDBio *Aspergillus*-specific IgG and IgM can be used in the GAFFI diagnostic algorithm, supporting a previous report from Uganda (27). However, the algorithm relies mainly on chest radiograph, which would have missed one CPA patient who had CPA-suggestive imaging findings demonstrated on CT scans. Clinicians thus need to consider *Aspergillus*-specific antibody testing in successfully treated PTB patients who return with new or persistent respiratory symptoms without radiological progression on chest radiograph because about 50% have CPA (9,17,30). Most of these cases are usually presumed to be treatment failure, PTB relapse or reinfection but studies show some may actually have CPA (9,17,30). The current finding corroborates a recent study in Ghana, where CPA was present in 50% of patients with presumed recurrent PTB (17). Although *Aspergillus* culture was positive in eight more patients at T_1, none had symptoms or previous chest radiograph suggestive of CPA.

The major limitation of our study was that our sample size was small and may not be sufficiently representative. Validation in a larger population will carry more statistical weight. Also, some of the patients lost-to-follow-up may have developed CPA and thus we may have underreported the frequency of this problem, as in other studies with a significant proportion of study subjects lost to follow up.

5.5. CONCLUSION

The present study indicates that CPA may develop during and after completing anti-TB treatment among new bacteriologically confirmed PTB patients. *Aspergillus*-specific antibody testing is instrumental in screening patients prior to performing CT scans to confirm cases of CPA in resource-constrained settings where advance imaging is mostly unavailable or expensive to access. We recommend the validation of these findings in a larger study. Additionally, subsequent studies may also consider investigations at 3 months or half of the duration into treatment. This will be important to contribute to efforts to identifying strategies for early detection of CPA cases particularly in high PTB burden settings to minimize inappropriate retreatments for PTB and late presentations with aspergilloma.

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CHAPTER 6: STUDY 4– INVASIVE ASPERGILLOSIS AMONG HAEMATOLOGICAL MALIGNANCY PATIENTS IN GHANA

ABSTRACT

Invasive aspergillosis (IA) among haematological malignancy patients is rarely diagnosed or studied in many African countries. *Aspergillus* galactomannan (GM) enzyme immunoassay (EIA) utilized in aiding diagnosis is not readily accessible in Ghana. Previous studies have evaluated the IMMY sōna® *Aspergillus* GM lateral flow assay (LFA) and suggested it as a potential alternative to the GM EIA. We aimed to use the LFA in international (EORTC/MSGERC) definitions to obtain preliminary data on IA among patients with haematological malignancies in Ghana with a focus on the prevalence and antifungal prophylaxis. We conducted a pilot study among patients with haematological malignancies at the Korle-Bu Teaching Hospital, Ghana using the LFA, culture and computed tomography scan to screen for and classify IA cases according to international definitions. A total of 56 adult patients were recruited including acute leukaemia 14 (25.0%), chronic leukaemia 38 (67.9%), and lymphoma 4 (7.1%). Nine (16.1%) patients had a history of severe neutropenic episodes. All patients were on at least one chemotherapy drug. Three (5.4%) (95% CI, 0.4 – 10.4%) patients met the criteria for IA, comprising two probable IA in acute myeloid leukaemia and one possible IA in non-Hodgkin's lymphoma and constitutes one of five (20%) patients with ongoing severe neutropenia. The LFA was diagnostic in two IA patients. The IA cases were among 49 (87.5%) patients who did not receive antifungal prophylaxis. Proactive diagnostic approaches to IA and effective antifungal prophylaxis may be significant in the management of haematological malignancy patients with severe neutropenia in Ghana.

Keywords: invasive aspergillosis, haematological malignancy, Ghana, *Aspergillus* galactomannan, neutropenia, antifungal prophylaxis

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6.1 INTRODUCTION

The outcomes of patients with haematological malignancies are negatively affected by invasive fungal infections (IFIs) commonly invasive aspergillosis (IA) (1). IA is a potentially lethal infection and the major host factor for IA is the depth and duration of neutropenia (2,3). IA is acquired by inhalation of spores of *Aspergillus* species and over 80% of infections involve the lungs (4). Globally, the incidence of IA among patients with haematological malignancies is estimated between 4-11% (5). The case fatality rate attributed to IA is reported to reach 80% and mostly highest in the short-term after diagnosis (6). The very few studies from Africa are mostly from North Africa with two major studies in Tunisia reporting probable and possible IA rates of 9.9-15.2% and 2.2-12.4% respectively (2,3).

As with many other SFIs, diagnosis and research of IA in Africa is challenging due to inadequate awareness, low index of suspicion and insufficient diagnostic capacity. For example, implementing the recently re-issued diagnostic criteria for IA by the European Organization for Research and Treatment of Cancer (EORTC) /Mycoses Study Group Education and Research Consortium (MSGERC) may be difficult, particularly when *Aspergillus* galactomannan (GM) enzyme immunoassay (EIA) used in aiding diagnosis is not accessible (7–10). Additionally, fungal culture and histopathology are far from routine requests while very limited molecular detection for *Aspergillus* is undertaken on the continent (7,8). The recent introduction of lateral flow assays (LFA) for *Aspergillus* antigen could transform testing availability in Africa.

One of these assays is the CE-marked IMMY sōna® *Aspergillus* GM LFA. Evaluation studies of this LFA reports 87.5-100.0% sensitivity, 95.5-98.0% specificity and had a qualitative agreement of 89% with the well-established Bio-Rad *Aspergillus* GM EIA (11–13). Although early diagnosis and prompt antifungal treatment is often associated with decreased mortality rates, it can be an expensive practice and prevention would be preferred (14). Antifungal prophylaxis is highly recommended and is the standard of care in many centres despite occasional breakthrough infections (15,16). However, prophylaxis can alter GM diagnostic performance and requires consideration when testing GM. The practice of antifungal prophylaxis in many African settings including Ghana is unknown.

The aim of this pilot study was to use the IMMY sōna® *Aspergillus* GM LFA in the updated EORTC/MSGERC definitions to obtain preliminary data on IA in Ghana with a focus on the prevalence and antifungal prophylaxis practice.

6.2 METHODS

Patients with haematological malignancies were consecutively recruited from the Haematology Day Care, Korle Bu Teaching Hospital (KBTH) from August to November 2022. The KBTH is the largest tertiary care and national referral medical facility in Ghana and the Department of Haematology attends to patients across Ghana and the West African sub-region. Adults (≥ 18 years) newly diagnosed with a haematological malignancy and those with active haematological malignancy receiving treatment or relapsed, or remission patients were included after obtaining informed consent. Patients with aplastic anaemia were excluded as it represents a heterogenous group and not listed as a host factor among the haematological malignancy group in the EORTC/MSGERC definitions. Recruitment was done during different phases of diagnosis and management as follows; a) within a week of new diagnosis, b) within a week of relapse, c) prior to starting chemotherapy d) anytime during chemotherapy and e) end of chemotherapy. All types of chemotherapy drugs were considered.

Demographic data of patients and relevant clinical information including type of haematological malignancy, disease stage, treatment phase, previous and/or current severe neutropenia (≤ 0.5 x 10^9 cells/l) and antifungal prophylaxis were collected from medical records and documented onto a questionnaire. About 3 ml of venous blood and sputum was obtained from each patient into a serum separator tube and universal container, respectively. Samples were collected at the time of recruitment. Another venous blood sample was scheduled for collection within the next 7 days of collecting the first blood sample. If nasal aspirate, cerebrospinal fluid (CSF) or BAL were collected during routine clinical care, aliquots were obtained, where possible, for IMMY sōna® *Aspergillus* GM LFA. After sample collection, patients had a CT scan of chest, when clinically indicated either at the Radiology Department, KBTH or when necessary outsourced. Additionally, because CT scan abnormalities can precede a positive GM assay, patients with symptoms had CT scan undertaken for research purposes (17,18). If patients had nasal or neurologic symptoms, CT scan of sinus and brain were conducted. All CT scans were reviewed by a radiologist blinded to clinical and laboratory data to assess abnormalities associated with IA.

Direct examination of samples using potassium hydroxide (KOH) and lactophenol cotton blue (LPCB) was performed on sputum samples and nasal aspirate, CSF or BAL when obtained. Subsequently, fungal culture was carried out on these clinical samples by inoculating appropriate portions on a Sabouraud Dextrose agar (SDA) plate, incubated at 37°C for about one week and observed daily. Phenotypic methods were used for identification of fungi by macroscopic examination and microscopic analysis by the cellophane tape mount method. For non-culture-

based assays, serum or nasal aspirate, CSF and BALF was analysed using the IMMY sōna® *Aspergillus* GM LFA following the manufacturer's instructions.

Cases of IA were classified as proven, probable, and possible based on the newly updated 2020 EORTC/MSGERC definitions(9). The study was approved by the Institutional Review Board of the Korle-Bu Teaching Hospital (STC/IRB/00058/2020) and the University Research and Ethics Committee of the University of Manchester (Ref: 2022-13962-25109). Written informed consent was obtained from all participants.

The data was analysed with SPSS version 25 (IBM, Armonk, NY, USA) with a $P < 0.05$ considered statistically significant using Chi-square. Summary statistics were presented using frequency as numbers and percentages for all categorical variables. Median was calculated for non-normally distributed continuous variables.

6.3 RESULTS

Fifty-six patients comprising 26 (46.4%) males and 30 (53.6%) females with a median age of 47 years (range, 25-63 years) were included. The distribution of the underlying haematological malignancies is shown in Table 6.2. Twenty-nine (52.0%) patients recruited were on different known stages of chemotherapy. Only nine (16.1%) had a history of severe neutropenia within the last three months. Antifungal prophylaxis was given to seven (12.5%) patients, six (85.7%) fluconazole and one (14.3%) itraconazole. IMMY sōna® *Aspergillus* GM LFA was positive in 2 (3.6%) patients and 14 *Aspergillus* spp were isolated from 11 patients (19.6%). Out of the 27 patients who had CT scans, abnormal findings were observed in six (22.3%) patients, out of these 3 (11.1%) were associated with IA, according to the EORTC/MSGERC definitions.

Table 6.1: Definition for proven, probable, or possible IA extracted from 2020 EORTC/MSGERC definitions (9)

	Proven	**Probable IA**	**Possible IA**
Host factor	Haematological malignancy	Haematological malignancy	Haematological malignancy
Clinical feature	CT scan^{+}	CT scan^{+}	CT scan^{+}
Mycology evidence	Histopathology^{+} or culture^{+} (sterile specimen)	GM^{+} and/or culture $^{+}$ (sterile or unsterile specimen)	GM^{-} and culture^{-}

+ = EORTC/MSGERC defined features present or positive; - = negative

Three (5.4%) (95% CI, 0.4 – 10.4%) patients met the criteria for IA, including two (3.6%) probable and one (1.8%) possible IA case according to the EORTC/MSG definitions (Table 6.1). AML and non-Hodgkin's lymphoma was the underlying condition or host factor in two and one IA patients respectively. All the three IA patients had pulmonary disease.

Table 6.2: Demographics and Clinical Characteristics of Patients Categorized into IA and non-IA Group

Variables	**Total (N=56) (%)**	**IA (N=3) (%)**	**No evidence of IA (N=53) (%)**	***P* value**
Gender				
Male	26 (46.4%)	2 (7.7%)	24 (92.3%)	
Female	30 (53.6%)	1 (3.3%)	29 (96.7%)	0.757
Median Age, (Range)	47 (24 – 63)	54 (32 – 63)	46 (24-61)	0.133
Malignancy type				
ALL	4 (7.1%)	1 (25%)	3 (75%)	
AML	10 (17.9%)	2 (20%)	8 (80%)	
CLL	10 (17.9%)	0	10 (100%)	
CML	28 (50.0%)	0	28 (100%)	
NHL	4 (7.1%)	0	4 (100%)	0.333
Disease stage				
Newly diagnosed leukaemia	6 (10.7%)	0	6 (100%)	
Ongoing therapy	38 (67.9%)	2 (5.3%)	36 (94.7%)	
Remission	8 (14.3%)	0	8 (100%)	0.333
Relapse	4 (7.1%)	1 (25%)	3 (75%)	
Treatment phase				

Consolidation	4 (7.1%)	1 (25%)	3 (75%)	
Induction	10 (17.9%)	1 (10%)	9 (90%)	
Maintenance	12 (21.4%)	0	12 (100%)	
Salvage	3 (5.4%)	0	3 (100%)	
Unknown	27 (48.2%)	1 (3.7%)	26 (96.3%)	0.333
Antifungal prophylaxis	7 (12.5%)	0	7 (100%)	<0.001
History of neutropenia	9 (16.1%)	3 (33.3%)	6 (66.7%)	<0.001
Laboratory findings				
Absolute neutrophil count (mean×10^9)	4.64	0.45	8.29	<0.001
Positive GM LFA	2 (3.6%)	2 (100%)	0	0.500
Aspergillus culture	11 (19.6%)	2 (18.2)	9 (81.8)	0.333
A. flavus	5 (8.9%)	1 (20%)	4 (80%)	
A. fumigatus	4 (7.1%)	1 (25%)	3 (75%)	
A. niger	5 (8.9%)	0	5 (100%)	0.333
CT scan findings				
Lesion	4 (7.1%)	2 (50%)	2 (50%)	0.333
Halo sign	2 (3.6%)	2 (100%)	0	0.333
Consolidation	2 (3.6%)	2 (100%)	0	<0.001
Pleural effusion	1 (1.8%)	1 (100%)	0	0.500
Ground glass opacities	4 (7.1%)	1 (25%)	3 (75%)	0.333

Overall, CT scan was diagnostic in the three IA cases and the *Aspergillus* GM LFA was diagnostic in two IA cases (Table 6.3). None of the cases could be proven as biopsy or sterile samples were not

obtained for histological/cytological analysis and fungal culture. *Aspergillus fumigatus* and *Aspergillus flavus* were identified as the etiological agents in IA cases.

Table 6.3: EORTC/MSGERC classification of all patients

EORTC/MSGERC classification	**Frequency, *n***
Probable IA	**2**
EORTC/MSGERC imaging findings	2
Positive *Aspergillus* GM LFA	2
Positive *Aspergillus* culture	2
A. fumigatus	1
A. flavus	1
Possible IA	**1**
EORTC/MSGERC imaging findings	1
Positive *Aspergillus* GM LFA	0
Positive *Aspergillus* culture	0
No evidence of IA	**53**
non-EORTC/MSGERC imaging findings	3
normal imaging	21
Positive *Aspergillus* GM LFA	0
Positive *Aspergillus* culture	9
A. fumigatus	3
A. niger	5
A. flavus	4
No growth	44

All IA patients had a previous history of severe neutropenia within the last two months, but only one was neutropenic at the time of the study. Thus, among patients with a history of severe neutropenia, IA was described in 33.3% (3/9). None of the IA patients was on antifungal prophylaxis with IA occurring in 6.1% (3/49) of patients not on antifungal prophylaxis. Of the IA patients, only one who was classified as probable IA was critically ill and died within one-month post-IA diagnosis, although IA was not cited as the cause of death.

6.4 DISCUSSION

To the best of our knowledge this is the first prospective study attempting to evaluate the epidemiology of IA among haematology patients in Ghana and West African sub-region (19). The diagnostic challenges associated with IA makes it difficult to adequately investigate, especially in many developing countries where awareness is inadequate and access to relevant tools and medical infrastructure are insufficient. In this pilot study, we report a significant rate of 5.4% (95% CI, 0.4 – 10.4%) of IA among patients with haematological malignancies. The findings of the study support the belief that IA may probably be an important cause of morbidity and mortality in patients with haematological malignancy in Africa as reported from other continents (5).

The prevalence rate from the present study falls within the 4-11% rate reported in a global systematic review and meta-analysis (5). However, it is critical to mention that there was no case of proven IA in our study. This is because histopathology or cytopathology which is one of the requirements for classification of proven cases according to the EORTC/MSGERC definitions is rarely performed (20). For example, in our study, only one patient had biopsy collected for histological analysis which was negative for fungal elements. Additionally, no sterile specimen was collected and thus the role of direct examination or culture in defining a proven case of IA was limited.

The study also shows the *Aspergillus* GM LFA test could detect about two-thirds of IA cases when used alone. This is particularly important because definitive diagnosis can be challenging, although early and accurate diagnosis is crucial due to the high mortality associated with IA. This rapid test thus may be a critical tool for aiding the diagnosis of IA in centres without CT scan or other sophisticated techniques to demonstrate the evidence of *Aspergillus* infection as previously reported (12). The simple and rapid nature of the assay makes it easy to incorporate it into many resource-constrained settings. Recently, the *Aspergillus* GM LFA was reported to have performed better than the popular Bio-Rad *Aspergillus* GM EIA (11). Also, there is a newly developed galactofuranose detection in urine to further simplify the diagnosis of IA (21,22). Moreover, the CT scan features are not specific to IA. In our study, although halo sign, consolidation and pleural

effusion were abnormalities noted in only IA, there are several differential diagnoses including tuberculosis. Nevertheless, several factors may result in the assay being falsely positive, including age, prior food taken, exposure to certain antibiotics and immunoglobulins, co-morbidities and impaired intestinal lining and these factors must always be considered in interpretation of results (23,24).

Severe neutropenia for at least ten days or more is a major leading host factor strongly linked with IA among active haematological malignancy patients on treatment (9,25,26). Although this finding is largely corroborated by many studies, our rate was much higher (10,27). This could be mainly because only one (1.8%) patient in our study had received a mould-active prophylaxis (itraconazole). The primary antifungal used in prophylaxis influences the incidence of IA and is commonly reported to decrease the rate of occurrence of SFIs, albeit not the overall survival (28,29). Systematic reviews and guidelines recommend posaconazole as the antifungal of choice for minimizing exposure to SFIs among haematological malignancy patients particularly severe neutropenic AML patients (9,16,26,30). Unfortunately, this antifungal is not readily accessible in Ghana, and was observed only one patient received itraconazole, a mould-active antifungal prophylaxis while many received fluconazole which is generally not active against moulds. Although there is no specific policy or guideline for prophylaxis the current practice involves providing prophylaxis to patients experiencing febrile neutropenia (personal communication).

There are several limitations to our study. To begin with, this is a single-centre study in a national referral hospital where guidelines for the diagnosis and treatment of SFIs at the Haematology Department were non-existent and thus most of the research diagnostic approaches were not easy to implement. Secondly, the centre has no dedicated haematology ward, creating challenges in accessing critically ill patients on admission and in following up with serial *Aspergillus* GM screening. Also, the findings cannot be generalised due to the small sample size of our study. A larger study with an increased awareness and improved understanding of IA among haematological malignancies is required to generate more reliable epidemiological data.

6.5 CONCLUSION

In conclusion, our study shows that IA may occur at significant rates among patients with haematological malignancy in Ghana, particularly among those with AML and history of neutropenia and could contribute to morbidity and mortality. Also, *Aspergillus* GM LFA may improve early diagnosis and diagnostic-driven targeted antifungal therapy as baseline CT scan and antifungal prophylaxis, particularly, mould-active agents are not routinely utilised in these settings.

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CHAPTER 7: STUDY 5– SPECTRUM AND AETIOLOGY OF FUNGAL INFECTIONS IN GHANA: A 10-YEAR RETROSPECTIVE HISTOMOLECULAR STUDY

ABSTRACT

Fungal infections have gained attention in recent times as a significant cause of morbidity and mortality worldwide. Comparatively, the epidemiology of fungal infections in several countries in Africa has not been extensively described. In Ghana, a recent survey estimated about 4% of the Ghanaian population are affected by 'serious' fungal infections, although the specific pathogens involved were not usually clarified. This was due to the absence of epidemiological studies and inadequate laboratory diagnostic capacity generally restricted to histopathology. The aim of this study was to describe the spectrum and aetiology of histopathologically diagnosed fungal infections in Ghana. We retrospectively reviewed reports from 2012 to 2021 from three major pathology laboratories in Ghana to identify reports indicating the detection of fungal elements, then extracted demographics and clinical details, and subsequently obtained tissue blocks to identify causative organisms by pan-fungal PCR and DNA sequencing. Over the study period, 107 cases were found. No specific temporal trend was observed. The majority (53.3%) of the cases were females and the median age was 41 years. The most frequently affected site was the sinonasal area (34%). The type of fungal infection was determined for 58 cases, comprising aspergillosis (21 cases), candidiasis (14 cases), dermatophytosis (six cases), mucormycosis (three cases), two cases each of chromoblastomycosis, histoplasmosis, eumycetoma, entomophthoromycosis, sporotrichosis and Malassezia and a single case each of cryptococcosis and onychomycosis. Out of the 95 tissue blocks retrieved, fungal DNA was detected in 44 (46%) cases and in 24 (54.5%) were successfully sequenced but in only seven was the fungal aetiology identified to species level. Histopathology and molecular analysis were concordant in five of seven (71.4%) cases to the genus level. Of the 53 cases with clinical information, only seven (13.2%) requests suspected a fungal infection. There is probably a wide spectrum of fungal infections in Ghana including those caused by rare yeasts and moulds. Improving laboratory diagnostic capacity by complementing histopathology with serology, culture, and molecular methods could enhance accurate detection of fungal infections.

Keywords: fungal infection, Ghana, histopathology, molecular analysis

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7.1 INTRODUCTION

Fungal infections comprising cutaneous, subcutaneous, and invasive infections have gained significant attention in recent times as a major cause of morbidity and mortality globally. The recent launch of the first ever WHO Fungal Priority Pathogen List and the additions of some subcutaneous fungal infections to the WHO Neglected Tropical Diseases list have been instrumental in championing efforts to improve diagnosis of fungal infections across the world (1,2). Presently, the epidemiology of fungal infections in several countries in Africa has not been extensively described. In Ghana, the only attempt previously was a survey that estimated about 4% of the Ghanaian populace probably affected by major fungal infections (3). The survey could not report on the fungal aetiology in many instances due to the absence of epidemiological studies and inadequate laboratory diagnostic capacity. In Ghana, laboratory testing for SFIs particularly has historically been restricted to histopathology given the absence of conventional culture and direct microscopy methods and contemporary antigen-antibody and molecular tests (3,4). Furthermore, there is no specific surveillance programme for any fungal infection in Ghana. The diversity of the fungal infections affecting Ghanaians and their causative organisms are thus unclear. The only available data has comprised case reports and small case series that rarely reported on aetiological agents (3,4).

Analysing laboratory data is a common method of evaluating the epidemiology of infectious diseases and studies suggest histopathology is probably the most common approach in detecting fungal infections in Ghana and many African settings (3–6). Moreover, histopathology is a critical requirement in confirming fungal infections, that is, allowing the designation confidence of 'proven'. Although, histopathology cannot reliably identify most causative fungi to genus or species level, it can classify *Coccidioides, Paracoccidioides, Sporothrix, Histoplasma* and *Blastomyces* at least to genus level (7).

Review of histopathology reports has been previously used to evaluate the epidemiological parameters including prevalence and trends of single or spectrum of fungal infections in some African countries such as Nigeria (8–10), Togo (11,12) and recently Uganda (13). The aim of this study was to evaluate the trend, spectrum, and clinical suspicion of histopathologically diagnosed fungal infections while identifying their causative organism by molecular analysis on archived tissue blocks.

7.2 METHODS

This was a multi-centre retrospective study conducted to review the histopathology reports of three major public histopathology laboratory services providers in Ghana, namely the Department

of Pathology, Korle-Bu Teaching Hospital; Cellular Pathology Unit, Ghana Standard Authority and J.M Wadhwani Department of Anatomical Pathology, 37 Military Hospital from 2012 to 2021 to identify reports indicating the presence of fungal elements. These histopathology laboratories provide diagnostic services to attached hospitals and many hospitals from all regions across Ghana. The histopathology report records were manually reviewed at the KBTH and 37 Military Hospital. At the Ghana Standard Authority reports were electronically searched using the following keywords: fungi, fungal element (s), fungal bodies, hyphae, yeast, pseudohyphae and spores. Secondarily, distinctive morphological appearance of fungal elements such as spherules, sclerotic/muriform/medlar/copper penny bodies and grains were looked for. Cases were selected from the general population irrespective of underlying condition or site of sample collection. The sociodemographic and clinical details including age, gender, site of tissue collection and clinical suspicion for fungal infection were extracted from laboratory records. Additionally, the type of histological stains used, appearance fungal elements or features seen, and fungal infection diagnosed by examining pathologists were all extracted from laboratory reports. The corresponding archived tissue blocks of positive cases were then retrieved for molecular analysis to identify the aetiological agents to genus or species level.

Formalin-fixed paraffin-embedded (FFPE) tissue blocks were re-cut, re-stained with fungal stain, periodic acid Schiff (PAS) and re-examined by the author (BKO) and a consultant pathologist (IE) for confirmation of the presence of fungal elements, when possible. For DNA extraction, tissue blocks were cut into 15 µm sections and five to 10 sections were collected into a 1.5 ml microcentrifuge tube to obtain approximately 25 mg of tissue for DNA extraction. Tissue sections were deparaffinized, digested, and DNA extracted using Quick-DNA FFPE miniprep kit (Zymo Research Corporation, Irvine, California, USA) following the manufacturer's instructions. Five microliters of extracted DNA from the sample was used for pan-fungal PCR. The pan-fungal PCR assay implemented was an ITS gene hemi-nested PCR using an in -house developed protocol. PCR assay was performed on a Maxygene II Thermal Cycler (Axygen Scientific, Union City, California, Spain). PCR products were purified and outsourced to a private genomics products and service provider, Inqaba Biotech for sequencing in South Africa.

Ethical approval for this study was indirectly obtained from the Institutional Review Committee of the KBTH (STC/IRB/00058/2020) and 37 Military Hospital (37MH-IRB/FP/IPN/551/21) as part of the entire 'Epidemiology of Invasive Fungal Infections in Ghana' project but was generally deem not to require full ethics approval. However, appropriate administrative permission was obtained from all three institutions involved in study.

Data was statistically analysed using the Statistical Products and Services Solutions (SPSS), version 25 (IBM Corp, Armonk, New York, USA). We summarized statistics of variables (sex, age, clinical and histopathology characteristics) using frequencies, percentages, median and interquartile range where applicable. The distribution of cases according to specific years for each centre was analysed.

7.3 RESULTS

Over the 10-year study period from 2012 to 2021, we found 107 cases of histopathological reports revealing the presence of fungal elements or structures (see Appendix 13) for details on the 107 cases). These comprised 91 deep fungal infections and 16 superficial and muco-cutaneous infections. The number of cases per year among the three centres ranged from 3 to 16 with the highest number of cases recorded in 2013 (Figure 7.1). There was no identifiable pattern recognized in the number of cases recorded over the 10-year period. Most cases were from the Ghana Standard Authority and KBTH, accounting for 60 (56.1%) cases and 39 (36.4%) respectively.

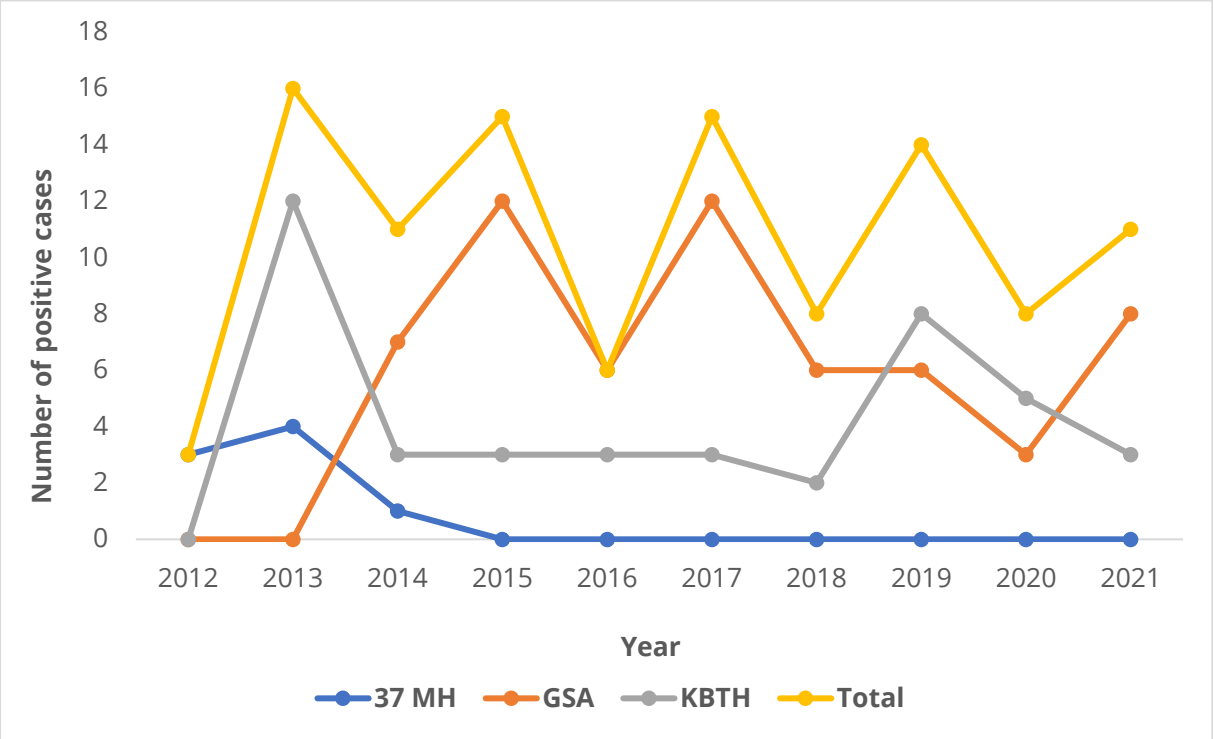

Figure 7.1: Trend of fungal infections diagnosed by histopathology over 10 years

The spectrum of fungal infection was relatively broad with determination of the type of fungal infection made for 58 (54.2 %) cases (Figure 7.2). The diagnosed fungal infections included

aspergillosis (21 cases), candidiasis (14 cases), dermatophytosis (six cases), mucormycosis (three cases) and chromoblastomycosis, histoplasmosis, eumycetoma, entomophthoromycosis/phycomycosis, sporotrichosis and *Malassezia* infection (two cases each). The remaining fungal infections implicated were cryptococcosis and onychomycosis, a single case each.

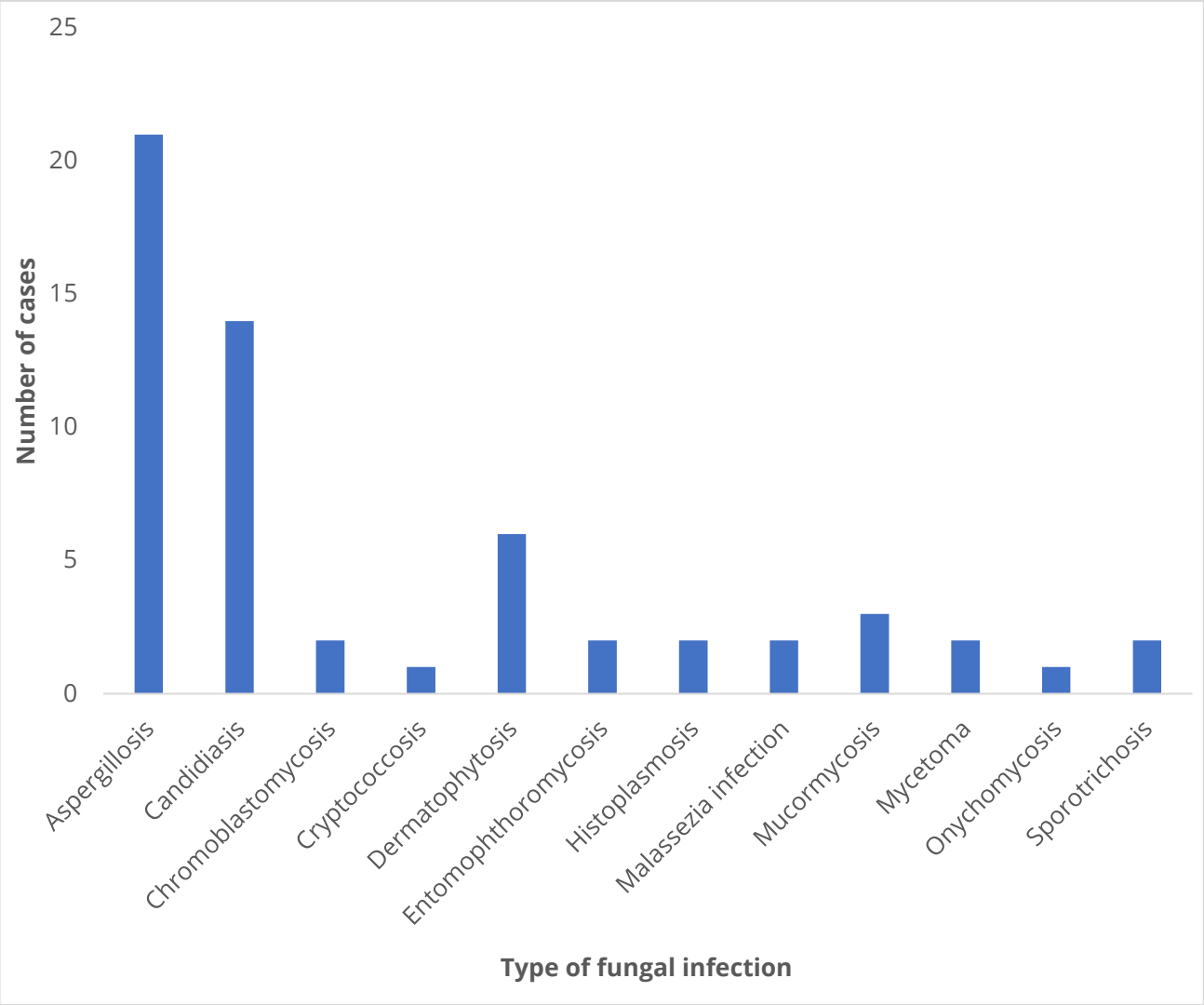

Figure 7.2: Number of cases of different types of fungal infections

Clinical information was available for 53 cases, but only in seven (14.6%) cases was there suspicion for fungal infection by clinicians showing that majority of the cases were a clinical surprise. Most cases were diagnosed in females 57 (53.3%). Age ranged from 4 to 86 years with a median age of 41 years (IQR, 31-55). Regarding the sites involved, the nose or nasal regions were the commonest

with 34 cases mostly affected by aspergillosis (18) and mucormycosis (3), followed by GIT (19), limbs (17) and the skin (7) and other body sites had less than five cases (Appendix 12).

A major observation made in this study was that special fungal stains were rarely used, that is, PAS (16 cases), Grocott-Gomori methenamine silver (GMS) (three cases) and unspecified special stain (three cases) with the remaining 85 cases diagnosed on H&E alone. There were both regular fungal morphologies (yeasts and hyphae) and special fungal structures such as sclerotic/muriform/medlar/copper penny bodies (Figures 7.3 and 7.4). Generally, the majority (87 cases) of the fungal structures detected was not adequately described with several scanty descriptions such as fungal bodies, fungal cyst, fungal elements, hyphae, and yeast. This observation was consistent among the three centres.

Out of the 107 cases, 95 blocks were retrieved for molecular analysis. Fungal DNA was detected in 44 (46%) and 24 amplicons were successfully sequenced but only seven had good chromatographs and allowed for identification to species level by comparing the sequences obtained with the GenBank database via the National Center for Biotechnology Information (NCBI) platform (Table 7.1). The remaining 18 cases were mixed infections requiring further sequencing. There were two discordant findings between histopathology and molecular identifications.

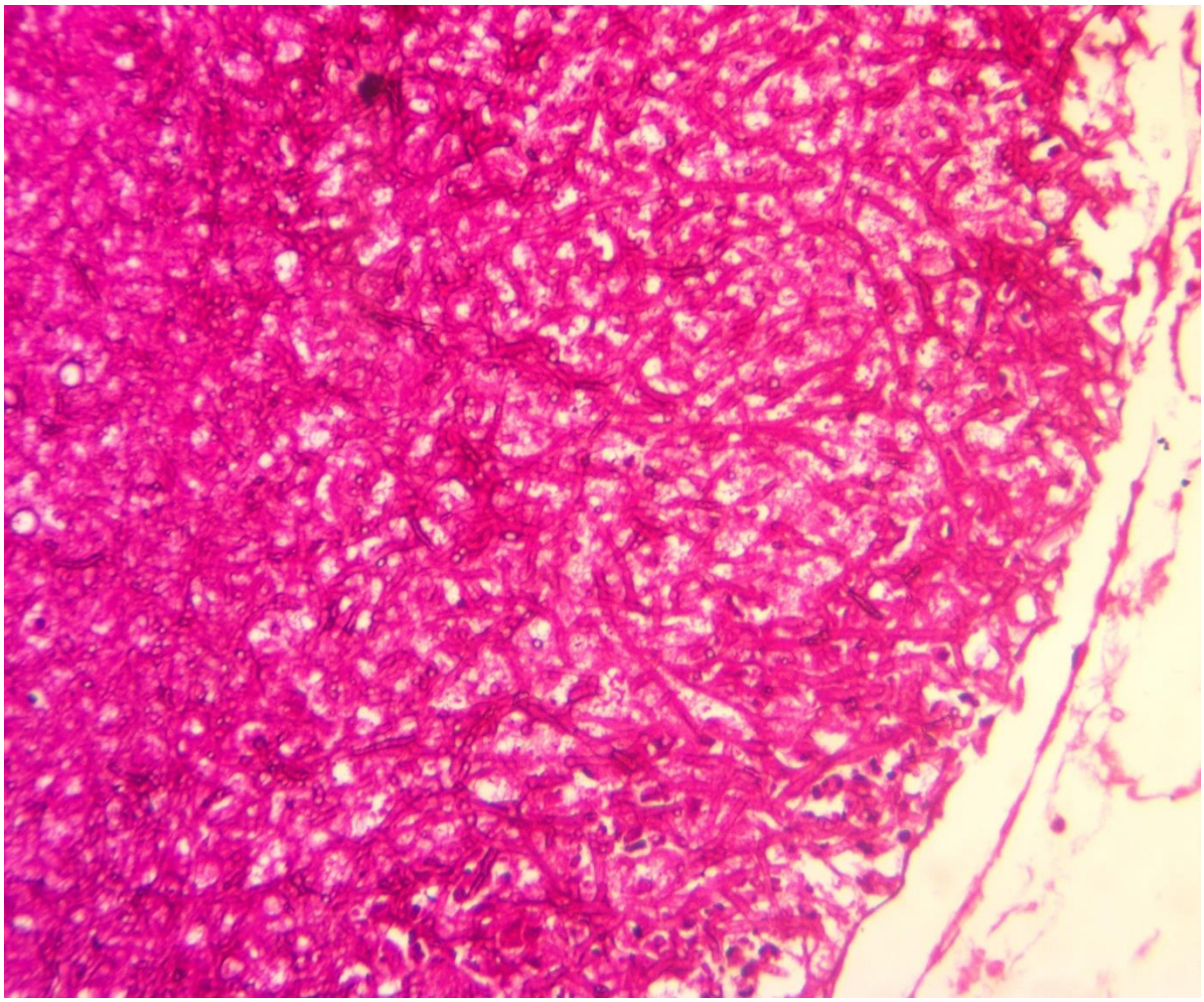

Figure 7.3: H&E-stained section of a left antral mass from a 55-year-old female with epistaxis and chronic headache showing regular acute branching dichotomous septate hyphae typical of *Aspergillus* species.

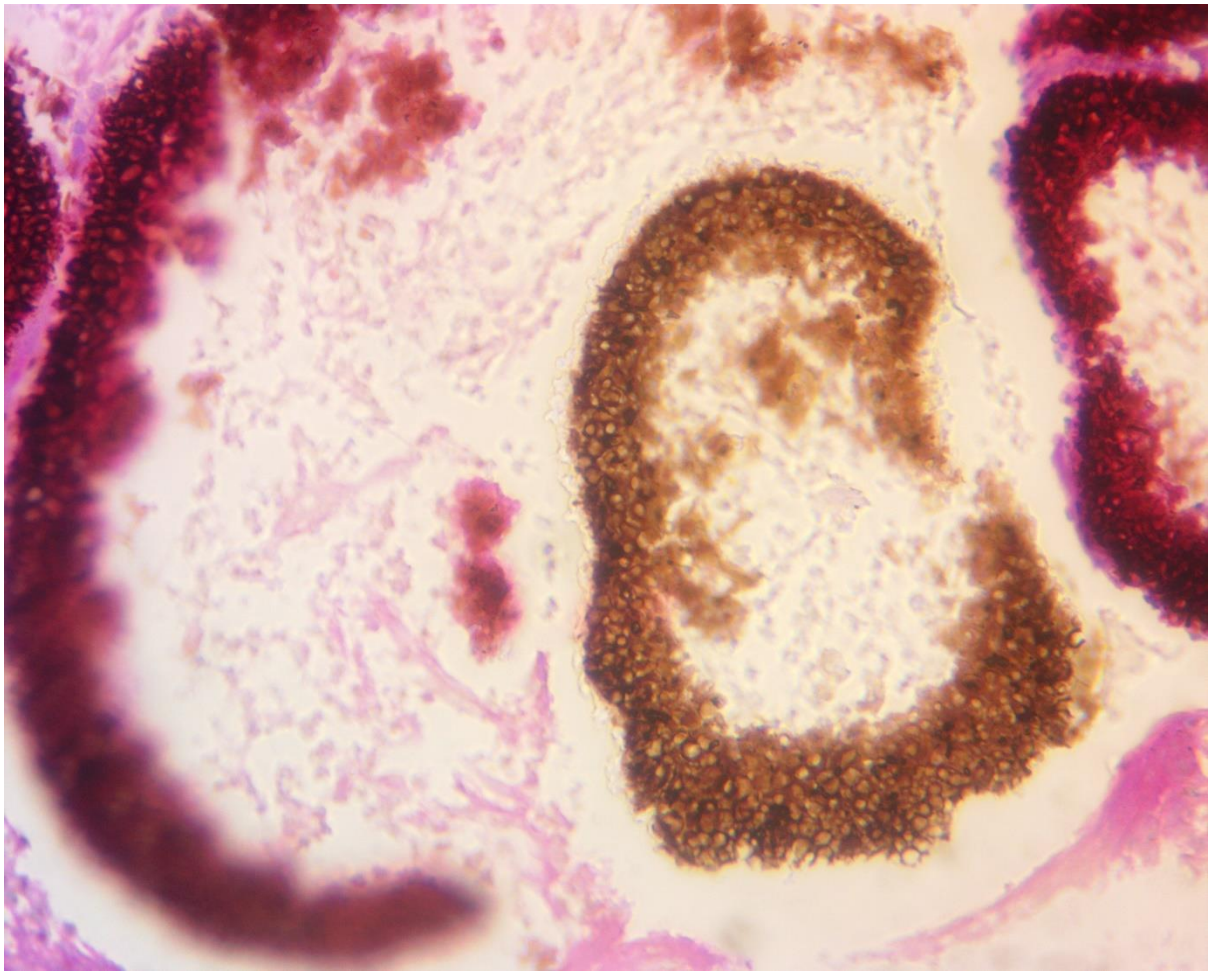

Figure 7.4: PAS-stained section of a left foot infected ganglion from a 49-year-old male showing a ring of aggregates of pigment-producing and double contoured spherical structures known as sclerotic bodies which are consistent with chromoblastomycosis.

Table 7.1: Comparison of histopathology and molecular identification of seven cases

Study ID	Sample site	Histopathology ID	Molecular ID
FHM006	Sinonasal	Sporotrix *spp*	*Aspergillus flavus*
FHM010	GIT	*Candida spp*	*Candida guillermondii*
FHM017	Limb/elbow	*Aspergillus spp*	*Aspergillus fumigatus*
FHM033	Sinonasal	*Aspergillus spp*	*Aspergillus sydowii*
FHM036	GIT/appendix	*Candida spp*	*Candida albicans*
FHM041	Sinonasal	*Aspergillus spp*	*Trichoderma spp*
FHM046	Scalp	*Not stated*	*Trichoderma spp*

7.4 DISCUSSION

The study aimed at highlighting the epidemiology of fungal infections diagnosed by histopathology in Ghana with a special focus on the trend, spectrum, and aetiology. Over the 10-year study period, from 2012 to 2021, there were 107 cases recorded, including 91 deep fungal infections among the three histopathology laboratories. There were diverse fungal infections reported and this finding offers a new narrative about the epidemiology of fungal infections in Ghana, particularly deep fungal infections. This is extremely important in the Ghanaian clinical setting particularly considering the critical role of histopathology in aiding diagnosis and in most instances the commonly available means of diagnosis (4).

Compared to a survey analysing the burden of fungal infections in Ghana, the present study reveals a broader scope of the spectrum of fungal infections seen in Ghana to include some uncommonly reported infections (3). These include mostly subcutaneous fungal infections such as eumycetoma, chromoblastomycosis, sporotrichosis and entomophthoromycosis. In a review of mycetoma in West Africa, there was no indigenous case reported from Ghana (14). Only two studies have previously reported entomophthoromycosis, specifically, basidiobolomycosis, in Ghana, but the specific aetiology could not be confirmed as diagnosis was made with histopathology (15,16). It is, however, noteworthy, some of these reported infections may have been misdiagnosed by examining pathologists. This was evident in two of the seven finalized cases. Notwithstanding, these reported cases suggest the possible existence of a broad spectrum of fungal infections including endemic fungal infections. Enhanced immigration and international travels are likely to increase the diversity of fungal infections in the future (7,14). The nasal region was the site frequently diagnosed with deep fungal infection by histopathology, probably because samples from these areas are relatively easy to collect in comparison to sampling internal organs.

The fact that most of the cases were seen at the KBTH and Ghana Standard Authority is probably due to long serving pathology consultants with at least 30 years of experience supporting those centres. Additionally, unlike the 37 Military Hospital which seldom received samples outside their facility and the Greater Accra region, these centres process samples from several private and public hospitals within and outside the Greater Accra region from all regions of the country.

In the era of growing antifungal resistance and expansion of rare species of moulds and yeasts as causative agents of fungal infections, accurate identification of aetiological agents of infections has become more important now than ever. Identifying the right cause of infection allows for the right choice of antifungal medications to ensure the right treatment is offered to ensure good clinical outcomes. In view of this, in the absence of routine fungal culture, carrying out further molecular

investigations on tissue blocks which histopathology analysis demonstrated the presence of fungal elements will go a long way to influence drug options while providing data on emerging or rare species causing human infections (17). Despite the increasing use of molecular methods on FFPE to aid species level identification in many clinical mycology laboratories, the methodology or procedures of available assays are not extensively standardized and validated (18–22). Meanwhile, the 46% PCR positivity rate recorded in our study compares with a 41% rate reported for another study that worked on samples stored for up to 10 years (23). However, when stored up to 7 years, rates between 51-63% have been reported (24). Moreover, this can be an expensive and equipment intensive venture and implementation in the laboratory settings in Ghana is difficult.

The use of fungal stains is strongly recommended by guidelines and experts to improve the detection of fungal elements or structures in tissues during histopathological analysis (7,25). In the present study, we observed that main fungal stains, that is, PAS and GMS were rarely used. Other special fungal stains such as Alcian blue (commonly for *Cryptococcus* spp), mucicarmine (commonly for *Cryptococcus* spp (15), *Blastomyces* spp and *Rhinosporidium* spp) and Fontana-Masson (mainly for dematiaceous fungi) were not used at all (26). Attempting to diagnose invasive forms of fungal infections with only H&E as noted in 79% cases of the study, is generally difficult and frequently associated with reduced sensitivity. Although fungi can be seen with conventional H&E stain, special stains enhance the detection of fungi in tissues. These stains also allow for narrowing identification of possible aetiologies and aid in preliminary diagnosis for example for cryptococcosis. The diverse and inharmonious ways of describing and reporting fungal structures including the use of broad descriptions such as yeasts, hyphae, fungal bodies, and fungal elements reveals the inadequate training in reporting on fungal elements in tissue. Detailed description of fungal structures or elements could provide hints to clinicians on likely aetiologies which could play a role in the treatment decisions.

Limitations

The other major histopathology laboratory at the Komfo Anokye Teaching Hospital serving significant number of hospitals in the Middlebelt and Northern parts of Ghana, was not included in the study. This facility probably receives significant samples from rural areas where agricultural activities were profound and could have implicated some implantation fungal infections. Some tissue blocks could not be retrieved. Moreover, some blocks only allowed for a few sections to be cut, so a second opinion on the presence of fungal elements could not be done for all cases. Next generation sequencing on successfully amplified DNA could not be done due to time and financial constraints; this resulted in the identification and finalization of only seven cases**.**

7.5 CONCLUSION

Overall, we retrospectively evaluated the trend, spectrum, and aetiology of histopathologically diagnosed fungal infections in Ghana. The findings highlight a possible underdiagnosis, low index of clinical suspicion, diverse fungal infections including a few not previously reported and rare aetiological agents identified by molecular methods. There is the need to improve awareness among clinicians, sensitize and train pathologists, support histopathology analysis with molecular assays for accurate identification of fungal pathogens and explore antigen-antibody tests and culture. Finally, prospective epidemiological studies are required to better appreciate the burden of the major spectrum fungal infections outlined.

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CHAPTER 8: SUMMARY AND CONCLUSION

8.1 OVERVIEW: FROM ESTIMATION TO ACTUALITY

As mentioned, and extensively illustrated in the introductory chapter (Chapter 1) of this thesis, SFIs in general have garnered significant attention globally in the past three decades than ever before, especially, in the last 2 years. This has been due to wide and systematic advocacy and sensitization by national professional bodies and international organisations such as ISHAM, GAFFI, ECMM and MSGERC. Additionally, studies on the prevention, epidemiology, diagnosis, and management of SFIs have expanded. The findings have particularly transformed the scope of diagnosis and treatment of SFIs, reducing the time to diagnosis and treatment initiation. This is of utmost importance because SFIs are usually strenuous to diagnose, and their diagnosis are not straightforward. Moreover, classical testing methods such as direct microscopy, culture and histopathology are not very sensitive and have longer TAT; consequently, most SFIs were diagnosed during autopsy. Early diagnosis and prompt antifungal treatment has thus been a mirage for clinicians. Additionally, most SFIs have a poor prognosis when diagnosis is delayed. The major advancements in SFI diagnosis have been the evolution of antigen-antibody, biomarker, and molecular assays with high sensitivity, specificity, and shorter test runs. Some require non-invasive or straightforward invasive specimen sampling methods such as lumbar puncture. In recent times, the antigen-antibody assays, mostly designed originally as EIA to run on manual or automated platforms, are transitioning to LFA platforms which are easier to be implemented in both developed and resource-limited settings. It is opined by experts that the simplified ways of testing via RDTs will improve access to essential fungal testing and encourage research around the globe, particularly, in resource-limited settings where classical methods are not available at all or widely inaccessible.

The recent expanding awareness about SFIs globally, has extended to many African countries. Nonetheless, the continuous lack of access to essential fungal diagnostics has meant that the epidemiology of SFIs in these countries has been extremely limited, albeit advancing steadily (1). High quality local epidemiological studies provide a good guide to those most at-risk patient groups and the potential value of diagnosis. Like many African countries, very little is known about SFIs in Ghana and the literature on SFIs have been scanty. Information on the frequency, pattern, types of SFIs, endemicity, causative organisms, resistance to antifungal drugs and mechanisms of antifungal drug resistance are not known. Indeed, the available literature prior to 2019 was generally sporadic case reports or series (Chapter 1, Table 1.3) (2). Notwithstanding, the patient groups at major risk to SFIs including patients with HIV, TB and haematological malignancy had a

significant population and continue to expand in Ghana. Moreover, the global epidemiology of SFIs has been evolving in the past few decades, with rising cases, emergence of new pathogens and at-risk patient groups, as well as previously non-pathogenic fungi noted to become pathogenic. In 2019, the author led the first major attempt to generate a more broad and systematic epidemiological data on SFIs in Ghana (2). This was done using rates of SFI in major at-risk patient groups from other African settings or beyond, at-risk group population and general population data in a model developed by the LIFE and GAFFI to estimate the incidence or prevalence of selected SFIs. This burden estimate has been extremely significant and yielded several multiplying effects including setting the stage for increasing awareness, producing preliminary figures for advocacy, highlighting existing knowledge and skill gaps, and ultimately birthing the current work presented in this thesis. Despite the profound implications for diagnostics and care enumerated above from the published paper, it was not persuasive enough to signal the need for improvements in diagnosis. The primary hesitation focussed on the methodology, which did not present a convincing appeal to both low and high-level stakeholders because true fungal infection rates for Ghana were not available for the burden estimates. However, an important implication from the few SFI case reports and series was insufficient awareness, low index of clinical suspicion and lack of access to fungal diagnostics which need to be improved to drive progress in improving diagnosis and epidemiology (3–6).

The main aim of this thesis was to explore the incorporation of fungal RDTs in clinical diagnostic algorithms to drive SFI diagnosis in Ghana. Additionally, this research examined the trend, types, and presentations of histopathologically diagnosed fungal infections as well as using histomolecular techniques to confirm and identify their aetiological agents. The focus was to take advantage of the emerging and expanding phenomenon of fungal RDTs by initiating their use in real clinical settings in Ghana to detect SFIs in relevant at-risk groups. On the other hand, as histopathology has been demonstrated as the major means of diagnosing fungal infections (particularly, SFIs) this thesis ought to review histopathology reports to evaluate histopathology-diagnosed fungal infections. Since histopathology cannot reliably identify aetiological agents, histomolecular analysis was employed for definitive identification of the causative organisms in tissues reported to have fungal elements.

Three major patient groups at high risk for SFIs, that is, HIV, TB and haematological malignancy were the focus for the four prospective studies using four different RDTs. The selection of these three patient groups was guided by the SFI burden estimate study (2). Moreover, at the global, regional, and various national levels, the same patient groups were found to be among the most

vulnerable for SFIs (7). The prospective studies comprised three cross-sectional studies (cryptococcosis and histoplasmosis in HIV, CPA in presumed TB, and IA in haematological malignancy) and one follow-up study (CPA in confirmed TB). A retrospective study was then implemented to identify histopathology diagnosed fungal infections between 2012 and 2021, review their demographics and clinical characteristics from laboratory records and obtain archived tissue blocks for histomolecular analysis to identify their aetiological agents.

The first four paper chapters (Chapters 3 - 6) in this thesis generally convey the main findings of the thesis, that is an improved case detection of SFI championed by RDTs. The work also starts to unravel the relatively dynamic epidemiology of SFI in Ghana. It systematically and practically demonstrates the feasibility and essential role RDTs can play when incorporated into diagnostic algorithms to advance the detection of SFI, as widely speculated in literature. Particularly, they expound how these RDTs can be used to raise the suspicion of SFIs and then supplemented by other confirmatory radiological and laboratory investigations to make a definitive diagnosis. For the first time, for some infections, it has provided direct evidence of information on aetiological agents of SFI in Ghana, by both culture and molecular identification. Overall, the findings show how RDTs can be advantageous in providing a positive narrative about SFI epidemiology in African settings with the potential of saving money and lives. Furthermore, a review of histopathology records and post-histopathology molecular analysis (histomolecular assays) strategically highlight the historical dynamics of the diverse epidemiological features of fungal infections diagnosed at histopathology. The outcomes of the diagnostic approaches implemented throughout this research, contribute to the efforts to develop strategies for reducing the number of SFI cases that are missed or misdiagnosed in African settings. This thesis additionally provides a real-world diagnostic framework which could motivate SFI research in African settings, to overturn the current narrative of rarity of SFIs in these settings. The overall aspiration is that patients suffering from SFIs will receive appropriate treatment, improve clinical outcomes, and reduce attributable mortality.

8.2 INTER-RELATING THE RESEARCH QUESTIONS AND MAIN FINDINGS

This section attempts to revisit the rationale and key research questions and link them with the main findings as conveyed in each of the paper chapters. Further connections with the broad indications and implications are reflected on. Specifically, this is to outline the extent to which the research objectives defined from knowledge gaps in the introductory chapter (Chapter 1) were realized. The general question sought to be answered by this research was: how RDTs can be

utilized to initiate SFI diagnosis and how the dynamics of epidemiology can be appreciated to guide present diagnostic interventions in African settings? To answer this question, studies were strategically designed based on specific research questions and key objectives and then worked through to find the answers in tandem with the five paper chapters as follows.

How frequent is CM among different categories of people living with HIV in Ghana? How common is histoplasmosis in people living with HIV in Ghana? Can the OIDx Histoplasma LFA be a suitable alternative to the recommended IMMY Histoplasma EIA in detecting histoplasmosis in an African setting?

The main objective of the first study of this thesis was to unravel how commonly SFIs complicates people living with HIV, the patient group most often affected by SFIs in Africa. The focus was on CM and histoplasmosis which are common opportunistic fungal infections. This objective was central in the study (Chapter 3); aiming to determine the frequency of two main SFIs (CM and histoplasmosis) among HIV-infected patients in Ghana using CrAg LFA for CM and IMMY *Histoplasma* EIA and/or OIDx *Histoplasma* LFA for histoplasmosis. Antigen testing was complemented with confirmatory testing by conventional methods of direct microscopy, culture, and histopathology. The study reported a prevalence of 2.7% (95% CI, 0.1 – 5.3%) for CM and 4.7% (95% CI, 0.7 – 8.7%) for histoplasmosis with a median CD4 count of 204.5 cells/uL among the positive cases. Unlike CM, this is the first prospective epidemiology study on histoplasmosis in West Africa and the 4.7% (95% CI, 0.7 – 8.7%) incidence identifies histoplasmosis as a significant opportunistic fungal infection in Ghana. For CM, the point prevalence of 2.7% (95% CI, 0.1 – 5.3%) is slightly higher than the average rate from previous studies of 2% (8–10).

Admitting that the reported rates are small, nonetheless the higher incidence of histoplasmosis over CM is unusual and uncommon particularly in African settings. This is because CM, the commonest clinical manifestation of cryptococcosis, has the highest burden and mortality in HIV-infected patients in sub-Saharan Africa (11). It can also be argued that the introduction and expanded access to CrAg LFA screening and research is the major contributing factor to the popularity of CM. Despite the declining caseload of CM, mortality is on the increase, fuelled by a combination of many patients presenting late and failure of anti-retroviral therapy (11,12). A ground-breaking finding in the treatment of CM, involving a single high-dose liposomal amphotericin B alongside flucytosine and fluconazole could improve treatment outcomes (13). On the part of histoplasmosis, because diagnosis has mainly depended on conventional methods in

times past and their availability and accessibility are limited in several African countries, it has been neglected for a long time. Two studies in Uganda and Tanzania found lower rates of *Histoplasma* antigenemia (14,15) but relatively higher rates was reported from Cameroon (16). Literature reviews found many cases, including those affecting children (17,18). In recent times, however, this narrative is changing with increasing research in histoplasmosis with both conventional and more importantly contemporary assays such as antigen tests and molecular tests. This suggests the need for a relook at the true burden of HIV-associated histoplasmosis in HIV in Africa. In Ghana, for instance, the current HIV guidelines places little attention on histoplasmosis as an HIV opportunistic infection, in contrast to CM. Although antigen tests for CM and histoplasmosis are both listed on the WHO Model List of Essential In Vitro Diagnostic Assays and recommended by WHO CM and histoplasmosis guidelines, the LFA platform is recommended for CM and EIA for histoplasmosis.

The second study objective was to compare the diagnostic performance of a *Histoplasma* LFA from OIDx and the recommended *Histoplasma* EIA from IMMY since the current recommended platform for *Histoplasma* antigen testing is EIA. The OIDx *Histoplasma* LFA, is newly introduced and the only second LFA for *Histoplasma* EIA after the MiraVista *Histoplasma* LFA (which is not available commercially). The clinical and diagnostic performance of both LFAs is good, however unlike the MiraVista *Histoplasma* LFA, the OIDx *Histoplasma* LFA is more likely to be easily distributed and widely accessible on the continent of Africa (19). This study is the first using freshly collected urine samples to assess the diagnostic and clinical efficacy of the OIDx *Histoplasma* LFA. Although, the number of positive cases in this study was small, the OIDx *Histoplasma* LFA was positive in urine for all the confirmed cases of histoplasmosis. In contrast, many false positive results were noted in serum samples with the OIDx *Histoplasma* LFA and a similar observation was reported in a recent study in Trinidad (20). By extension the OIDx *Histoplasma* LFA can be a useful screening and diagnostic tool for histoplasmosis, particularly in settings where the EIA platforms are not available.

How common is CPA among patients presenting with TB-like symptoms? How to utilize the Aspergillus-specific IgG and IgM LFA to aid CPA diagnosis in Ghana? What are the common aetiological agents of aspergillosis in Ghana?

The objective of this study was to determine the prevalence of CPA in patients with presumed new PTB and PTB relapse. In other words, this study particularly sought to find the proportion of patients suspected or presumed to have PTB, that had CPA instead. This study was necessary

because of the similarities noted with the symptoms and radiological features of PTB and CPA. Without routine fungal testing, there is a high likelihood that many cases of CPA are being misdiagnosed as PTB. Moreover, of the many approaches utilized in studies of CPA in PTB particularly across TB endemic settings, this study approach has not been exploited. The study found that among patients suspected and being investigated for PTB, about four of every 100 (3.7%) without previous PTB had CPA while one in two (50%) of those previously treated for PTB had CPA (see Chapter 4). This finding brings a different dimension to the known association between CPA and PTB. These findings can be used to develop strategic screening programmes to enhance early detection of CPA cases, especially among patients who had previously been successfully treated for PTB in high TB burden settings. Our finding of about 50% (95% CI, 28 – 72%) of cured PTB patients who re-present with symptoms having CPA is similar to the findings in Vietnam and India (21,22). Of course, this will require a cost-effectiveness analysis prior to implementation to understand the benefits over the financial implications, especially as long-term oral antifungal therapy can be costly and is not funded by most agencies or governments. As the number of cases of PTB remain steadily high in Ghana and exposure to anti-TB medication expands, the number of survivors that could be affected by CPA cannot be downplayed. Drawing on the backbone of the thesis, that is, application and implementation of RDTs, in this case the *Aspergillus*-specific IgG and IgM assay as part of regular TB care. The RDT was diagnostic in all but one CPA patient, which shows it has value in improving CPA diagnosis in conjunction with medical imaging, particularly chest CT scan.

One common missing piece of information on aspergillosis cases reported from Ghana, is the silence on the species of *Aspergillus* involved. Using conventional fungal culture, this research isolated and identified species of *Aspergillus* associated with CPA. This is important to appreciate the predominant clinical isolates of *Aspergillus* species in Ghana: *A. fumigatus,* which are more often reported from Europe and *A. flavus* and *A. niger* more from LMIC, including Africa (23). Few data have examined the performance of the *A. fumigatus*-specific IgG and IgM LFA in cases of CPA caused by other *Aspergillus* species, although one UK study indicated a reduction of sensitivity by about 9% (24). This data requires replication in other settings, particularly in LMICs. In the era of azole resistance and emerging environmental linkage due to agricultural use of azoles in fungicides, species and azole susceptibility patterns have become increasingly important (25,26).

What is the incidence of CPA following the end of anti-TB treatment and 6 months post-treatment in bacteriologically confirmed PTB patients?

Very few longitudinal studies have investigated CPA during or after treatment for PTB. Published studies had recruited patients from both bacteriologically confirmed and/or smear negative PTB patients; none has restricted their focus to bacteriologically confirmed PTB. To answer this precise question, a continuation of the initial study on CPA (Chapter 3) was undertaken to determine the incidence of CPA among the patients that had new bacteriologically confirmed PTB and received anti-TB treatment. Two time points were selected, that is, at the end of treatment and 6-month post-treatment. Unlike the end of treatment time point which has been previously studied, 6-month after treatment was novel. Here again, the *Aspergillus*-specific IgG and IgM LFA played a pivotal role. Further investigations, particularly CT scan was done only when the RDT was positive to confirm the presence of radiological abnormalities frequently seen in CPA. The trend of an increasing rate of CPA development over time found by this study corroborates with similar studies in Uganda and Indonesia (27,28). It reveals that PTB patients may develop CPA just around the time of successfully completing their anti-TB medication course and the months after. The overall rate of CPA within 12 months of starting anti-TB therapy was 10.7%, admittedly in a small cohort of 28 patients. Some of these patients may have had cured PTB and subsequently been affected by CPA. Overall, an appropriate integration of *Aspergillus*-specific antibody screening into the post-TB care provision for instance during and after the end of anti-TB treatment can contribute to the early detection of CPA in high PTB burden settings. The risk for CPA extends for many years (up to 30) post-TB treatment in other countries (29), but further longitudinal data is required.

How frequently does IA occur among patients with haematological malignancies in Ghana? Can the sona Aspergillus GM LFA reliably be diagnostic for IA? How common is antifungal prophylaxis practiced in patients with haematological malignancies in Ghana?

To answer these sub-research questions broadly, the study detailed in Chapter 6 was pursued to generate epidemiological and clinical data on IA among patients with haematological malignancy using the *Aspergillus* GM LFA as the central diagnostic tool. The *Aspergillus* GM LFA was diagnostic in two out of three cases diagnosed as probable and possible IA. This suggests that in the absence of the widely recommended GM EIA, this RDT can be implemented in appropriate diagnostic algorithms to aid the diagnosis of IA. IA is an uncommon and seldomly suspected secondary infection in this patient group in SSA. The published body of data for IA in haematological

malignancy is missing significant contributions from SSA. Nonetheless, a few cases of IA have been reported from other at-risk groups from Africa (30). It is argued by experts that the incidence of IA in haematological malignancy patients could be at least similar as reported in the developed countries. In industrialised nations, IA rates are lower when antifungal prophylaxis is used, so the incidence may be higher in Africa, where anti-mould prophylaxis is (probably) barely used as noted in our study (Chapter 6). However, considering the low survival rate of IA patients in Africa, it is difficult to confirm this supposition in the absence of data including a pragmatic screening approach. It is very likely many of these patients die without the diagnosis of IA, and can only be detected at post-mortem, if done.

This study further evaluated the antifungal prophylaxis regime in Ghana. An effective mould-active prophylaxis is a well-established strategy to reduce the incidence of IA (31–34). In many advanced clinical settings, prophylaxis is a standard routine that is regularly monitored and revised periodically based on research. Among several mould-active antifungal agents used for prophylaxis, systematic reviews and meta-analysis studies point to posaconazole as the most effective in reducing the rate of IA occurrence. Along this line, expert consensus and guidelines has thus strongly recommended posaconazole for the first-line drug of choice for prophylaxis. Posaconazole is unavailable in Ghana. Contrarily, the study identified a sub-optimal anti-fungal practice, in terms of the frequency and choice of anti-fungal drug. Antifungal prophylaxis was not usually prescribed, and the most common agents used were fluconazole, which have no anti-mould activity and occasionally itraconazole which has a lower efficacy in preventing invasive mould infection including IA (32–34).

What is the fungal infection incidence pattern in Ghana? What types of fungal infections are diagnosed in Ghana? Are some rare moulds or yeast or endemic fungal infections diagnosed in Ghana?

Fungal infections, ranging from deep seated cutaneous to subcutaneous and internal organ infections are not extensively reported on or studied in Ghana. As reviewed in Chapter 1, histopathology is the most accessible laboratory approach in making a laboratory diagnosis of fungal infections in the absence of other techniques such as culture, serology, and molecular analysis. To appreciate the real-world epidemiological, clinical and laboratory dynamics of fungal infections, evaluating histopathologically diagnosed cases is probably the most appropriate. This study was undertaken to review histopathology reports in the last decade (2012 to 2021)**.** This excluded autopsy reports. Over the 10-year period, over which this retrospective study was undertaken, 107 cases were identified among three major histopathology laboratories. This

almost certainly emphasises the huge under-diagnosis or under-recognition of fungal infections in general in Ghana. Moreover, unlike tumour or malignancy, tissue samples are infrequently collected for histopathology when infectious agents are suspected. Specific biopsies to diagnose a fungal infection are rarely done. Besides, the practice of infectious disease as a sub-specialty of internal medicine is a recent development in Ghana. Furthermore, the expertise and experience necessary to accurately detect these fungal infections during histopathological examination is limited.

The analysis of the number of cases per year over the period revealed no specific increasing or decreasing trend. This is an irregular observation, as reviews and new studies hint at a rising trend of cases in other countries. Notwithstanding, this is plausible in a setting where awareness is inadequate and clinical suspicion for cases is insufficient. The spectrum of infections revealed was diverse including both common infections and those not previously reported from Ghana in literature such as, chromoblastomycosis, sporotrichosis and mycetoma. The commonest fungal infections per the reports retrieved was fungal rhinosinusitis caused by *Aspergillus* and Mucorales. The histomolecular analysis of FFPE retrieved to identify causative organisms was only completed for seven cases. Molecular identification for the cases identified to species level reported rare species such as *Aspergillus sydowii*. Additionally, cases suggested to be caused by *Sporothrix* spp and *Aspergillus* spp by histopathology were confirmed to *Aspergillus* flavus and *Trichoderma* spp respectively by histomolecular analysis. The integration of histomolecular analysis of FFPE blocks as applied in the study has the potential to improve the final histopathology diagnosis. This is particularly crucial in the era of constant emergence of novel fungal pathogens and antifungal resistance.

The table below (Table 8.1) is a modification of Table 1. 8 (Chapter 1), and accordingly updated to summarise the main findings answering the specific research questions along with corresponding papers status.

Table 8. 1: Overview of research questions, research objectives and main findings

Chapter	Study/Paper	Research questions	Research objectives	Main findings
3	*Cryptococcal and Histoplasma Antigen Screening among People with HIV in Ghana and Comparative Analysis of OIDx Histoplasma Lateral Flow Assay and IMMY Histoplasma Enzyme Immunoassay* *Published*	a. How frequent is CM among different categories of people living with HIV in Ghana? b. How common is histoplasmosis in people living with HIV in Ghana? c. Can the OIDx *Histoplasma* LFA be a suitable alternative to the recommended IMMY *Histoplasma* EIA in detecting histoplasmosis in an African setting?	a. To determine the frequency of CM and histoplasmosis among HIV-infected patients in Ghana. b. To compare the performance of OIDx *Histoplasma* LFA and the reference IMMY *Histoplasma* EIA in detecting histoplasmosis.	Histoplasmosis is probably a significant HIV-associated fungal infection as cryptococcosis in Ghana, with prevalence rates of 4.7% (95% CI, 0.7 – 8.7%) and 2.7% (95% CI, 0.1 – 5.3%) respectively. The CrAg LFA and OIDx *Histoplasma* LFA were both diagnostic for the respective confirmed cases of CM and histoplasmosis. The OIDx *Histoplasma* LFA performed very well in comparison to IMMY *Histoplasma* EIA, with a 98.4% concordance.
4	*Chronic Pulmonary Aspergillosis is Common Among Patients with Presumed Tuberculosis Relapse in Ghana* *Published*	a. How common is CPA among patients presenting with TB-like symptoms? b. How to utilize the Aspergillus-specific IgG and IgM LFA to aid CPA diagnosis in Ghana? c. What are the common aetiological agents of aspergillosis in Ghana?	a. To determine the prevalence of CPA in patients with presumed new TB and TB relapse. b. To evaluate the use *Aspergillus*-specific IgG and IgM assay in Ghana. c. To identify the common aetiological agents of aspergillosis in Ghana	CPA should be considered a relevant differential diagnosis of PTB, particularly relapse PTB. Among presumed new PTB and relapse PTB, CPA was diagnosed in 3.7% (95% CI, 0.5 – 6.9%) and 50% (95% CI, 28 – 72%) respectively and overall, 9.7% (95% CI, 5.0 – 14.4%). The *Aspergillus*-specific IgG and IgM LFA was diagnostic in 93% of CPA cases. *A. fumigatus and A. flavus* were the common isolates from CPA patients.
5	*Importance of Aspergillus-specific antibody screening for diagnosis of chronic pulmonary aspergillosis after tuberculosis treatment: a prospective follow-up study in Ghana*	a. What is the incidence of CPA following the end of anti-TB treatment and 6 months post-treatment in bacteriologically confirmed PTB patients?	a. To determine the incidence of CPA among confirmed PTB patients receiving anti-TB treatment at two timepoints, at the end of treatment and 6-month post-treatment.	The rate of CPA development increase over time, after completing anti-TB treatment with an overall incidence of 10.7% (90% CI, 1.1 – 20.3%). The incidence rates of CPA at the end of anti-TB treatment and 6-month afterwards were 3.0% and 7.4% respectively. Again, the *Aspergillus*-

	Published			specific IgG and IgM test was an indicator for CPA in all diagnosed cases. The SGRQ score got worse for CPA patients, and those who failed anti-TB treatment or had PTB relapse.
6	*Invasive aspergillosis among haematological malignancy patients in Ghana: A pilot study on at the national referral hospital* *Published*	a. How frequently does IA occur among patients with haematological malignancies in Ghana? b. Can the sona *Aspergillus* GM LFA reliably be diagnostic for IA? c. How common is antifungal prophylaxis practiced in patients with haematological malignancies in Ghana?	a. To generate epidemiological and clinical data on IA among patients with haematological malignancy in Ghana. b. To evaluate the clinical efficacy of *Aspergillus* GM LFA in the diagnosis of IA among haematological malignancy patients in Ghana. c. To evaluate the practice of antifungal prophylaxis in patients with haematological malignancies in Ghana	IA prevalence was revealed to be 5.4% (90% CI, 0.4 – 10.4%) in leukaemia, representing 20% of neutropenic patients. The *Aspergillus* GM LFA was diagnostic in two out of the three patients who met the criteria for IA. Only a handful of patients received antifungal prophylaxis, with just one patient receiving a mould-active antifungal agent.
7	*Trend, spectrum, and aetiology of fungal infections in Ghana: a 10-year retrospective study of histopathologically diagnosed cases* *Not published*	a. What is the fungal infection incidence pattern in Ghana? b. What types and presentations of fungal infections are diagnosed in Ghana? c. Are some rare moulds or yeasts, or endemic fungal infections diagnosed in Ghana?	a. To evaluate the trend and spectrum of fungal infections in Ghana. b. To confirm aetiological agents of histopathologically diagnosed fungal infections with histomolecular analysis. c. Compare histopathology suggested aetiological agents and molecular identified agents	Overall, 107 cases were found. More than one-third of the cases affected the nasal area. The spectrum of infections was diverse including common (aspergillosis and candidiasis), endemic (chromoblastomycosis and sporotrichosis) and rare (entomophthoromycosis and eumycetoma) infections. Fungal stains were rarely used. Out of the seven cases with histomolecular identification of aetiology, two were at variance with fungi suggested at histopathology. Clinical suspicion for fungal infection was very low.

8.3 REFLECTIONS ON THESIS CONTRIBUTIONS AND IMPACT

Mycology in the medical laboratory and in clinical practice has been grossly neglected in Ghana. This thesis has made a substantive contribution to the epidemiological data on SFIs in Ghana. It also makes practical and policy impact as well as applied contributions to the field of mycology in Ghana and Africa, specifically and worldwide, generally. This is done by stimulating the much-needed conscious efforts to design and implement strategies to improve the status quo of prevention, diagnosis, and treatment of SFI as well as the education, training, and research in clinical/medical mycology. The contributions and impact of the thesis are discussed under four themes.

8.3.1 Sensitization and Advocacy

This thesis is the first broad diagnostic and epidemiology project on SFIs in Ghana and one of the major elements of the implementation of this project was to increase the awareness among healthcare professionals. The project involved a multidisciplinary team of healthcare professionals including physicians, medical laboratory scientists, nurses (general and public health) and pharmacists. Among the physicians, the sub-specialties that played various roles in the project are infectious diseases, pulmonology, clinical microbiology, haem-oncology, radiologist, and pathologist. All cadres of the clinical and healthcare team through their engagement in the project were exposed to the real-case scenarios of approaches to screening and confirming diagnosis of SFIs. Prior to the commencement of patient recruitment and throughout the period of the project, a series of sensitization meetings were organised with major participation by the clinical team contributing to the project. Furthermore, as part of the project set up, specific talks and workshops were given to some of the clinical team members to improve their understanding on the diagnosis of the SFIs to be studied. One such meeting led to the coming together of healthcare professionals, academics, and researchers with interest in fungal infections and medical mycology to form the Ghana Medical Mycology Group (now registered as the Ghana Medical Mycology Society). Throughout the duration of the PhD, many of such sensitization and capacity building meetings were organized (Appendix 14). This was mainly done in collaboration with the Fungal Infections Kare Initiative (FIKI) Ghana (also previously founded by the author in 2019) and later championed through the Ghana Medical Mycology Society. It was evident that knowledge about SFIs was poor, as confessed by some of the participating physicians, medical laboratorians and nurses who revealed hearing such as 'aspergillosis', 'cryptococcosis' and 'histoplasmosis' for the first time. This is not surprising because a previous survey of the knowledge on fungal infections among health sciences students including medical, medical laboratory, nursing and pharmacy students showed

many of them knew little about fungal infections particularly SFIs. Aside from healthcare professionals, during routine health talks given to TB patients and their relatives or caregivers, aspergillosis sessions were added for them to be aware of the relationship between TB and CPA. Furthermore, the assigned roles of these professionals included engaging patients with Participant Information Sheet, clinical assessment for SFIs, examining histopathology slides for fungi, confirming laboratory diagnosis of SFIs, and taking final clinical decisions afforded them the opportunity to familiarize and acquaint themselves with the practice of clinical mycology. Throughout its 3-year implementation period, this project has enhanced awareness about SFIs, particularly the Infectious Diseases/HIV clinic, Respiratory/Chest Diseases Unit and Haematology Unit of the KBTH, which was the main project site.

In terms of advocacy, the thesis has generated very relevant epidemiological data that can serve as a critical tool to make a case for more attention to SFIs. To effectively advocate for an improvement of the status quo, in this case, for improved awareness, diagnosis and treatment of SFIs in Ghana, one needs a baseline epidemiological data to strategically push this forward. This would then allow for the development and design of strategic and implementation plans that are measurable. At the policy level, the project caught the attention of some stakeholders at the Ghana Health Service, through the Clinical Laboratory Unit under the Institutional Care Division. This had led to the Head of Clinical Laboratory Unit developing an interest in contemplating a nationwide laboratory training exercise to advance the skills and laboratory capacity of laboratorians in fungal diagnosis.

8.3.2 Diagnosis

The thesis has provided and initiated an extensive view of the diagnosis and epidemiology of SFIs in Ghana. The HIV-associated CM and histoplasmosis study (Chapter 3), which advanced the research testing support beyond the study period, introduced, and enabled reliable access to testing for opportunistic fungal infections outside superficial and muco-cutaneous infections. CrAg LFA and *Histoplasma* antigen EIA/LFA tests requests increased and expanded from the main HIV clinic to also include other departments/units and wards at the KBTH (data to be retrospectively analysed and published). Previously, CrAg tests had been outsourced to a private medical laboratory that used the latex agglutination assay but was often associated with a longer TAT. The CrAg LFA proved to be simpler, easily implementable at the HIV clinic laboratory or physician's office and results reported in less than 24 hours. Unlike the CrAg, the *histoplasma* antigen has not been tested before either in-house or outsourced. *Histoplasma* antigen testing has a critical role in the diagnosis of histoplasmosis particularly disseminated histoplasmosis and Ghanaian clinicians

through this study were made to appreciate this. They also managed cases of proven infection, from confirming diagnosis to treating and following up. The fact that histoplasmosis appeared to be such an important SFI in HIV patients came as a surprise to the physicians.

In terms of the *Aspergillus* IgG and IgM testing to aid the diagnosis of CPA (Chapter 4 and 5), implementation of the test enhanced efforts of clinicians in detecting cases. The prior practice was to make the diagnosis based on the radiological appearance of a fungal ball on either CXR or CT scan. This approach has the disadvantage of delaying diagnosis as radiological abnormalities, such as fungal ball is a late presentation of CPA. Moreover, only ~40% of patients with CPA have fungal balls, many of which are not visible on a CXR. Increased levels of *Aspergillus*-specific IgG have been established by several studies as a key element in CPA diagnosis and allows for the early detection of CPA. Specifically, in the second CPA study (Chapter 5), a positive *Aspergillus*-specific IgG and IgM LFA was used as an indication for ordering a CT scan to confirm radiological features suggestive of CPA. Another added advantage offered by *Aspergillus*-specific IgG testing is the important role it plays in monitoring treatment, and this is particularly important as radiological abnormalities seldom improve despite clinical improvement. Once the research study had been completed, *Aspergillus*-specific IgG and IgM LFA was continued to be used at the Chest Clinic OPD and extended to the wards and Respiratory/Asthma Clinic (for ABPA) from which a high positivity rate was observed (data to be retrospectively analysed and published).

The pilot study using *Aspergillus* GM LFA in IA (Chapter 6), was the first introduction of such a test to Ghana and the first study of IA as a complication of haematological malignancy at the Haematology Department of KBTH. A major limitation to the successful implementation of *Aspergillus* antigen testing, is the early deaths of many patients at highest risk. General improvement of the immediate management of acute leukaemia will be required, including rapid diagnosis of IA. In this case therefore, testing was not translated into routine clinical practice as observed in the case of CrAg LFA, *Histoplasma* antigen EIA/LFA and *Aspergillus*-specific IgG and IgM LFA (Chapters 3, 4 and 5).

The main diagnostic achievement of the histopathology and histomolecular study was the enhanced understanding of the relevance of frequently using fungal stains (particularly PAS and GMS) to improve detection of fungi in tissue and allowing for definitive identification of fungal aetiologies. Additionally, a more coordinated and consistent format of describing fungal elements or structure was expounded. This is important for assisting attending clinicians in deciding on a more precise fungal diagnosis and thus factoring it into the choice of antifungal drugs.

Overall, all fungal laboratory diagnostic tools utilized in the project, both antigen-antibody assays and histomolecular tests had a subsequent transformational impact towards improving diagnosis of fungal infections in Ghana.

8.3.3 Epidemiology

As this thesis provides the foremost epidemiological data on SFIs in Ghana, it has contributed to the global literature on SFIs with contributions from Ghana and Africa. Prior to the present study, there have been three prospective studies on CM or CA, one retrospective study and a single case report (Chapter 1, Table 1.3). The point prevalence rate of 2.7% (95% CI, 0.1 – 5.3%) reported for CM or CA is comparable to the approximately 2-3% rates previously found by three other studies (8–10). Collectively, these data corroborate suggestions that the CM burden in Ghana is probably lower compared to other SSA countries. On the other hand, the study on HIV-associated histoplasmosis, the first ever in Ghana, revealed that the incidence of histoplasmosis is probably a significant HIV-associated fungal infection as cryptococcosis. The actual numbers of cases were small (despite enrolling 150 people) and needs confirmation and extension to other parts of Ghana. Nonetheless, this finding is likely to serve as an eye-opener for studies of HIV-associated fungal infections in Africa as a whole and encourage more studies on HIV-associated histoplasmosis particularly with the advent of simple and easy diagnostics such as *Histoplasma* LFA utilized in the present study. Some experts consider that, with such expanded studies and testing as currently available for CrAg LFA, the true burden of HIV-associated histoplasmosis may be determined as it is widely thought to be underestimated now.

CPA was an unfamiliar term and rarely used in Ghana, but a well-known condition frequently presenting as a post-PTB complication. A single case has been reported from Ghana as pulmonary aspergillosis (3). The cross-sectional CPA study (Chapter 3) exploited a novel approach for investigating CPA by focusing on suspected or presumed PTB patients (both new and relapse). This showed 9.7% (95% CI, 5.0 – 14.4%) of patients undergoing investigation for PTB had CPA, 3.7% (95% CI, 0.5 – 6.9%) for new and 50% (95% CI, 28 – 72%) for relapse. It establishes the approach could be one of the effective strategies that can be further assessed and implemented to screen for CPA in PTB which helps to prevent delayed diagnosis in high TB settings. Additionally, the follow-up CPA study (Chapter 5) revealed prevalence rates of CPA at the end of anti-TB treatment and 6-month post treatment to be 3.3% and 7.4%, respectively. In our burden estimate published prior to this thesis started in 2019, we had estimated a prevalence of 12,620 cases of which 50% were thought to be in those cured of PTB (2). Remodelling using these data from Ghana and additional outcome data from other countries, as exemplified in India (35), would suggest an

annual incidence of CPA of 9,800 and a 5 year prevalence of 24,180 cases. Follow-up studies in CPA are limited and specifically few had investigated at the end of anti-TB treatment, and none had thus far looked at 6-month post-treatment. The present study expands the emerging approaches to the design of longitudinal studies to understand the rates of CPA incidence during, at the end and after anti -TB treatment. The 2021 WHO estimates for TB incidence in Ghana is 45,000 cases (93% PTB) and 15,700 deaths (36). The mortality in HIV-infected people was 57% and in non-HIV patients was 31.2%. One possible explanation for the high mortality is misdiagnosis of CPA as PTB and the wrong treatment being given.

The incidence of IA in any of the common underlying conditions such as haematological malignancy, transplant, HIV, or ICU people has been rarely studied in Ghana or many African settings. In Ghana, only one case of disseminated IA has been reported in a young male adult on prolonged antibiotics and systemic steroids (6). In the pilot study conducted, an IA prevalence rate of 5.4% (90% CI, 0.4 – 10.4%) was reported among patients with haematological malignancy, and 20% in those who were neutropenic. Although a small study, this is a significant contribution to documenting leukaemia-associated IA from the West African sub-region, as most studies are from North Africa (37,38).

Across the aspergillosis studies (Chapter 4,5 and 6), the different species of *Aspergillus* involved were reported. Unlike environmental and plant *Aspergillus* isolates which are commonly reported, this represents to the best of the author's knowledge the first collection of clinical isolates of *Aspergillus* species from Ghana, reporting a total of 86 *Aspergillus* species. Of the two reported cases of aspergillosis (one each of CPA and IA), the causative agent was not reported to the species level.

As shown in Table 1. 3 (Chapter 1), the reported spectrum of SFIs affecting the Ghanaian population is limited and the studies (mainly case reports and series) have reported few types of fungal infections. In the histopathology-histomolecular study (Chapter 7), a broad range of fungal infections ranging from the common types (candidiasis and aspergillosis) and included some rarely diagnosed infections (entomopthoromycosis and eumycetoma) and those with geographical restriction or endemic infections (chromoblastomycosis and sporotrichosis). This re-affirms the popular assertions that the effect of migration and international travels means any fungal infection can be diagnosed anywhere. Furthermore, the molecular identification revealed some rare yeast and mould as causative agents of fungal infections. This is a relevant advancement on the types of fungal pathogens reported from Ghana because previous studies are silent on confirmed culture or molecular identification.

8.3.4 Training and Research

The project also made a significant contribution to the upgrade of the knowledge and skills of clinicians, nurses, laboratory scientists and pharmacists involved in the project (and extended to other healthcare professionals at the KBTH) on laboratory diagnosis and treatment of SFIs. The pre-recruitment lectures, discussions, and training events have had a positive impact on the understanding of the general management of SFIs. Post-recruitment and throughout the duration of the five studies, targeted meetings or seminars were periodically organised to impart specific knowledge and skills (Appendix 14). In terms of the knowledge and skills that were conveyed, for clinicians it included when to request and how to interpret results of fungal RDTs (CrAg LFA, *Histoplasma* EIA/LFA, *Aspergillus* IgG and IgM LFA and *Aspergillus* GM LFA) and for laboratory scientists, inoculating fungal culture and examining (macroscopic and microscopic) growth.

The project has stimulated and encouraged research in clinical/medical mycology or on SFIs in Ghana, particularly at the Department of Medical Microbiology, UGMS and Department of Medicine and Therapeutics (Chest Diseases Unit and Fevers Unit), KBTH. The project purposefully demonstrated the research demands and revealed opportunities to expand studies in clinical mycology in Ghana. Thus, there are studies that were inspired by this project that have been published or presented at a conference (Appendix 15). Other studies are presently ongoing and at different stages of planning and execution including the following: *Pneumocystis jirovecii* pneumonia in HIV using *Pneumocystis jirovecii* immunofluorescence kit, *Aspergillus*-specific antibody positivity rate in chronic respiratory disorders, CPA among clinically diagnosed PTB patients, ABPA in severe asthma patients, and effect of fungal stains (PAS and GMS) on detection of fungi in histopathological examination. Moreover, academics and researchers from other universities and tertiary hospitals have expressed interest in replicating some of the studies at their centres.

8.4 LIMITATIONS

As this project was the first of its kind in Ghana, in terms of conducting diagnostic and epidemiological studies on different SFIs simultaneously, some potential shortfalls were anticipated and subsequently experienced during implementation of the studies. The limitations as discussed below, while admittedly not negating findings from the studies, they most importantly highlight opportunities for future research that requires exploration.

For the HIV study, the absence of routine CD4 testing provision was a major challenge to structuring a strict guideline-adherent design. CD4 count has historically been the main guide for investigating opportunistic infections in HIV including SFIs. Specifically, the lower the CD4 count, the higher risk of HIV patients affected by opportunistic infections including SFIs. The current WHO guidelines on HIV-associated cryptococcal disease recommends screening for CrAg among HIV patients with CD4 counts less than 100 cells/μl. Similar testing guidelines exist for HIV-associated histoplasmosis. Unfortunately, in the past few years, CD4 counts have become uncommon, and testing has declined substantially with treatment monitoring now focused more on viral load testing. This phenomenon is not peculiar to Ghana, and very common in many African settings. Only a handful of recruited patients had recent CD4 count data available from their medical records. A complete or substantial CD4 count data would have also allowed for stratification of patient's risk levels and proportions of CrAg or *Histoplasma* Ag positivity. It would have also enabled an appropriate contextual comparison with findings from other studies. Moreover, it is possible for some of the patients without CD4 count records to have higher values and have lower risk to SFI and lower the prevalence rate determined. Although an attempt was made to offer CD4 testing to recruited patients this was impeded by procurement and logistics difficulties in acquiring the test kits due to COVID-19 challenges. Additionally, the small numbers of positive cases means that the reported rates have a wide interval between upper and lower confidence limits and so a precise population estimates cannot be guaranteed.

Another obstacle encountered in the two CPA studies was the incomplete acquisition of all CXR and CT scan films. The presence of abnormal radiological features plays an important role in the definition and classification of CPA. Despite the many similarities in radiological features between CPA and PTB, a few findings are more frequently associated with CPA (fungal ball, pleural thickening and paracavitary fibrosis), and others less so (lymphadenopathy, miliary pattern and pleural effusion). Patients recruited into the CPA studies, had been clinically assessed by clinicians at primary centres from different parts of Ghana and subsequently referred for Xpert MTB testing at the KBTH. A significant number of patients had already had CXR done for them within one or two weeks prior to the referral, and it was inappropriate to have them do a new CXR. Instead, determined efforts were made to retrieve the films of previously taken CXR for review by the research radiologist. Unfortunately, not all films from such patients could be retrieved and they had to be excluded in the final analysis. Some of these patients had a positive *Aspergillus*-specific antibody and probably with the relevant abnormal chest radiograph features would have met the criteria for CPA and affected the reported prevalence rate.

The number of patients recruited in the CPA II and IA studies were relatively small. The CPA II study was a follow-up on the main CPA I study which unfortunately had very few new bacteriologically confirmed PTB patients. Indeed, although similar studies had larger sample sizes, they included all patients treated for PTB, whereas the present study focussed only on bacteriologically confirmed PTB patients, excluding clinically diagnosed PTB patients. The drawback of the small sample size is that it does not allow for accurate comparisons and a confident generalisation in the wider PTB population.

As extensively explained in Chapter 6, the IA study was planned as a pilot study to assess the feasibility of implementing a robust and integrative clinical investigation and understand the potential challenges. Notwithstanding, the findings from the study are important, and have laid the appropriate foundation for a larger definitive study, pending the acquisition of new funding.

Classifying IA as a proven case requires histopathological examination of tissues revealing fungi or isolating pathogenic fungi from sterile clinical samples. Although histopathology laboratory services are routinely available at the KBTH, tissue samples are rarely collected mainly due to the cost involved in collecting samples and histopathological processing because patients pay out of pocket. Furthermore, for ethical restrictions, invasive sampling was excluded for only research laboratory investigations. The same applies to collection of sterile samples, and thus culture was only carried on sputum samples. The lack of these criteria limited classification of proven cases. Likewise, bronchoscopy and antigen detection on bronchoscopy fluid might have supported the diagnosis but was not available and is infrequently done in Ghana.

In the histopathology-histomolecular study a major challenge experienced was poor storage of archived tissue blocks. This was particularly observed at two of the histopathology laboratories involved. This resulted in some of the blocks not being retrieved. For those that were retrieved, the prevailing poor storage conditions of tissue blocks may have affected the success of DNA extraction and amplification. This is one of the possible reasons for the low PCR success rate. Additionally, because histomolecular assays are not standardized, replication of evaluated procedures was a constraint, and thus, an in-house PCR had to be designed and implemented. However, because the in-house method utilized has not been evaluated with proven cases, its efficiency was not guaranteed, and it is unclear if it affected the PCR and DNA sequencing success rate. Also, because the histopathology-histomolecular study was not originally planned and budgeted for, there was limited funds available for its full implementation particularly to the next generation sequencing level, and so only seven cases were completed. This is because compared

to Sanger sequencing, which was used, next generation sequencing has been extensively reported to be more robust in yielding quality sequences that easily allows for identification.

8.5 FUTURE RESEARCH ON SFIs

Building on the findings from this thesis and the highlighted limitations as discussed in detail above, there is the need to progress the positive impacts by conducting additional future studies. This will refine the current data and provide a deeper insight into understanding of the status of SFIs in Ghana and West Africa. These important future studies are outlined below under five thematic areas based on underlying conditions or at-risk patient groups and the general population.

8.5.1 HIV and SFIs

Primary

HIV-associated cryptococcal disease studies in Ghana utilizing CrAg have consistently reported a relatively low burden (2-3%) but all these studies were conducted in Accra and Kumasi, the two largest cities in Ghana. Only one was influenced by CD4 count and was a retrospective study. A cost analysis survey suggests routine CrAg screening and pre-emptive fluconazole treatment is cost-effective in settings with a cryptococcal antigenaemia prevalence rate of at least 1.4%. Considering the diverse approaches to CrAg screening investigations with many deviating from the WHO HIV-associated cryptococcal disease management guidelines there is the need for a single centre or coordinated national multi-centre CrAg screening programme following updated recommendations. With this, a broad comprehensive understanding of the burden will be appreciated and direct the next steps towards designing and implementing a national screening programme.

This thesis consolidates the widespread proposition that histoplasmosis is probably endemic in Africa particularly the West African sub-region. However, its burden and general epidemiology is not well studied, and the topic has remained neglected. In a review of histoplasmosis in Africa, histoplasmosis caused by *Histoplasma capsulatum* var *duboisii* (African histoplasmosis) was more dominant in West Africa. The emergence of *Histoplasma* antigen RDTs for aiding laboratory diagnosis of histoplasmosis is believed to improve case detection in conjunction with histopathology and culture but its efficacy for Africa histoplasmosis is unclear. Unfortunately, the thesis could not confidently determine diagnosed cases were caused by either *capsulatum* or *duboisii* variant of *Histoplasma capsulatum* and the cultures were negative due limitations as discussed above. Thus, the role of RDTs in African histoplasmosis diagnosis remains uncertain. An

evaluation of *Histoplasma* antigen EIA/LFA in diagnosing African histoplasmosis using proven cases is worthy of investigation in future.

Secondary

Beyond cryptococcosis and histoplasmosis, *Pneumocystis jirovecii* pneumonia is known to have a substantial burden in HIV patients. Although previously thought to be uncommon in African settings, a recent review suggests the contrary. Low case detection and underestimation was reportedly linked to poverty and increasing country GDP was associated with increasing case numbers (39). Notwithstanding, as in many countries in Africa, the true burden of laboratory confirmed cases in Ghana is unknown although clinically diagnosed cases are often recorded. Traditionally, laboratory diagnosis is by direct examination of respiratory specimens such as sputum and BAL but is not very sensitive. Immunofluorescence and PCR are contemporary diagnostic tools for *Pneumocystis jirovecii* pneumonia, but PCR is the most recommended presently. None of these techniques are presently not routinely available and going forward, the impact of laboratory diagnosis in the management of *Pneumocystis jirovecii* pneumonia in HIV needs to be evaluated. It may be of greatest value for stopping potentially toxic high dose cotrimoxazole and corticosteroids, if tests are negative and particularly helpful in babies with pneumonia, which were not addressed in this thesis.

8.5.2 TB and SFIs

Primary

The thesis found a 50% prevalence rate of CPA among 20 presumed PTB relapse patients. It can be rightly argued that the sample size involved was small and thus a generalisation to the larger group of patients in this category may be inaccurate. In view of this, a larger study, probably with a multicentre approach is essential to substantiate the present findings. Also based on the rate reported herein, routine screening for *Aspergillus*-specific IgG for previously treated PTB patients returning to care with TB-like symptoms is an appropriate call. Therefore, a cost-effectiveness analysis study of this screening strategy is also important.

Secondary

In the thesis, among the probable CPA-PTB co-infection patients, it was observed that *Aspergillus* -specific IgG and IgM was only positive when the MTB burden was low. It is unclear what this means, whether it is a coincidence or an existing phenomenon. Although, the argument was made of the frequent false positivity of Xpert MTB in previously treated PTB patients in the thesis (Chapter 4-

Discussion), it is also possible that they are indeed there were true dual CPA-PTB infections, but presence of *Aspergillus* suppressed the load of MTB. This hypothesis requires exploration.

Learning from the findings on HIV-associated histoplasmosis from the thesis, it may be prudent to replicate this in the TB population. This is because histoplasmosis is commonly misdiagnosed as TB in both immunocompromised (particularly HIV) and immunocompetent patients with chronic pulmonary disease and usually described as smear-negative TB. The histoplasmosis and TB interrelation is widely held to be common in high HIV burden settings and has been extensively studied in Latin American countries where histoplasmosis is reported to be at least as common as TB in HIV. Recently, two studies conducted in Nigeria reported significant rates of histoplasmosis among PTB patients either with or without HIV corroborating with the suggestion that histoplasmosis overlaps HIV and TB in SSA (40–42).

8.5.3 Haematological malignancy and SFIs

Secondary

As already mentioned, and discussed throughout the thesis, IA study among patients with haematological malignancy was a pilot study. The study highlighted some relevant challenges but has also exposed clinicians to IA as a complication in caring for haematological malignancy patients. This serves as the foundation for a large study that would involve collection and analysis of tissue and sterile samples to enable classification of proven cases of IA. Risk stratification of patients will be crucial to success, given that stable outpatients with chronic leukaemia are at low risk of IA.

8.5.4 Fungal Histopathology

Primary

The fifth study found fungal rhinosinusitis as the frequently diagnosed fungal infections by histopathology. Furthermore, there were cases of fungal skin neglected tropical diseases (NTDs) recorded, that is, chromoblastomycosis, eumycetoma and sporotrichosis. However, neither of these two fungal infection groups have been investigated in Ghana previously.

Secondary

The findings from the histopathology-histomolecular study (Study 5) which focused on only pre-mortem cases probably hint a similar scenario or more could be revealed at post-mortem analysis knowing that clinical suspicion is insufficient in these settings. Hence, a retrospective review of histopathological reports of post-mortem cases could complement the present findings.

8.5.5 General

Primary

The work reported in this thesis documents, for the first time, the aetiological agents of SFIs, particularly, aspergillosis (CPA and IA) from Ghana. The data generated is an important initial step towards recognizing the common species of *Aspergillus* involved in infections and then going further to explore azole resistance frequency. Certainly, this is important in these times, where azole resistance is on the rise. The next step to progress this, is to evaluate the molecular identification, antifungal susceptibility profile and mechanisms of azole resistance of *Aspergillus* spp isolated from the CPA and IA studies. The molecular characteristics of clinical isolates can also be compared with archived environmental isolates.

Secondary

Patients admitted to ICU wards represent another major risk factor for SFI mainly candidiasis/candidemia, and aspergillosis. Although conventional blood culture tests are available, they are not sufficiently sensitive to detect fungi due to sample volume and media used. Additionally, collection of invasive samples is not routine. Similarly, patients with severe asthma can have their symptoms worsen by exposure to fungi particularly *Aspergillus* spp. and result in conditions classified as ABPA or SAFS. A study evaluating the burden of these conditions among Ghanaian asthma patients will be far-reaching and could probably improve care provision for those with severe asthma.

8.6 RECOMMENDATIONS

This section elaborates the potential implications of the outcomes of the project in driving relevant transformation in policy formulations, clinical practice, education in the health sciences and post-qualification training. This is discussed under two broad grounds, namely policy and practice, and education and training.

8.6.1 Policy and Practice

As demonstrated throughout the thesis, there is a general inadequate awareness and insufficient attention to SFIs by key stakeholders and policymakers including institutions like the Ministry of Health, Ghana Health Service, Teaching Hospitals and Medical/Health Research Institutions. Through engagements with the relevant special programme agencies on which the thesis

impinges including the National AIDS/STI Control Programme, National TB Control Programme, and Neglected Tropical Diseases Programme it was evident there was little knowledge on how SFIs complicate the main patient groups these programmes focus on. The initial engagement with these institutions and agencies generated considerable interest. This could be further enhanced and progressed towards formulating policies that allow integration of fungal infections and clinical mycology practice into the healthcare system for routine healthcare provision in Ghana. As a starting point, care for SFIs relevant to specific disease programmes can be progressively integrated at least at the tertiary hospitals. Prior to implementation, there is the need to undertake cost-effectiveness studies or an economic analysis to ensure financial viability of this approach. Organisations that can champion these courses include the GMMS and FIKI-Ghana, which have indeed played a key role in advancing awareness of fungal infections in Ghana. The summary report on the outcomes from the thesis can serve as a critical advocacy tool, as epidemiological numbers are important in driving political and financial will to improve the status quo.

The current Ghana National HIV Management Guidelines does not specifically mention or significantly highlight histoplasmosis as an opportunistic fungal infection as it is done for cryptococcosis. Contrarily, the thesis reveals that histoplasmosis may have a higher burden than cryptococcosis in patients living with HIV. Although, more studies may be required to substantiate this proposition, it is nonetheless appropriate to point out histoplasmosis is a significant complication of HIV in Ghana, particularly in patients with AHD. This action will encourage clinicians to broaden their diagnostic scope to include histoplasmosis as a differential diagnosis. Also, patients diagnosed with CM could not access the recommended first-line drugs, amphotericin B and flucytosine, which are not stocked in any hospital pharmacy in Ghana. With expanded testing probably influenced by the thesis, there should be institutional policy reforms to make these WHO-listed Essential Medicines promptly available. Similar arguments can be made for histoplasmosis and amphotericin B, as itraconazole, which is widely available and easily accessible, is not recommended as first line therapy for severe and disseminated histoplasmosis. This situation befell one of the cases in the thesis (Chapter 3- Results); the patient should have been placed on amphotericin B. Ensuring these drugs are available and accessible at reasonable prices will save lives.

CPA and PTB have similar symptoms and radiological findings and may be confidently differentiated by laboratory testing. Although PTB is commonly regarded as a differential diagnosis of CPA, the reverse is rarely considered. However, the present revelation that one in two of previously treated PTB patients presenting to care with TB-like symptoms may have CPA

requires a review of the management of post-TB lung disease. In view of the substantial burden noted, routine screening for *Aspergillus*-specific IgG when providing post-TB lung disease care, particularly, those experiencing TB-like symptoms should be strongly considered.

SFIs commonly have similar clinical manifestations to other infectious diseases or malignancies and thus laboratory diagnosis is the bedrock of making a definitive diagnosis. Current medical mycology laboratory practice is sub-optimal in Ghana and routinely available tests are direct microscopy (skin scraping in KOH for fungal elements and CSF India ink for yeasts). Fungal culture, antigen-antibody tests and molecular assays are not or only rarely accessible across Ghana even in the tertiary hospitals. Direct microscopy and antigen-antibody testing are easy to implement with minimal technical training. At least at the tertiary hospital level there is the need to have mycology laboratories and mycology benches respectively to offer these diagnostic testing services. To the best of my knowledge all RDTs and the histomolecular assays implemented in the thesis were not routinely available in any hospital in Ghana and it is hoped that they can be sustained at the KBTH at least, with relevant engagement of key stakeholders and policy makers. With appropriate adequate training and practice, fungal culture can be initiated at the tertiary hospitals. Thankfully, the COVID-19 pandemic has expanded the capacity of many research/reference and hospital laboratories to undertake molecular testing routinely and fungal PCR can be integrated. The Noguchi Memorial Institute for Medical Research has, over the years, acted as the premier national reference laboratory, investigating outbreaks and recently led the COVID-19 diagnostic testing. Unfortunately, they lack a Mycology Department and like the National Public Health Reference Laboratories and hospital laboratories do not offer diagnostic services. This thesis makes a significant case for the establishment of mycology departments or units to bolster the preparedness for future fungal outbreaks.

8.6.2 Education and Training

An assessment of the knowledge and awareness of clinicians and other healthcare professionals involved in the project prior to sensitization and training sessions revealed the majority to be poorly educated and not well-informed about fungal infections, particularly, SFIs. To improve this requires a foundational change in health science education and a relook at the current curriculum to update it with thorough teaching and learning of clinical mycology. Although the curricula of some tertiary institutions have sufficient content on clinical mycology, they are allocated limited teaching hours. From the author's experience during his undergraduate degree, a scheduled medical mycology laboratory practical captured in the curriculum did not take place but was only

taught theoretically. Moreover, the experience of the teaching faculty is probably inadequate because there are very few mycology trained academics.

The index of suspicion for SFI as observed in the thesis was generally low, and most of the laboratory-diagnosed cases were by chance and not clinically suspected. With strategic advocacy efforts, coupled with adequate and appropriate education and training, clinicians could start 'to think fungi'. In addition to clinical suspicion, with an anticipated expansion of access to RDTs and requests for RDT testing, clinicians must be trained in the accurate interpretation of RDTs results. RDTs, as purposefully advanced throughout the thesis are not used alone in laboratory diagnosis, but complemented by conventional methods (direct microscopy, culture, and histopathology) and molecular assays. To build diagnostic capacity, a training workshop for medical laboratorians on direct microscopy and culture are required. Similarly, pathologists should be trained on the diverse forms of fungal elements and structure that can be seen in tissues and how to appropriately describe them to guide clinicians in making the appropriate choice of antifungal agents.

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APPENDICES

Appendix 1: Published part of 'Literature Review' / Chapter 1 paper/ Paper 1

DOI: 10.1371%2Fjournal.pntd.0010111

PLOS NEGLECTED TROPICAL DISEASES

REVIEW

Histoplasmosis in Africa: Current perspectives, knowledge gaps, and research priorities

Bright K. Ocansey[1]*, Chris Kosmidis[1,2], Martin Agyei[3], Améyo M. Dorkenoo[4,5], Olusola O. Ayanlowo[6], Rita O. Oladele[7], Tchin Darre[8], David W. Denning[1]

1 Division of Infection, Immunity and Respiratory Medicine, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom, 2 National Aspergillosis Centre, Wythenshawe Hospital, Manchester University NHS Foundation Trust, Manchester, United Kingdom, 3 Department of Internal Medicine, School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, 4 Department of Biology and Basic Sciences, Faculty of Health Sciences, University of Lomé, Lomé, Togo, 5 Division of Laboratories, Ministry of Health and Public Hygiene, Lomé, Togo, 6 Department of Medicine, College of Medicine, University of Lagos, Lagos, Nigeria, 7 Department of Medical Microbiology & Parasitology, College of Medicine, University of Lagos, Lagos, Nigeria, 8 Department of Pathology, University Teaching Hospital of Lomé, Lomé, Togo

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OPEN ACCESS

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Editor: Nalesh Premapragasan Govender, National Institute for Communicable Diseases, Johannesburg, South Africa, SOUTH AFRICA

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Competing interests: The authors have declared that no competing interests exist.

Abstract

Background

Histoplasmosis is a chronic granulomatous disease caused by the thermally dimorphic fungus *Histoplasma capsulatum*. The 2 variants *Histoplasma capsulatum* var. *capsulatum* (Hcc) and *Histoplasma capsulatum* var. *duboisii* (Hcd) causes infection in humans and commonly termed classical or American histoplasmosis and African histoplasmosis, respectively. *Histoplasma capsulatum* var. *farciminosum* (Hcf) affects equines. In recent times, there have been heightened sensitization on fungal infections such as histoplasmosis in Africa, aimed at improving awareness among relevant stakeholders, particularly healthcare workers. This effort is expected to be paralleled with increased detection of both classical and African histoplasmosis, which has remained underdiagnosed over the years. In this narrative review, we describe the current perspectives of histoplasmosis in Africa, identify knowledge gaps, and suggest research priorities.

Methods

A PubMed, Google Scholar, and Africa Journal Online (AJOL) literature search was conducted for studies on histoplasmosis in Africa between 2000 and 2020. Histoplasmosis essays in medical mycology textbooks were also consulted. This narrative review was prepared from the data gathered.

Findings

In the past 2 decades, histoplasmosis in general has seen a relative increase in case detection in some Africa countries, probably attributable to the gradually increasing medical

Appendix 2: Ethical approvals and Introduction letter from Ghana

In case of reply the number
And the date of this
Letter should be quoted
My Ref. No. KBTH/MD/68/20
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21st May, 2020

BRIGHT OCANSEY
SCHOOL OF BIOLOGICAL SCIENCES
UNIVERSITY OF MANCHESTER
UK

SCIENTIFIC AND TECHNICAL COMMITTEE APPROVAL
PROTOCOL IDENTIFICATION NUMBER: KBTH-STC 00058/2020

The Korle Bu Teaching Hospital Scientific and Technical Committee (KBTH-STC), on 21st May, 2020 approved your submitted study protocol.

TITLE OF PROTOCOL: "Epidemiology of neglected serious fungal diseases in Ghana"

PRINCIPAL INVESTIGATOR: Bright Ocansey

This approval requires that you **forward your approved document to Korle Bu Teaching Hospital – Institutional Review Board (KBTH-IRB) for the ethical aspect of the proposal to be assessed before the project can be initiated.**

This STC approval is valid till 28th October, 2020
You may, however, request extension of the approval period, or renewal as the case may be, should the study extend beyond the stated period.

Upon completion, you are required to submit a final report on the study to the STC. This is to enable the STC ensure among others that, the project has been implemented as per the approved protocol. You are also required to inform the KBTH-STC and Research Directorate of any publications that may emanate from the research findings.

Kindly note that, should the need arise, the KBTH-STC or IRB may institute appropriate measures to satisfy itself that study is being conducted according to the highest scientific and ethical standards.

Please note that any modification to the study protocol without Scientific Technical Committee (STC) approval renders this approval invalid.

Sincere regards,

Prof. G. Obeng Adjei
Chairman, KBTH-STC

Cc: The Chairman, KBTH-IRB

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11th June, 2020

BRIGHT OCANSEY
SCHOOL OF BIOLOGICAL SCIENCES
UNIVERSITY OF MANCHESTER, UK

NEGLECTED SERIOUS FUNGAL DISEASE IN A TERTIARY HOSPITAL IN GHANA

KBTH-IRB /00058/2020

Investigator: Bright Ocansey

The Korle Bu Teaching Hospital Institutional Review Board (KBTH IRB) reviewed and granted approval to the study entitled: "Neglected Serious Fungal Disease in a Tertiary Hospital in Ghana"

Please note that the Board requires you to submit a final review report on completion of this study to the KBTH-IRB.

Kindly, note that, any modification/amendment to the approved study protocol without approval from KBTH-IRB renders this certificate invalid.

Please report all serious adverse events related to this study to KBTH-IRB within seven days verbally and fourteen days in writing.

This IRB approval is valid till 30th May, 2021. You are to submit annual report for continuing review.

Sincere regards,

DR. DANIEL ANKRAH
VICE CHAIR (KBTH-IRB)
FOR: CHAIR (KBTH-IRB

Cc: The Chief Executive Officer, KBTH

The Director of Medical Affairs, KBTH

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11th June, 2020

BRIGHT OCANSEY
SCHOOL OF BIOLOGICAL SCIENCES
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INSTITUTIONAL APPROVAL: KORLE BU TEACHING HOSPITAL-SCIENTIFIC AND TECHNICAL COMMITTEE/INSTITUTIONAL REVIEW BOARD (KBTH-STC/IRB/00058/2020

Following approval of your study entitled: "Neglected Serious Fungal Disease in a Tertiary Hospital in Ghana" by the Korle Bu Teaching Hospital-Scientific and Technical Committee/Institutional Review Board.

I am pleased to inform you that institutional approval has been granted for the conduct of your study in Korle Bu Teaching Hospital.

Please contact the Head of Department to discuss the commencement date of the study.

Please note that. this institutional approval is rendered invalid if the terms of the Institutional Reviewed Board/Scientific and Technical Committee approval are violated.

Sincere regards,

Dr. Harry Akoto
Dep. Director of Medical Affairs
For: Director of Medical Affairs

Cc: The Chief Executive
Korle Bu

MEDICAL DIRECTORATE
KORLE BU TEACHING HOSPITAL

11th June, 2020

LETTER OF INTRODUCTION – BRIGHT OCANSEY
"NEGLECTED SERIOUS FUNGAL DISEASE IN A TERTIARY HOSPITAL IN GHANA"

I have the pleasure to introduce to you the above-named Investigator from School of Biological Sciences University of Manchester, UK. Bright Ocansey sought and has been granted approval to conduct a study entitled: "Neglected Serious Fungal Disease in a Tertiary Hospital in Ghana".

He is to contact you to discuss the commencement date of the study.

Please verify his identity with a Government issued National ID card and accord him the needed assistance.

Attached is the Scientific and Technical Committee and Institutional Review Board approval, which specifies the terms.

Sincere regards,

Dr. Harry Akoto
Dep. Director of Medical Affairs
For: Chief Executive

DISTRIBUTION

1. The Head, Dept. of Medicine, Korle Bu
2. The Head, Korle Bu Blood Bank

NBTS/RES-76/RDAL-02

04 February, 2021

Bright Ocansey,
University of Manchester

Dear Bright Ocansey,

Re: Research Protocol (NBSGRD/201410/02) "Neglected Serious Fungal Diseases in a Tertiary Hospital in Ghana."

Thank you for your letter seeking approval to conduct the above study at the Southern Zonal Blood Centre (SZBC) of the National Blood Service (NBS). You provided the following documents for consideration:

- Project Proposal
- Ethical Clearance by the Korle-Bu Institutional Review Board (IRB)
- Completed Online Registration Form
- Letter of Introduction
- Letter Requesting for Permission

These documents have been reviewed by the relevant NBSG reviewers and your request has been approved. Approval is conditional upon:

- Continued adherence to NBS approved operating procedures.
- Adherence to all ethical requirements.
- Provision of notification of when the data collection commences and ends.

You have requested to collect **2mls** of ninety (90) de-identified (anonymized) blood samples to be used as controls for your research study, from the Laboratory Department of the Southern Zonal Blood Centre by 30th March, 2021.

It is mandatory that the Research and Development Department of the NBS review manuscripts or other publications that emanate from this study. You will therefore be required to submit such reports or proceedings to the NBS ahead of dissemination or publication.

You are required to submit a copy of the final report on this study once it is completed.

Yours sincerely,

Dr. Lucy Asamoah-Akuoko
Head, Research & Development

Cc: Head Laboratory, SABC
Research Officer, R&D

In case of reply the number
And the date of this
Letter should be quoted
My Ref. No. KBTH/IRB/00821
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31st August, 2021

BRIGHT OCANSEY
SCHOOL OF BIOLOGICAL SCIENCES
UNIVERSITY OF MANCHESTER, UK

RE-REQUEST FOR INSTITUTIONAL REVIEW BOARD APPROVAL OF PROTOCOL AMENDMENT
"EPIDEMIOLOGY OF INVASIVE FUNGAL INFECTIONS IN A TERTIARY HOSPITAL IN GHANA"

Reference your letter dated 27th August, 2021 seeking for amendment of the research protocol.

The Korle Bu Teaching Hospital Institutional Review Board (KBTH IRB) reviewed and granted approval to an amendment of study protocol entitled: "Epidemiology of serious Fungal Infections in a Tertiary Hospital in Ghana" to "Epidemiology of Invasive Fungal Infections in a Tertiary Hospital in Ghana".

KBTH-IRB /00058/2020

Investigator: Bright Ocansey

This amendment requires that you comply with all other terms as specified in the original IRB approval of 11th June, 2020 and amendment/extension of 15th July, 2021.

Please report all serious adverse events related to this study to KBTH-IRB within seven days verbally and fourteen days in writing.

This IRB approval is valid till 30th June, 2022. You are to submit annual report for continuing review.

Sincere regards,

DR. DANIEL ANKRAH
VICE CHAIR (KBTH-IRB)
FOR: CHAIR (KBTH-IRB

Cc: The Chief Executive Officer, KBTH
The Director of Medical Affairs, KBTH

Institutional Review Board
37 Military Hospital
Neghelli Barracks
ACCRA

Tel: 059 1759506
Email: irbmilhosp@gmail.com

02 February 2022

ETHICAL CLEARANCE

37MH-IRB/FP/IPN/551/21

On 01 February 2022 the 37 Military Hospital (37MH) Institutional Review Board (IRB) approved your protocol.

TITLE OF PROTOCOL: Epidemiology of Invasive Fungal Infections in Ghana

PRINCIPAL INVESTIGATOR(s): BRIGHT OCANSEY

Please note that a final review report must be submitted to the Board at the completion of the study.

Please report all serious adverse events related to this study to 37MH-IRB within seven (7) days verbally and fourteen (14) days in writing.

This certificate is valid till 31 January 2023.

DR EDWARD ASUMANU
(37MH-IRB, Vice Chairman)

INSTITUTIONAL REVIEW BOARD
02 FEB 2022
37 MILITARY HOSPITAL · ACCRA

Cc: Brig Gen NA Obodai
Commander, 37 Military Hospital

Appendix 3: Ethical approvals from University of Manchester

Research Governance, Ethics and Integrity
2nd Floor Christie Building
The University of Manchester
Oxford Road
Manchester
M13 9PL
Tel: 0161 275 2206/2674
Email: research.ethics@manchester.ac.uk

MANCHESTER 1824
The University of Manchester

Ref: 2020-9372-16067

21/07/2020

Dear Mr Bright Ocansey, Dr Chris Kosmidis, Prof David Denning

Study Title: Screening for invasive fungal diseases among ART-experienced HIV patients using non-culture-based assays

University Research Ethics Committee 2

I write to thank you for submitting the final version of your documents for your project to the Committee on 10/07/2020 16:37 . I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation as submitted and approved by the Committee.

COVID-19 Important Note

If you are conducting research with a data collection methodology that involves face-to-face contact (i.e. interviews, focus groups, psychological experiments, tissue sampling, and any other research procedure requiring face-to-face contact) you must switch to data collection via Skype, telephone or an alternative digital platform.

Please note, you do not need to seek a formal amendment to your existing ethical approval to make these changes provided your consent procedures remain the same (i.e. if you are still obtaining written consent but the form is returned by post or email). If you are choosing an alternative consenting procedure, please submit a formal amendment to your ethical approval via the usual process.

If switching your data collection to digital or electronic means is not possible (i.e. human tissue studies) then you must suspend all research activity until further notice unless doing so will have critical impacts on research participants (i.e. affect their wellbeing or care).

Please also consider whether you need to submit an amendment to extend your dates of data collection, due to postponed fieldwork or other research activities. If you need to seek an extension, you must do so before the end date as listed on your approved ethics application/last approved amendment or within 3 months of this date.

Researchers who wish to continue with face-to-face data collection during this period will require specific approval from the Research Governance, Ethics and Integrity Team. Such approval will only be given if 1) the researcher is a member of staff or PGR, 2) the research is specifically related to the Covid-19 situation and data collection has to take place at the present time, or 2) there are exceptional reasons for the continuation of face-to-face data collection (i.e. critical impacts on the wellbeing or care of research participants).

Please see https://www.staffnet.manchester.ac.uk/rbe/ethics-integrity/ethics/ for further details

Please see below for a table of the title, version numbers and dates of all the final approved documents for your project:

Document Type	File Name	Date	Version
Default	Study 2 Questionnaire V2	09/04/2020	2
Additional docs	NEGLECTED SFD IN A TERTIARY HOSPITAL IN GHANA IRB v4	11/06/2020	4
Consent Form	Study 2 Consent for V4	22/06/2020	4
Participant Information Sheet	Study2 Information Sheet V5	22/06/2020	5
Additional docs	FINAL TWI Study2 Consent Form V1	06/07/2020	1
Additional docs	FINAL TWI Study2 Information Sheet V1	06/07/2020	1
Additional docs	UG Medical Microbiology permission letter	06/07/2020	1
Data Management Plan	Study 2 Premortem IFD DMP v4	08/07/2020	4
Additional docs	KBTH IRB STC 1	09/07/2020	1
Additional docs	KBTH IRB STC 2	09/07/2020	1
Additional docs	KBTH IRB STC 4	09/07/2020	1
Additional docs	KBTH IRB STC 3	09/07/2020	1
Additional docs	KBTH IRB STC 5	09/07/2020	1
Additional docs	Study 2 responses v2	09/07/2020	2

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MANCHESTER 1824
The University of Manchester

Ref: 2020-9368-16168

28/07/2020

Dear Mr Bright Ocansey, Prof David Denning, Dr Chris Kosmidis

Study Title: Screening for CPA in a cohort of suspected TB patients in Ghana

University Research Ethics Committee 2

I write to thank you for submitting the final version of your documents for your project to the Committee on 23/07/2020 07:11 . I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation as submitted and approved by the Committee.

COVID-19 Important Note

Please ensure you read the information on the Research Ethics website in relation to data collection in the COVID environment as well as the guidance issued by the University in relation to face-to-face (in person) data collection both on and off campus.

A word document version of this guidance is also available.

Please see below for a table of the title, version numbers and dates of all the final approved documents for your project:

Document Type	File Name	Date	Version
Default	Study1 Questionnaire V2	14/04/2020	2
Data Management Plan	Study 1 DMP V3	30/04/2020	3
Additional docs	Letter (11) UREC CPA	15/06/2020	1
Participant Information Sheet	Study1 Information Sheet V6	22/06/2020	6
Participant Information Sheet	Study1 Information Sheet B V6	22/06/2020	6
Consent Form	Study1 Consent form B V6	22/06/2020	6
Additional docs	NEGLECTED SFD IN A TERTIARY HOSPITAL IN GHANA IRB v4	02/07/2020	4
Additional docs	FINAL TWI Study1 information Sheet V1	03/07/2020	1
Additional docs	FINAL TWI Study1 Consent form V1	03/07/2020	1
Additional docs	Translation authentication email	03/07/2020	1
Additional docs	UG Medical Microbiology permission letter	06/07/2020	1
Additional docs	KBTH IRB STC 1	09/07/2020	1
Additional docs	KBTH IRB STC 2	09/07/2020	1
Additional docs	KBTH IRB STC 3	09/07/2020	1
Additional docs	KBTH IRB STC 4	09/07/2020	1
Additional docs	KBTH IRB STC 5	09/07/2020	1
Additional docs	Study 1 responses v2	14/07/2020	2
Additional docs	UREC CPA 2	21/07/2020	1
Consent Form	Study1 Consent form V7	22/07/2020	7
Additional docs	Study 1 responses 2 v1	22/07/2020	1

This approval is effective for a period of five years however please note that it is only valid for the specifications of the research project as outlined in the approved documentation set. If the project continues beyond the 5 year period or if you wish to propose any changes to the methodology or any other specifics within the project, an application to seek an amendment must be submitted for review. Failure to do so could invalidate the insurance and constitute research misconduct.

You are reminded that, in accordance with University policy, any data carrying personal identifiers must be encrypted when not held on a secure university computer or

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Ref: 2022-13962-25109

23/08/2022

Dear Mr Bright Ocansey, Prof David Denning, Dr Chris Kosmidis

Study Title: Invasive aspergillosis among haematological malignancy patients at a tertiary hospital in Ghana

University Research Ethics Committee 2

I write to thank you for submitting the final version of your documents for your project to the Committee on 16/08/2022 06:47 . I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation as submitted and approved by the Committee.

Please note that your approved data collection window expires on 30/08/2022 , as per the information you provided in section D of your application. If you wish to extend this you must submit a formal amendment before this date or a new ethics application may be required. The maximum window for data collection the Committee is able to approve is 5 years from the date ethics approval is granted (5 years from 23/08/2022). If you wish to collect data beyond 5 years a new ethics application will be required.

Please ensure you review the Research Ethics website throughout the duration of your project to keep up to date on current UoM guidance and best practice.

Please see below for a table of the title, version numbers and dates of all the final approved documents for your project:

Document Type	File Name	Date	Version
Additional docs	UG Medical Microbiology permission letter	06/07/2020	1
Additional docs	Translation authentication email	10/07/2020	1
Additional docs	KBTH IRB STC 5	09/10/2020	5
Additional docs	KBTH IRB Amendment Approval	31/08/2021	1
Additional docs	EPIDEMIOLOGY OF INVASIVE FUNGAL INFECTIONS IN GHANA PROPOSAL IRB revised	30/01/2022	7
Default	New Study 4 Questionnaire v3	07/04/2022	3
Additional docs	Twi New Study4 Consent form V1	27/04/2022	1
Additional docs	Twi Study 4 information Sheet V1	27/04/2022	1
Additional docs	Letter	11/07/2022	1
Data Management Plan	New Study 4 DMP v2	28/07/2022	2
Participant Information Sheet	New Study 4 Information Sheet v4	29/07/2022	4
Consent Form	New Study 4 consent form v3	29/07/2022	3
Additional docs	Response to UREC comments	29/07/2022	1
Additional docs	Letter	12/08/2022	1
Additional docs	KBTH IRB Extension Approval	15/08/2022	1
Additional docs	Response to UREC comments 2	15/08/2022	1
Default	Distress protocol or debrief sheet -Not required	15/08/2022	1

This approval is only valid for the specifications of the research project as outlined in the approved documentation set.

If you wish to propose any changes to the methodology or any other specifics within the project, including the dates of data collection, an application to seek an amendment must be submitted for review. Failure to do so could invalidate the insurance and constitute research misconduct.

You are reminded that, in accordance with University policy, any data carrying personal identifiers must be encrypted when not held on a secure university computer or kept securely as a hard copy in a location which is accessible only to those involved with the research.

Reporting Requirements:

You are required to report to us the following:

1. Amendments: Guidance on what constitutes an amendment
2. Amendments: How to submit an amendment in the ERM system
3. Ethics Breaches and adverse events

Research Governance, Ethics and Integrity
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MANCHESTER 1824
The University of Manchester

Ref: 2020-9529-16112

16/07/2020

Dear Mr Bright Ocansey, , Dr Chris Kosmidis, Prof David Denning

Study Title: Invasive fungal diseases in Ghanaian HIV patients: An autopsy study

University Research Ethics Committee 5

I write to thank you for submitting the final version of your documents for your project to the Committee on 15/07/2020 16:42 . I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation as submitted and approved by the Committee.

COVID-19 Important Note

If you are conducting research with a data collection methodology that involves face-to-face contact (i.e. interviews, focus groups, psychological experiments, tissue sampling, and any other research procedure requiring face-to-face contact) you must switch to data collection via Skype, telephone or an alternative digital platform.

Please note, you do not need to seek a formal amendment to your existing ethical approval to make these changes provided your consent procedures remain the same (i.e. if you are still obtaining written consent but the form is returned by post or email). If you are choosing an alternative consenting procedure, please submit a formal amendment to your ethical approval via the usual process.

If switching your data collection to digital or electronic means is not possible (i.e. human tissue studies) then you must suspend all research activity until further notice unless doing so will have critical impacts on research participants (i.e. affect their wellbeing or care).

Please also consider whether you need to submit an amendment to extend your dates of data collection, due to postponed fieldwork or other research activities. If you need to seek an extension, you must do so before the end date as listed on your approved ethics application/last approved amendment or within 3 months of this date.

Researchers who wish to continue with face-to-face data collection during this period will require specific approval from the Research Governance, Ethics and Integrity Team. Such approval will only be given if 1) the researcher is a member of staff or PGR, 2) the research is specifically related to the Covid-19 situation and data collection has to take place at the present time, or 2) there are exceptional reasons for the continuation of face-to-face data collection (i.e. critical impacts on the wellbeing or care of research participants).

Please see https://www.staffnet.manchester.ac.uk/rbe/ethics-integrity/ethics/ for further details

Please see below for a table of the title, version numbers and dates of all the final approved documents for your project:

Document Type	File Name	Date	Version
Default	Study3 Questionnaire V1	15/04/2020	1
Data Management Plan	Study 3 DMP V6	18/05/2020	6
Additional docs	KBTH IRB STC 1	02/07/2020	1
Additional docs	KBTH IRB STC 2	02/07/2020	1
Additional docs	KBTH IRB STC 3	02/07/2020	1
Additional docs	KBTH IRB STC 4	02/07/2020	1
Additional docs	KBTH IRB STC 5	02/07/2020	1
Additional docs	UG Medical Microbiology permission letter	06/07/2020	1
Additional docs	UREC Study 3 Post-mortem IFD	06/07/2020	1
Additional docs	Translation authentication email	09/07/2020	1
Participant Information Sheet	Study3 Information Sheet V4	10/07/2020	4
Consent Form	Study3 Consent form V3	10/07/2020	3
Additional docs	Study 3 Information Sheet V4 TWI	10/07/2020	4
Additional docs	Study3 Consent form V3 2 TWI	10/07/2020	3
Additional docs	Study 3 responses v1	13/07/2020	1

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MANCHESTER 1824
The University of Manchester

Ref: 2020-9593-16127

23/07/2020

Dear Mr Bright Ocansey, , Dr Chris Kosmidis, Prof David Denning

Study Title: Establishing a Ghana registry for African histoplasmosis

University Research Ethics Committee 5

I write to thank you for submitting the final version of your documents for your project to the Committee on 17/07/2020 13:58 . I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation as submitted and approved by the Committee.

COVID-19 Important Note

Please ensure you read the information on the Research Ethics website in relation to data collection in the COVID environment as well as the guidance issued by the University in relation to face-to-face (in person) data collection both on and off campus.

A word document version of this guidance is also available.

Please see below for a table of the title, version numbers and dates of all the final approved documents for your project:

Document Type	File Name	Date	Version
Default	AH Case Report Form V2	25/04/2020	2
Default	AH Case Management Form V2	25/04/2020	2
Participant Information Sheet	Study4 Information Sheet V2	01/05/2020	2
Participant Information Sheet	Study4 Information Sheet V2	01/05/2020	2
Data Management Plan	Study 4 DMP V3	06/05/2020	3
Default	WHQOL-BREF questionnaire	13/05/2020	2
Additional docs	UG Medical Microbiology permission letter	06/07/2020	1
Additional docs	Letter (13)	06/07/2020	1
Participant Information Sheet	Study4 Information Sheet V3	08/07/2020	3
Consent Form	Study4 Consent form V2	08/07/2020	2
Additional docs	Study4 Consent form V2 2 TWI	08/07/2020	2
Additional docs	Study4 Information Sheet V3 2 TWI	08/07/2020	3
Additional docs	KBTH IRB STC 1	09/07/2020	1
Additional docs	KBTH IRB STC 2	09/07/2020	1
Additional docs	KBTH IRB STC 3	09/07/2020	1
Additional docs	KBTH IRB STC 4	09/07/2020	1
Additional docs	KBTH IRB STC 5	09/07/2020	1
Additional docs	Translation authentication email	09/07/2020	1
Advertisement	Study 4 Email V2	10/07/2020	2
Advertisement	Study 4 Social Media Postings V2	10/07/2020	2
Additional docs	Study 4 responses v2	17/07/2020	2

This approval is effective for a period of five years however please note that it is only valid for the specifications of the research project as outlined in the approved documentation set. If the project continues beyond the 5 year period or if you wish to propose any changes to the methodology or any other specifics within the project, an application to seek an amendment must be submitted for review. Failure to do so could invalidate the insurance and constitute research misconduct.

You are reminded that, in accordance with University policy, any data carrying personal identifiers must be encrypted when not held on a secure university computer or kept securely as a hard copy in a location which is accessible only to those involved with the research.

Appendix 4: Participant Information Sheets - English

Study 1

Screening for invasive fungal diseases in HIV patients using non-culture based assays

Participant Information Sheet (PIS)

You are being invited to take part in a research study to know how common invasive fungal diseases (these are fungal infections that affect internal organs and can cause serious illness) occur in Ghanaian HIV patients on antiretroviral therapy in a study being undertaken for the award of a PhD degree. Before you decide whether to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully before deciding whether to take part 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. Thank you for taking the time to read this.

About the research

- **Who will conduct the research?**

Bright Ocansey (bright.ocansey@postgrad.manchester.ac.uk)

Division of Infection, Immunity and Respiratory Medicine

School of Biological Sciences

Faculty of Biology, Medicine and Health Sciences

University of Manchester, UK

- **What is the purpose of the research?**

The purpose of this research is to determine how frequently some invasive fungal diseases may occur among Ghanaian HIV patients on antiretroviral therapy.

You have been approached either because you are experiencing acute illness, or because you may be included as a control without acute illness. Invasive fungal diseases are significant cause of illness in HIV patients, even those taking antiretroviral drugs. The study will enrol 150 HIV patients on antiretroviral therapy with complaints of acute illness and 75 HIV patients on antiviral therapy with no complaints (in a control group) to provide blood and urine samples for invasive fungal disease screening using rapid test kits. Screening will be done for 3 invasive fungal diseases namely, cryptococcosis and histoplasmosis.

Will the outcomes of the research be published?

It is expected that the study will determine the prevalence of invasive fungal diseases among HIV patients on antiretroviral therapy with complaints of acute illness. This will guide doctors on diseases they may look out for when caring for HIV patients. Laboratory results will be made known to you (including control group patients) and your doctor. The research data will be structured into a student thesis, published in journals, and presented at conferences or seminars.

- **Who has reviewed the research project?**

This study has been reviewed by The University of Manchester Research Ethics Committee, UK and Institutional Review Board of the Korle-Bu Teaching Hospital, Ghana.

Who is funding the research project?

This study is funded by CARIGEST SA and The University of Manchester.

What would my involvement be?

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

Your personal, medical, laboratory and drug data will be extracted from your patient folder onto a structured questionnaire. You will be asked to provide information that may be missing in your records. If you do not have a previously provided blood and urine available at the laboratory, a small blood sample will be collected from you and then be asked to provide urine. Syringe and needle will be used in collecting blood and may cause mild pain, bruising, or bleeding but is unlikely to occur or affect your health. Any discomfort will be attended to by your doctor. All research laboratory testing will be free. You may benefit from being diagnosed with an invasive fungal disease which could have been missed and then receive the right treatment. Your samples may be stored for subsequent research studies by the same or other research team.

You are expected to be involved for a maximum of one hour. This will involve 40 minutes for obtaining both blood and urine samples as part of routine care and 20 minute to assist complete the questionnaire. Laboratory testing will be done in batches, once every week. Laboratory results will be released to you and your doctor immediately they become ready approximately in one-week.

Will I be compensated for taking part?

You will be given a GHS 30.00 to compensate for the extra time spent at the clinic.

- **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. You will be allowed to say if you will or will not take part in the study after going through this information sheet and your questions answered. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason and without detriment to yourself. This does not affect your data protection rights. If you decide not to take part you do not need to do anything further.

Data Protection and Confidentiality

- **What information will you collect about me?**

In order to participate in this research project, we will need to collect information that could identify you, called "personal identifiable information". Specifically, we will need to collect:

- Name
- Date of birth
- Sex
- Occupation

- **Under what legal basis are you collecting this information?**

We are collecting and storing this personal identifiable information in accordance with data protection law which protect your rights. These state that we must have a legal basis (specific reason) for collecting your data. For this study, the specific reason is that it is "a public interest task" and "a process necessary for research purposes".

- **What are my rights in relation to the information you will collect about me?**

You have a number of rights under data protection law regarding your personal information. For example, you can request a copy of the information we hold about you.

If you would like to know more about your different rights or the way we use your personal information to ensure we follow the law, please consult our Privacy Notice for Research attached.

- **Will my participation in the study be confidential and my personal identifiable information be protected?**

In accordance with data protection law, The University of Manchester is the Data Controller for this project. This means that we are responsible for making sure your personal information is kept

secure, confidential, and used only in the way you have been told it will be used. All researchers are trained with this in mind, and your data will be looked after in the following way:

All information obtained from you will be used for the purpose of this study. All forms of data will be stored and held by the University of Manchester on a password protected internet drive. Confidentiality of the information provided by the participants will be ensured and safeguarded. Data will be anonymised, that is your name and any other identifying information will be removed and replaced with a random ID number and the assigned key will be stored on the University's secure server and only known to the research team that is principal investigator, academic supervisors and lead collaborators in Ghana. Research data will be retained for 5 years. Data set will be made publicly available and shared through the University of Manchester institutional repository. Personal information and consent forms will be terminated according to University of Manchester research data management policy.

When you agree to take part in a research study, the information about you may be provided to researchers running other research studies in this organisation. The future research will be of a similar nature to this research project and will concern only using previous data. Your information will only be used by this organisation and researchers to conduct research in accordance with The University of Manchester's Research Privacy Notice**.** This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of validating previous study, and cannot be used to contact you regarding any other matter. It will not be used to make decisions about future services available to you.

Potential disclosures:

If, during the study, we have concerns about your safety or the safety of others, we will inform your doctor, care team or family member. Also, research laboratory test results will be made known to you and your doctor for appropriate management when necessary.

Please also note that individuals from The University of Manchester or regulatory authorities may need to look at the data collected for this study to make sure the project is being carried out as planned. This may involve looking at identifiable data. All individuals involved in auditing and monitoring the study will have a strict duty of confidentiality to you as a research participant.

What if I have a complaint?

- **Contact details for complaints**

If you have a complaint that you wish to direct to members of the research team, please contact: Dr Peter Puplampu (pedpup@yahoo.com, +233 20 630 1551)

If you wish to make a formal complaint to someone independent of the research team or if you are not satisfied with the response you have gained from the researchers in the first instance then please contact

The Research Ethics Manager, Research Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing: research.complaints@manchester.ac.uk or by telephoning 0161 275 2674.

Or

Research and Development Unit, Medical Directorate, Central Admin Block, Korle-Bu Teaching Hospital, P. O. Box KB 77, Accra, Ghana, e-mail: rdo@kbth.gov.gh or Phone: + 233-302739510

If you wish to contact us about your data protection rights, please email dataprotection@manchester.ac.uk or write to The Information Governance Office, Christie Building, The University of Manchester, Oxford Road, M13 9PL at the University and we will guide you through the process of exercising your rights.

You also have a right to complain to the Information Commissioner's Office about complaints relating to your personal identifiable information Tel 0303 123 1113

Contact Details

If you have any queries about the study or if you are interested in taking part then please contact the researcher, BRIGHT OCANSEY (bright.ocansey@postgrad.manchester.ac.uk, +233 54 279 0540)

Study 2 and 3

Screening for chronic pulmonary aspergillosis in a cohort of TB patients in Ghana

Participant Information Sheet (PIS) A

You are being invited to take part in a research study to evaluate chronic pulmonary aspergillosis (which is a slow, progressive and destructive lung disease caused by *Aspergillus* species that affects immunocompetent and mildly immunosuppressed patients) as a possible misdiagnosis or complication of TB using simple diagnostic tools in a study being conducted for the award of a PhD degree. Before you decide whether to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully before deciding whether to take part 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. Thank you for taking the time to read this.

About the research

- **Who will conduct the research?**

Bright Ocansey (bright.ocansey@postgrad.manchester.ac.uk)

Division of Infection, Immunity and Respiratory Medicine

School of Biological Sciences

Faculty of Biology, Medicine and Health Sciences

University of Manchester, UK

- **What is the purpose of the research?**

The purpose of this research is to evaluate chronic pulmonary aspergillosis as a possible misdiagnosis or complication of TB using simple diagnostic tools.

You have been approached because your doctor suspects you may have TB considering your signs and symptoms. Previous studies suggest chronic pulmonary aspergillosis may be misdiagnosed as TB or worsen TB. The study will recruit 180 patients with suspected TB to provide blood and

sputum samples to look for *Aspergillus* species (the organism that causes chronic pulmonary aspergillosis) through antibody testing, microscopy, and culture. Chest x-ray will also be done to assess the status of your lung. Research laboratory tests and chest x-ray will be repeated at 6 and 12 months if you are treated for TB. Depending on your laboratory and x-ray reports during a follow-up visit, a CT scan may be done for you.

Will the outcomes of the research be published?

It is expected that the study will determine the occurrence of chronic pulmonary aspergillosis among patients with suspected TB and those managed for TB. This will guide doctors when caring for both new and old TB patients. Laboratory and imaging reports will be made known to you. The research data will be structured into a student thesis, published in journals, and presented at conferences or seminars.

- **Who has reviewed the research project?**

This study has been reviewed by The University of Manchester Research Ethics Committee, UK and Institutional Review Board of the Korle-Bu Teaching Hospital, Ghana.

Who is funding the research project?

This study is funded by CARIGEST SA and The University of Manchester.

<u>What would my involvement be?</u>

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

Your will be asked to answer questions on a structured questionnaire about yourself including basic information, occupation and health. Small blood sample will be collected from you and chest x-ray done for you. Syringe will be used in collecting blood and may cause mild pain, bruising, or bleeding but is unlikely to occur or affect your health. There is a very low risk for side effects from doing the x-ray. If you experience any discomfort a doctor will attend to you. If you are having a productive cough, a portion of the sputum sample you will provide for routine TB test will be used for research testing, so your TB test will be done irrespective of your consent decision. Laboratory and x-ray tests will be free. You may benefit from being diagnosed with chronic pulmonary aspergillosis which could have been missed and so you will receive the right treatment.

You are expected to be spend a maximum of 75 minutes. This will involve 15 minutes for answering questionnaire and 60 minutes to provide blood and sputum and take chest x-ray.

Laboratory testing will be done in batches, once every week. Laboratory results and x-ray reports will be given to you and your doctor immediately they are ready. The total duration of the study is 12 months. If you are placed on TB drugs, during your routine follow-up visit at 6 and 12 months, you will provide blood and sputum (if you are having productive cough) and take chest x-ray. The time needed for each follow-up visit will be one hour. Depending on your laboratory and x-ray reports, that is an abnormal x-ray suggestive of chronic pulmonary aspergillosis and a positive *Aspergillus* antibody test during a follow-up visit (either at 6 or 12 months), a chest CT scan may be done for you. Samples may be retained, and you may be approached by the research team or it's collaborators for further studies and your contact details will be kept so you will be provided with summary feedback of research findings when the study is completed.

Will I be compensated for taking part?

You will be given a GHS 30.00 per follow-up visit to compensate for your travel expenses and extra time spent at the clinic.

- **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. You will be allowed to say if you will or will not take part in the study after going through this information sheet and your questions answered. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason and without detriment to yourself. However, it will not be possible to remove your data from the project once it has been anonymised as we will not be able to identify your specific data. This does not affect your data protection rights. If you decide not to take part you do not need to do anything further.

Data Protection and Confidentiality

- **What information will you collect about me?**

In order to participate in this research project we will need to collect information that could identify you, called "personal identifiable information". Specifically, we will need to collect:

- Name
- Date of birth or age
- Sex
- Occupation

- Residential details

➢ **Under what legal basis are you collecting this information?**

We are collecting and storing this personal identifiable information in accordance with data protection law which protect your rights. These state that we must have a legal basis (specific reason) for collecting your data. For this study, the specific reason is that it is "a public interest task" and "a process necessary for research purposes".

➢ **What are my rights in relation to the information you will collect about me?**

You have a number of rights under data protection law regarding your personal information. For example you can request a copy of the information we hold about you.

If you would like to know more about your different rights or the way we use your personal information to ensure we follow the law, please consult our Privacy Notice for Research attached.

- **Will my participation in the study be confidential and my personal identifiable information be protected?**

In accordance with data protection law, The University of Manchester is the Data Controller for this project. This means that we are responsible for making sure your personal information is kept secure, confidential and used only in the way you have been told it will be used. All researchers are trained with this in mind, and your data will be looked after in the following way:

All information obtained from you will be used for the purpose of this study. All forms of data will be stored and held by the University of Manchester on a password protected internet drive. Confidentiality of the information provided by the participants will be ensured and safeguarded. Data will be anonymised, that is your name and any other identifying information will be removed and replaced with a random ID number and the assigned key will be stored on the University's secure server that will only be known to the research. Research data will be retained for 5 years. Data set will be made publicly available and shared through the University of Manchester institutional repository.

When you agree to take part in a research study, the information about you may be provided to researchers running other research studies in this organisation. The future research will be of a

similar nature to this research project and will concern only using previous data. Your information will only be used by this organisation and researchers to conduct research in accordance with The University of Manchester's Research Privacy Notice**.** This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for further similar studies and cannot be used to contact you regarding any other matter. It will not be used to make decisions about future services available to you.

Potential disclosures:

If, during the study, we have concerns about your safety or the safety of others, we will inform your doctor, care team or family member. Also, laboratory test results and chest x-ray reports will be made known to your doctor for appropriate management.

Please also note that individuals from The University of Manchester or regulatory authorities may need to look at the data collected for this study to make sure the project is being carried out as planned. This may involve looking at identifiable data. All individuals involved in auditing and monitoring the study will have a strict duty of confidentiality to you as a research participant.

What if I have a complaint?

- **Contact details for complaints**

If you have a complaint that you wish to direct to members of the research team, please contact: **Dr Jane Afriyie-Mensah (jafriyiemensah@yahoo.com, +233 20 630 1108)**

If you wish to make a formal complaint to someone independent of the research team or if you are not satisfied with the response you have gained from the researchers in the first instance then please contact

The Research Ethics Manager, Research Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing: research.complaints@manchester.ac.uk or by telephoning 0161 275 2674.

Or

Research and Development Unit, Medical Directorate, Central Admin Block, Korle-Bu Teaching Hospital, P. O. Box KB 77, Accra, Ghana, e-mail: rdo@kbth.gov.gh or Phone: + 233-302739510

If you wish to contact us about your data protection rights, please email dataprotection@manchester.ac.uk or write to The Information Governance Office, Christie

Building, The University of Manchester, Oxford Road, M13 9PL at the University and we will guide you through the process of exercising your rights.

You also have a right to complain to the Information Commissioner's Office about complaints relating to your personal identifiable information Tel 0303 123 1113

Contact Details

If you have any queries about the study or if you are interested in taking part then please contact the researcher, BRIGHT OCANSEY (bright.ocansey@postgrad.manchester.ac.uk, +233 54 279 0540)

Screening for chronic pulmonary aspergillosis in a cohort of TB patients in Ghana

Participant Information Sheet (PIS) B (Control Group)

You are being invited to take part in a research study to evaluate chronic pulmonary aspergillosis (which is a slow, progressive, and destructive lung disease caused by *Aspergillus* species that affects immunocompetent and mildly immunosuppressed patients) as a possible misdiagnosis or complication of TB using simple diagnostic tools in a study being conducted for the award of a PhD degree. Before you decide whether to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully before deciding whether to take part 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. Thank you for taking the time to read this.

About the research

- **Who will conduct the research?**

Bright Ocansey (bright.ocansey@postgrad.manchester.ac.uk)

Division of Infection, Immunity and Respiratory Medicine

School of Biological Sciences

Faculty of Biology, Medicine and Health Sciences

University of Manchester, UK

- **What is the purpose of the research?**

The purpose of this research is to evaluate chronic pulmonary aspergillosis as a possible misdiagnosis or complication of TB using simple diagnostic tools.

You have been approached because you are eligible and about to voluntarily donate blood. Previous studies suggest chronic pulmonary aspergillosis almost always occur in people with a history of chronic respiratory disease. The study will recruit 90 blood donors to provide blood to check the performance of the antibody testing to be used in diagnosing chronic pulmonary aspergillosis in patients suspected to have TB.

Will the outcomes of the research be published?

It is expected that the study will determine the occurrence of chronic pulmonary aspergillosis among patients with suspected TB and those managed for TB. This will guide doctors when caring for both new and old TB patients. The research data will be structured into a student thesis, published in journals and presented at conferences or seminars.

- **Who has reviewed the research project?**

This study has been reviewed by The University of Manchester Research Ethics Committee, UK and Institutional Review Board of the Korle-Bu Teaching Hospital, Ghana.

Who is funding the research project?

This study is funded by CARIGEST SA and The University of Manchester.

What would my involvement be?

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

Small blood will be collected from your blood sample collected from you during donation and so you may not provide a different blood after you have given consent.

You are expected to be spend the routine blood bank time.

Your contact details will be kept so that you will be provided with summary feedback of research findings when the study is completed

Will I be compensated for taking part?

Please, we are sorry unfortunately there is no compensation for your involvement aside the normal incentives for donating blood

- **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. You will be allowed to say if you will or will not take part in the study after going through this information sheet and your questions answered. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason and without detriment to yourself. This does not affect your data protection rights. If you decide not to take part you do not need to do anything further.

Data Protection and Confidentiality

- **What information will you collect about me?**

We will not collect any information about you.

- **Under what legal basis are you collecting this information?**

We are collecting and storing this personal identifiable information in accordance with data protection law which protect your rights. These state that we must have a legal basis (specific reason) for collecting your data. For this study, the specific reason is that it is "a public interest task" and "a process necessary for research purposes".

- **What are my rights in relation to the information you will collect about me?**

You have a number of rights under data protection law regarding your personal information. For example you can request a copy of the information we hold about you.

If you would like to know more about your different rights or the way we use your personal information to ensure we follow the law, please consult our Privacy Notice for Research attached.

- **Will my participation in the study be confidential and my personal identifiable information be protected?**

In accordance with data protection law, The University of Manchester is the Data Controller for this project. This means that we are responsible for making sure your personal information is kept secure, confidential, and used only in the way you have been told it will be used. All researchers are trained with this in mind, and your data will be looked after in the following way:

All information obtained from you will be used for the purpose of this study. All forms of data will be stored and held by the University of Manchester on a password protected internet drive. Confidentiality of the information provided by the participants will be ensured and safeguarded. Data will be anonymised, that is your name and any other identifying information will be removed and replaced with a random ID number and the assigned key will be stored on the University's secure server that will only be known to the research. Research data will be retained for 5 years.

Data set will be made publicly available and shared through the University of Manchester institutional repository.

When you agree to take part in a research study, the information about you may be provided to researchers running other research studies in this organisation. The future research will be of a similar nature to this research project and will concern only using previous data. Your information will only be used by this organisation and researchers to conduct research in accordance with The University of Manchester's Research Privacy Notice**.** This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for further similar studies, and cannot be used to contact you regarding any other matter. It will not be used to make decisions about future services available to you.

Potential disclosures:

If, during the study, we have concerns about your safety or the safety of others, we will inform your doctor, care team or family member.

Please also note that individuals from The University of Manchester or regulatory authorities may need to look at the data collected for this study to make sure the project is being carried out as planned. This may involve looking at identifiable data. All individuals involved in auditing and monitoring the study will have a strict duty of confidentiality to you as a research participant.

What if I have a complaint?

- **Contact details for complaints**

If you have a complaint that you wish to direct to members of the research team, please contact: **Dr Jane Afriyie-Mensah (jafriyiemensah@yahoo.com, +233 20 630 1108)**

If you wish to make a formal complaint to someone independent of the research team or if you are not satisfied with the response you have gained from the researchers in the first instance then please contact

The Research Ethics Manager, Research Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing: research.complaints@manchester.ac.uk or by telephoning 0161 275 2674.

Or

Research and Development Unit, Medical Directorate, Central Admin Block, Korle-Bu Teaching Hospital, P. O. Box KB 77, Accra, Ghana, e-mail: rdo@kbth.gov.gh or Phone: + 233-302739510

If you wish to contact us about your data protection rights, please email dataprotection@manchester.ac.uk or write to The Information Governance Office, Christie Building, The University of Manchester, Oxford Road, M13 9PL at the University and we will guide you through the process of exercising your rights.

You also have a right to complain to the Information Commissioner's Office about complaints relating to your personal identifiable information Tel 0303 123 1113

Contact Details

If you have any queries about the study or if you are interested in taking part then please contact the researcher, BRIGHT OCANSEY (bright.ocansey@postgrad.manchester.ac.uk, +233 54 279 0540)

Study 4

Screening for invasive aspergillosis among haematological malignancy patients at the Korle-bu Teaching Hospital

Participant Information Sheet (PIS)

You are being invited to take part in a research study to know how common the infection invasive aspergillosis (this is serious deep-seated illness caused by a fungus which is everywhere in the environment and known as *Aspergillus*) occur among patients with blood cancer in a study being undertaken for the award of a PhD degree. Before you decide whether to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully before deciding whether to take part 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. Thank you for taking the time to read this.

About the research

- **Who will conduct the research?**

Bright Ocansey (bright.ocansey@postgrad.manchester.ac.uk)

Chris Kosmidis

David Denning

Division of Evolution, Infection and Genomics

University of Manchester, UK

Local Research Team

Benjamin Otoo, Noguchi Memorial Institute of Medical Research, University of Ghana, Ghana

Abraham Lamptey, Korle-Bu Teaching Hospital, Ghana

Enoch Mensah, University of Ghana Medical School, Ghana

Hafisatou Gbadamosi, Korle-Bu Teaching Hospital, Ghana

- **What is the purpose of the research?**

The purpose of this research is to determine how common invasive aspergillosis are likely to occur among blood cancer patients in Ghana.

You have been approached either because you have been newly diagnosed with or being treated for blood cancer. Invasive aspergillosis has been found to be a significant cause of illness in blood cancer patients mostly in developed countries, even in those who no longer have signs of cancer. The study will enrol 65 blood cancer patients to provide blood, sputum and to do CT scan for invasive aspergillosis screening using an international guideline.

Will the outcomes of the research be published?

It is expected that the study will determine the prevalence of invasive aspergillosis among blood cancer patients. This will guide doctors on diseases they may look out for when caring for blood cancer patients. Laboratory results will be made known to you and your doctor. The research data will be put together in a student thesis, published in journals and presented at conferences or seminars.

- **Who has reviewed the research project?**

This study has been reviewed by The University of Manchester Research Ethics Committee, UK and Institutional Review Board of the Korle-Bu Teaching Hospital, Ghana.

Who is funding the research project?

This study is funded by CARIGEST SA and The University of Manchester.

<u>What would my involvement be?</u>

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

Your personal, medical, laboratory and drug data relevant to the study will be extracted from your patient folder onto a structured questionnaire. You will be asked to provide information that may

be missing in your records. A small blood sample will be collected from you and then be asked to provide sputum. Syringe and needle will be used in collecting blood and may cause mild pain, bruising, or bleeding but is unlikely to occur or affect your health. Additionally, you will take one or two CT scans depending on your symptoms. Any discomfort will be attended to by your doctor. All research laboratory and imaging tests will be free. You may be diagnosed with an invasive aspergillosis which could have been missed and then receive the right treatment. However, kindly be aware that partaking in the study do not involve any form of treatment from the research team.

Your blood sample may be stored in Ghana for subsequent research studies in future by the same or other research team.

You are expected to be involved for a maximum of one hour. This will involve about 10 minutes to assist complete the questionnaire, about 10 minutes for obtaining both blood and sputum samples as part of routine care and about 40 minutes to do CT scan (s). Laboratory testing will be done in batches, once every week. All test results will be released to you and your doctor immediately they become ready approximately in one-week.

Will I be compensated for taking part?

You will be given a GHS 45.00 (£5.00) in cash to compensate for the extra time spent at the hospital.

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

It is up to you to decide whether to take part. You will be allowed to say if you will or will not take part in the study after going through this information sheet and your questions answered. If you do decide to take part, you will be given this information sheet to keep and will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time without giving a reason and without detriment to yourself. This does not affect your data protection rights. If you decide not to take part, you do not need to do anything further.

Data Protection and Confidentiality

- **What information will you collect about me?**

To participate in this research project, we will need to collect information that could identify you, called “personal identifiable information”. Specifically, we will need to collect:

- Name
- Date of birth
- Sex
- Occupation

➢ **Under what legal basis are you collecting this information?**

We are collecting and storing this personal Identifiable information in accordance with data protection law which protect your rights. These state that we must have a legal basis (specific reason) for collecting your data. For this study, the specific reason is that it is "a public interest task" and "a process necessary for research purposes".

➢ **What are my rights in relation to the information you will collect about me?**

You have several rights under data protection law regarding your personal information. For example, you can request a copy of the information we hold about you.

If you would like to know more about your different rights or the way we use your personal information to ensure we follow the law, please consult our Privacy Notice for Research attached.

- **Will my participation in the study be confidential and my personal identifiable information be protected?**

In accordance with data protection law, The University of Manchester is the Data Controller for this project. This means that we are responsible for making sure your personal information is kept secure, confidential and used only in the way you have been told it will be used. All researchers are trained with this in mind, and your data will be looked after in the following way:

All information obtained from you will be used for the purpose of this study. All forms of data will be stored and held by the University of Manchester on a password protected internet drive. Confidentiality of the information provided by the participants will be ensured and safeguarded. Data will be anonymised, that is your name, and any other identifying information will be removed and replaced with a random ID number and the assigned key will be stored on the University's secure server and only known to the research team that is principal investigator, academic supervisors and research team in Ghana. Research data will be retained for 5 years. Data set will be made publicly available and shared through the University of Manchester institutional

repository. Personal information and consent forms will be terminated according to University of Manchester research data management policy.

When you agree to take part in a research study, the information about you may be provided to researchers running other research studies in this organisation. The future research will be of a similar nature to this research project and will concern only using previous data. Your information will only be used by this organisation and researchers to conduct research in accordance with The University of Manchester's Research Privacy Notice**.** This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of validating previous study and cannot be used to contact you regarding any other matter. It will not be used to make decisions about future services available to you.

Potential disclosures:

If, during the study, we have concerns about your safety or the safety of others, we will inform your doctor, care team or family member. Also, research laboratory test results will be made known to you and your doctor for appropriate management when necessary.

Please also note that individuals from The University of Manchester or regulatory authorities may need to look at the data collected for this study to make sure the project is being carried out as planned. This may involve looking at identifiable data. All individuals involved in auditing and monitoring the study will have a strict duty of confidentiality to you as a research participant.

What if I have a complaint?

- **Contact details for complaints**

If you have a complaint that you wish to direct to members of the research team, please contact: **Chief Investigator- David Denning** david.denning@manchester.ac.uk**, +44 7802 482193)**

If you wish to make a formal complaint to someone independent of the research team or if you are not satisfied with the response you have gained from the researchers in the first instance, then please contact.

The Research Ethics Manager, Research Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL, by emailing: research.complaints@manchester.ac.uk or by telephoning 0161 306 8089.

Or

Research and Development Unit, Medical Directorate, Central Admin Block, Korle-Bu Teaching Hospital, P. O. Box KB 77, Accra, Ghana, e-mail: rdo@kbth.gov.gh or Phone: + 233-302739510

If you wish to contact us about your data protection rights, please email dataprotection@manchester.ac.uk or write to The Information Governance Office, Christie Building, The University of Manchester, Oxford Road, M13 9PL at the University and we will guide you through the process of exercising your rights.

You also have a right to complain to the Information Commissioner's Office about complaints relating to your personal identifiable information Tel 0303 123 1113

Contact Details

If you have any queries about the study or if you are interested in taking part then please contact the researcher, BRIGHT OCANSEY (bright.ocansey@postgrad.manchester.ac.uk, +233 54 2790 540)

Additional Information on COVID-19

Due to the current COVID-19 pandemic, we have made some adjustments to the way in which this research study will be conducted that ensures we are adhering to the latest government advice in relation to social distancing as well as taking all reasonable precautions in terms of limiting the spread of the virus. You should carefully consider all of the information provided below before deciding if you still want to take part in this research study. If you choose not to take part, you need to inform research team. If you have any additional queries about any of the information provided, please speak with a member of the research team.

Are there any additional considerations that I need to know about before deciding whether I should take part?

You are very unlikely to be exposed to any risk of contracting COVID-19 while partaking in the study because precautionary measures stipulated by the Clinic will be followed strictly.

What additional steps will you take to keep me safe while I take part?

Among the precautionary measures to keep you safe include encouraging frequent hand washing with soap and running water, disinfecting sample collection area regularly, booking small number of patients and providing nose masks to participants.

Is there any additional information that I need to know?

You are advised to adhere to appointment schedules as much as possible and communicate any challenges or concerns you may have with any member of the research team.

Additional data use

When necessary, you can call the Ghana Health Service Emergency number 112

What if the Government Guidance changes?

The current government guideline has no impact on the study, changes in future such as lock downs may mean any form of contact will be postponed.

What if I have additional queries?

If you have any queries about the study or if you are interested in taking part then please contact the researcher, BRIGHT OCANSEY (bright.ocansey@postgrad.manchester.ac.uk, +233 54 2790 540)

Appendix 5: Consent forms - English

Study 1

Screening for invasive fungal diseases in HIV patients using non-culture based assays

Consent Form

If you are happy to participate please complete and sign the consent form below

	Activities	Initials
1	I confirm that I have read the attached information sheet for the above study and have had the opportunity to consider the information and ask questions and had these answered satisfactorily.	
2	I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving a reason and without detriment to myself. I understand that it will not be possible to remove my data from the project once it has been anonymised and forms part of the data set. I agree to take part on this basis.	
3	I agree to my doctor being informed of my participation in this study.	
4	I agree to have a blood and urine sample taken for the research purpose as explained to me. I understand that the research using my sample will be for testing antigens of fungi.	
5	I understand that the sponsors of this study may make my blood and urine sample available to other researchers for studies in future and that this may include researchers abroad. I give permission for these individuals to have access to my sample, (but not any personal identifying information about me). I offer my sample as a gift.	

6	I understand that data collected during the study may be looked at by individuals from The University of Manchester or regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data.	
7	I agree that any anonymised data collected may be shared with researchers at other institutions.	
8	I agree that any data collected may be published in anonymous form in student thesis, academic books or journals.	
9	I agree that the researchers/researchers at other institutions may contact me in future about other research projects.	
10	I agree that the researchers may retain my contact details in order to provide me with a summary of the findings for this study.	
11	I agree that the researchers can inform my doctor about the test results	
12	I agree to take part in this study.	

Data Protection

The personal information we collect and use to conduct this research will be processed in accordance with data protection law as explained in the Participant Information Sheet and the Privacy Notice for Research Participants.

_____ _____ _____

Name of Participant Signature Date

_____ _____ _____

Name of the person taking consent Signature Date

(A copy each of the consent form will be made available to the participant, the research team (original), and for the care team)

Study 2 and 3

Screening for chronic pulmonary aspergillosis in a cohort of TB patients in Ghana

Consent Form

If you are happy to participate please complete and sign the consent form below

Item	Activities	Initials
1	I confirm that I have read the attached information sheet for the above study and have had the opportunity to consider the information and ask questions and had these answered satisfactorily.	
2	I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving a reason and without detriment to myself. I understand that it will not be possible to remove my data from the project once it has been anonymised and forms part of the data set. I agree to take part on this basis.	
3	I agree to my doctor being informed of my participation in this study	
4	I agree to provide blood and sputum sample for the research purpose as explained to me. I understand that my samples will be analysed for fungi. I also agree to do the chest x-ray and when necessary, the CT scan.	
5	I understand that the sponsors of this study may make my blood and sputum samples available to other researchers for studies in future and that this may include researchers abroad. I give permission for these individuals to have access to my sample, (but not any personal identifying information about me. I offer my sample as a gift).	
6	I understand that data collected during the study may be looked at by individuals from The University of Manchester or regulatory authorities, where it is relevant to	

	my taking part in this research. I give permission for these individuals to have access to my data.	
7	I agree that any anonymised data collected may be shared with researchers at other institutions.	
8	I agree that any data collected may be published in anonymous form in student thesis, academic books or journals.	
9	I agree that the researchers or researchers at the University of Manchester or other institutions may contact me in future about other research projects.	
10	I agree that the researchers may retain my contact details in order to provide me with a summary of the findings for this study.	
11	I agree that the researchers can inform my doctor about the test results	
12	I understand that my contact details will be kept so I will be provided with summary feedback of research findings when the study is completed	
13	I agree to take part in this study.	

Note: It is not compulsory to consent to items 7, 9 and 10

Data Protection

The personal information we collect and use to conduct this research will be processed in accordance with data protection law as explained in the Participant Information Sheet and the Privacy Notice for Research Participants.

_____	_____	_____
Name of Participant	Signature	Date

_____	_____	_____

Name of the person taking consent Signature Date

(A copy each of the consent form will be made available to the participant, the research team (original), and for the care team)

Screening for chronic pulmonary aspergillosis in a cohort of TB patients in Ghana

Consent Form (Control Group)

If you are happy to participate, please complete and sign the consent form below.

	Activities	Initials
1	I confirm that I have read the attached information sheet for the above study and have had the opportunity to consider the information and ask questions and had these answered satisfactorily.	
2	I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving a reason and without detriment to myself I agree to take part on this basis.	
3	I agree that a portion of my donated blood may be sampled for the research purpose as explained to me. I understand that my samples will be used to evaluate test performance.	
4	I understand that my contact details will be kept so I will be provided with summary feedback of research findings when the study is completed	

Data Protection

The personal information we collect and use to conduct this research will be processed in accordance with data protection law as explained in the Participant Information Sheet and the Privacy Notice for Research Participants.

_____ _____ _____

Name of Participant Signature Date

_____ _____ _____

Name of the person taking consent Signature Date

(A copy each of the consent form will be made available to the participant, the research team (original), and for the care team)

Study 4

MANCHESTER 1824
The University of Manchester

Screening for invasive aspergillosis among haematological malignancy patients at the Korle-bu Teaching Hospital

Consent Form

If you are happy to participate, please complete and sign the consent form below.

SNo.	Activities	Initials
1	I confirm that I have read the attached information sheet for the above study and have had the opportunity to consider the information and ask questions and had these answered satisfactorily.	
2	I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving a reason and without detriment to myself. I understand that it will not be possible to remove my data from the project once it has been anonymised and forms part of the data set. I agree to take part on this basis.	
3	I agree to my doctor being informed of my participation in this study.	
4	I agree to provide blood and sputum samples for the research purpose as explained to me. I understand that the research using my sample will be for testing a fungus called *Aspergillus*.	
5	I agree to my medical records being accessed to extract clinical details relevant to the study	
6	I understand that the sponsors of this study may make my blood sample available to other researchers for studies in future and that this may include researchers abroad. I give permission for these individuals to have access to my sample, (but not any personal identifying information about me). I offer my sample as a gift.	

7	I understand that data collected during the study may be looked at by individuals from The University of Manchester or regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data.	
8	I agree that any data collected may be published in anonymous form in student thesis, academic books or journals.	
9	I agree that the researchers can inform my doctor about the test results	
10	I agree to take part in this study.	

Optional consents

SNo.	Activities	Initials
1	I agree that any anonymised data collected may be shared with researchers at other institutions.	
2	I agree that the researchers, other researchers at the University of Manchester or researchers from other institutions may contact me in future about other research projects.	
3	I agree that the researchers may retain my contact details in order to provide me with a summary of the findings for this study.	

Data Protection

The personal information we collect and use to conduct this research will be processed in accordance with data protection law as explained in the Participant Information Sheet and the Privacy Notice for Research Participants.

_____	_____	_____
Name of Participant	Signature	Date
_____	_____	_____
Name of the person taking consent	Signature	Date

(A copy each of the consent form will be made available to the participant, the research team (original), and for the care team)

Appendix 6: Participant Information Sheets - Local dialect (Twi)

Study 1

Yareɛ nhwehwɛmu a yɛde non-culture based assays (yɛfa laboretri tɛst so de hwehwɛ sɛ abɔdeɛ nketewa a wɔmfa aniwa nhunu gye sɛ yɛhyɛ da yɛn wɔn) yɛ fa *invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) ho wɔ nnipakuo a wɔwɔ HIV yareɛ na wɔrenom nnuro a ɛbɛbrɛ HIV mmoawa no ase (ART)

Krataasini a yɛtwerɛ nnipa a wɔde wɔn ho bɛhyɛ nhwehwɛmu ho nsɛm agu so

Yɛreto nsa afrɛ wo sɛ bɛka adesua nhwehwɛmu bi a ɛbɛma yahunu sɛdeɛ *invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) taa yɛ Ghanafoɔ a wɔwɔ HIV yareɛ na wɔrenom nnuro a ɛbɛbrɛ HIV mmoawa no ase (ART), afa adesua bi yɛreyɛ de akɔgye PhD abɔdin bi.

Ansa na wobɛyɛ w'adwene sɛ wode wo de wo ho bɛhyɛ mu no, ɛho hia sɛ wote deɛ enti a wɔreyɛ saa nhwehwɛmu yi ne deɛ ɛbɛka ho. Mepa wokyɛw sɛ, nya berɛ kenkan nsɛm a ɛdidi soɔ yi yie ansa na wayɛ w'adwene sɛ wode wo ho bɛhyɛ mu. Sɛ wopɛ a, wobɛtumi ne nnipa afoforɔ adi ho nkɔmmɔ. Mepa wo kyɛw, sɛ biribi wɔ hɔ a wonte aseɛ a, anaa wohia nkyerɛkyerɛmu bio a, bisa. Meda wo ase sɛ woanyɛ berɛ rekenkean yei.

<u>Deɛ ɛfa nhwehwɛmu no ho</u>

- Hwan na ɔrebɛyɛ nhwehwɛmu no?

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- **Nhwehwɛmu no ho botaeɛ ne sɛn?**

Nhwehwɛmu no botaeɛ ne sɛ ɛbɛkyerɛ mprɛ dodoɔ a *invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) no bi taa ka Ghanafoɔ a wɔwɔ HIV yareɛ na wɔrenom nnuro a ɛbɛbrɛ HIV mmoawa no ase (ART).

Yaba wo nkyɛn ɛfiri sɛ worefa yareɛ a ano yɛ den mu, anaasɛ ɛfiri sɛ yɛde wo aka ho sɛ yɛde wo bɛyɛ ntotoho (control) kuo a wɔnni yadeɛ a ano yɛ den no bi. *Invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) yɛ farebae kɛseɛ a ɛde yareɛ brɛ wɔn a wɔwɔ HIV yareɛ na wɔrenom nnuro a ɛbɛbrɛ HIV mmoawa no ase. Adesua yi bɛtwerɛ nnipa 150 wɔn a wɔwɔ HIV yareɛ na wɔrenom nnuro a ɛbɛbrɛ HIV mmoawa no ase na wɔsan bɔ soboɔ sɛ wɔwɔ yareɛ a ano yɛ den, **ne** nnipa 75 a wɔwɔ HIV yareɛ na wɔrenom nnuro a ɛbɛbrɛ HIV mmoawa no ase na mmom wɔmmɔ soboɔ sɛ wɔwɔ yareɛ a ɛmu yɛ den (wɔbɛyɛ ntotoho [control] kuo), wɔbɛma mogya ne dwonsɔ nhwɛsoɔ a yɛde *rapid test kit* bɛyɛ nhwenhwɛmu afa *invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) ho. Yɛbɛyɛ nhwɛhwɛmu no afa *invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) no ho, ɛbi ne; *cryptococcosis, histoplasmosis* ne *aspergillosis*.

Wode deɛ ɛbɛfiri nhwehwɛmu no aba no bɛto dwa?

Yɛrehwɛ kwan sɛ adesua no bɛkyerɛ sɛdeɛ *invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) no aheta wɔ nnipa a wɔwɔ HIV yareɛ na wɔrenom nnuro a ɛbɛbrɛ HIV mmoawa no ase (ART) na wɔsan bɔ soboɔ sɛ wɔwɔ yareɛ a ano yɛ den. Yei bɛkyerɛ adokotafoɔ kwan afa nyarewa a wɔbɛtumi ahwɛ kwan sɛ wɔbɛhunu berɛ a wɔrehwɛ nnipa a wɔwɔ HIV yareɛ. Yɛde labɔretri nsunsuanesoɔ no bɛkyerɛ wo ne wo dɔkota (wɔn a wɔyɛ ntotoho kuo no nso ka ho bi).

Yɛbɛkyekyɛ nhwehwɛmu no nsɛmboano (data) no mu ayɛ bi adesua tiisiisi a wɔde to dwa wɔ adesuade nwoma (journal) mu ne nhyiamu anaa badwa sɛmina ase.

Hwan na ahwɛ nhwehwɛmu adwuma no mu agye atom?

The University Manchester Kɔmitii a ɛhwɛ Nneyɛe Pa a ɛfa Nhwehwɛmu adwuma ho so (Research Ethics Committee), UK ne Institutional Review Board a ɛwɔ Korle-Bu Teaching Hospital, Ghana ahwɛ adesua yi agye atom.

Hwan na wafa nhwehwɛma adwuma no ho ka?

CARIGEST SA ne The University of Manchester na afa saa adesua yi ho ka.

<u>Me ho a mede bɛhyɛ adesua no mu ne sɛn?</u>

Sɛ mede me ho hyɛ mu a ɛdeɛn nsɛm na wobɛbisa me?

Yɛbɛgye wo nsɛmboano a ɛfa wo, w'ayaresa, laboretri ne nnuro ho afiri w'ayaresa folda mu de agu nsɛmmisa a yahyehyɛ mu so. Yɛbɛtumi agye nsɛm bi a ayera afiri wo ho nsɛm a yatwerɛ ato hɔ mu. Yɛbɛgye wo mogya nhwɛsoɔ kakra bi na yasrɛ wo na wode wo dwansɔ aba. Yɛde siringye ne paneɛ na ɛbɛtwe wo mogya no, na ɛbia ɛbɛyɛ wo ya kakra, awɔtre wo honam, anaa mogya kakra bɛba nanso ɛrenkɔba sɛ ɛbɛha w'apɔmuden. Wo dɔkota bɛhwɛ asɔ ɔhaw biara ano. Nhwehwɛmu nyinaa a yɛbɛyɛ no laboretri no, worentua ho ka. Sɛ yɛfiri wo na yɛhunu sɛ wowɔ *invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) a anka ɛbɛtumi asie a, wobɛtumi anya mfasoɔ wɔ ayaresa a ɛfata a wobɛnya. Yɛbɛtumi de wo nhwɛsoɔ no asie de ama nhwehwɛmu kuo yi ara anaa afoforɔ de ayɛ nhwehwɛmu bio.

Wo ho a wode bɛhyɛ mu no no mmoro dɔnhwere baako. Sima 40 na yɛde bɛtwe mogya ne dwonsɔ nhwɛsoɔ no, sɛdeɛ daa daa ɔhwɛ teɛ no; yɛde sima 20 bɛboa ayiyi nsɛmmisa mboano (questionnaire) no ano awie. Yɛbɛyɛ laboretri nhwehwɛmu no akuo akuo, dapɛn biara prɛko. Sɛ, ɛbɛyɛ sɛ dapɛn baako akyi, na laboretri nsunsuanesoɔ no yɛ krado a, ntɛm pa ara no yɛde bɛma wo ne wo dɔkota.

Mɛnya akatua bi afiri me ho a mede bɛhyɛ mu no?

Yɛbɛma wo GHS 30.00 de apata wo berɛ a wobɛsɛe no wɔ ayaresabea hɔ no.

Sɛ mempɛ sɛ mede ho hyɛ mu anaa mesesa m'adwene a, deɛn na ɛbɛsi?

Ɛgyina wo ankasa wo so sɛ wode wo ho bɛhyɛ mu anaasɛ womfa wo ho nhyɛ mu. Sɛ wo kenkan sa nsɛmfua krataa yi wie na wonya wo nsɛmbisa no ho anoyie a, yɛbɛma wo kwan na woaka sɛ wode wo ho bɛhyɛ mu anaasɛ womfa wo ho nhyɛ mu. Sɛ woyɛ w'adwene sɛ wode wo ho bɛhyɛ mu a, yɛde saa nsɛmfua krataa yi bɛma wo asie na yasrɛ ama wode wo nsa ahyɛ penee krataa bi ase. Sɛ woyɛ w'adwene sɛ wode wo ho bɛhyɛ mu a woda so wɔ ho kwan sɛ wotwe wo ho firi mu berɛ biara a womma nkyɛrɛkyerɛmu biara na ɛremfa ɔhaw mmrɛ wo. Yei nha asɛdeɛ a wowɔ fa wo nsɛmbuano bambɔ ho. Sɛ woyɛ w'adewene sɛ womfa wo ho nhyɛ mu a, ɛho nhia sɛ woyɛ biribi foforɔ biara bio.

<u>Nsɛmbano ho bambɔ ne kokoam mu nsɛm</u>

Nsɛm bɛn na wobɛgye afa me ho?

Sɛ ɛbɛboa na wode wo ho ahyɛ nhwehwɛmu aduwuma yi mu nti, ɛsɛ sɛ yɛgye nsɛm a ɛbɛma yahyɛ wo nso, a yato din "nsɛm a ɛhyɛ obi nso" (personal identifiable information). Ne titire no, yɛbɛhia sɛ yɛbɛgye:

- Din
- Awoda
- Bɔbea
- Adwuma

Mmara bɛn so na woregyina agye saa nsɛm yi?

Yɛgyina nsɛmboa bambɔ mmara a ɛbɔ w'asɛdeɛ ho ban so na yɛregye nsɛm a a ɛhyɛ obi nso. Yeinom ka sɛ, ɛsɛ yɛgina mmara so (botaeɛ pɔtee) a yɛde regye wo nsɛmboa no. Wɔ saa adesua yi no, botaeɛ pɔtee ne sɛ "ɛyɛ ɔmanfoɔ yiedie adwuma" ne "kwan bi a ɛho hia na yɛfa so yɛ nhwehwɛmu botaeɛ nti"

Deɛn ne m'asɛdeɛ wɔ nsɛm a wobɛgye afa me ho no?

Nsɛmboa Bambɔ Mmara a ɛfa wo ho nsɛm ho no, ma wo asɛdeɛ pii. ɛbi ne sɛ, wobɛtumi abisa nsɛm a yagye afa wo ho no nhwɛsoɔ (copy).

Sɛ wopɛ sɛ wohunu biribi bio fa w'asɛdeɛ ahodoɔ ho anaa kwan a yɛfa so de wo wo ho nsɛm yɛ adwuma sɛdeɛ ɛbɛma yɛadi mmara no so a, yɛpa wo kyɛw, kenkane firi yɛn Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ a ɛka yei ho no.

Me ho a mede bɛhyɛ adesua no mu bɛyɛ kokoam adeɛ na me nsɛm a ɛhyɛ me nso mobɛbɔ (personal identifiable information) ho ban?

Sɛdeɛ nsɛmboa bambɔ mmara teɛ no, ɛyɛ The University of Manchester na ɛwɔ Nsɛmbano no so Tumi wɔ saa adwuma yi so. Yei kyerɛ sɛ ɛyɛ yɛn asɛdeɛ sɛ yɛhwɛ yie sɛ yɛde wo ho nsɛm no bɛsie kokoam na yɛde yɛ adwuma sɛdeɛ yaka akyerɛ wo sɛ yɛde bɛyɛ no. Yatete nhwehwɛmu adwumayɛfoɔ no nyinaa de wei ato wɔn adwene mu, na kwan a ɛdi soɔ yi so na yɛbɛfa ahwɛ wo nsɛmboa no so:

Yɛde nsɛm a yɛbɛgye afa wo ho nyinaa bɛyɛ adwuma afa saa adesua yi ho. Yɛde nsɛmboano biara bɛsie wɔ pendrive a internet password da so a ɛhyɛ The University of Manchester nsa. Kokoa mu nsɛm biara wɔn a wɔde wɔn ho bɛhyɛ mu no de bɛma no, yɛbɛhwɛ so yie na yabɔ ho ban. Yɛbɛyi biribiara a ɛbɛma obi ahu wo afiri nsɛmboano no ho, ɛbi ne sɛ, yɛbɛyi wo din ne nsɛm foforɔ biara obi bɛfa so ahunu wo afiri hɔ de ID nɔma a obiara nnim deɛ ɛbɛfiri aba ahyɛ ananmu, na yɛde safoa a yɛde bue ano no bɛsie wɔ Suapɔn no server a bambɔ da ho na nhwehwɛmu adwumayɛfoɔ kuo, wɔn ne nhwehwɛmufoɔ payin, nwomasua so hwɛfoɔ ne aboafoɔ akannifoɔ a wɔwɔ Ghana no nko ara na wɔnim. Yɛde nhwehwɛmu a ɛfa nsɛmboano no bɛsie mfie num. Yɛde nsemboano kuo (data set) no bɛtwo dwa na yafa The University of Manchester ahyehyɛdeɛ adekorabea so akyekyɛ mu.

Yɛbɛfa University of Manchester nsɛmboa nhyehyeɛ sohwɛ a ɛfa nhwehwɛmu ho (data management policy) de personal information ne penee nkrataa no ho adwuma aba awieɛ.

Sɛ wogye tom sɛ wode wo ho bɛhyɛ nhwehwɛmu adesua bi mu a, yɛbɛtumi de wo ho nsɛm no ama nhwehwɛmufoɔ a wɔreyɛ nhwehwɛnu adesua foforɔ wɔ saa dwumabea ha. Daakye nhwehwɛmu no su ne saa nhwehwɛmu adwuma yi bɛsɛ na deɛ ɛbɛyɛ ne sɛ ɛde nsɛmboano dada no nko ara na ɛbɛyɛ adwuma. Saa dwumabea yi ne nhwehwɛmufoɔ bɛfa The University of Manchester Kokoa mu Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ (Research Privacy Notice) na wɔde wo ho nsɛm bɛyɛ adwuma.

Saa nsɛm yi nna wo adi na yɛmfa nka nsɛm foforɔ ho wɔ kwan biara so a, ɛbɛma obi ahunu sɛ ɛyɛ wo. Wɔde nsɛm no bɛyɛ adwuma nkutoo a ne botaeɛ sɛ ɛbɛboa asi adesua dada bi so dua, na wɔntumi nnyina so mfrɛ wo wɔ asɛm biara ho. Wɔntumi nyina so mfa adwene wɔ daakye som bi a ɛbɛwɔ hɔ ama wo.

Berɛ a yɛbɛtumi ada kokoa mu nsɛm adi

Sɛ ɛkɔba sɛ adesua no mu no, yɛhyia ɔhaw fa wo bambɔ ho anaa afoforɔ bambɔ ho a, yɛbɛbɔ wo dɔkota, ahwɛfokuo anaa abusuafoɔ amaneɛ. Bio, sɛ ɛho hia a, yɛbɛma wo ne wo dɔkota ahunu laboretri nhwehwɛmu nsunsuanesoɔ wɔ ho nhwɛso pa.

Yɛpa wo kyɛw, hyɛ no nso sɛ ankorɛankorɛ firi The University of Manchester anaa nhyehyɛe sodifoɔ bɛtumi ahia sɛ wɔhwɛ nsɛmboano a yagyegye wɔ saa adesua yi ho ama wayɛ adwuma no sɛdeɛ wahyehyɛ ato hɔ no. Yei bɛtumi afa ho sɛ worehwɛ nsɛmboano a ɛda nso. Ankorɛankorɛ biara a woka adesua no nhwehwɛmu a ɛfa nokwaredie ne ahwɛfoɔ bɛyɛ won adwuma pɛpɛɛpɛ afa kokoa mu nsɛm a ɛfa wo ho sɛ obi a wode wo ho abɛhyɛ nhwehwɛmu mu.

<u>Na sɛ mewɔ ɔhaw bi ɛ?</u>

Nkyerɛkyerɛmu a ɛfa baabi a wode wo haw bɛkɔ.

Sɛ wonya ɔhaw bi na wopɛ sɛ nhwehwɛmu adwumakuo no aso te a, yɛpa wo kyɛw, kɔhu Dr Peter Puplampu wo (<u>pedpup@yahoo.com</u>, +233 20 630 1551).

Sɛ wopɛ sɛ wofa mmara kwan so de wo haw no kɔma obi a ɔnka nhwehwɛmu adwumakuo no ho anaasɛ w'ani nnye mmuaeɛ a wanya afiri nhwenhwɛmufoɔ ho berɛ a ɛdi kan no deɛ a, yɛpa wokyɛw, twerɛ kɔ:

The Research Ethics Manager, Research Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL, fa email so: research.complaints@manchester.ac.uk ana frɛ tetefon yi so: 0161 275 2674.

Anaa

Research and Development Unit, Medical Directorate, Central Admin Block, Korle-Bu Teaching Hospital, P. O. Box KB 77, Accra, Ghana, e-mail: rdo@kbth.gov.gh or Phone: + 233-302739510.

Sɛ wopɛ sɛ wo ne yɛn kasa fa w'asɛdeɛ a wowɔ fa wo nsɛmboano bambɔ ho a, yɛpa wo kyɛw fafa email yi so: dataprotection@manchester.ac.uk anaa terɛ kɔ The Information Governance Office, Christie Building, The University of Manchester, Oxford Road, M13 9PL at the University, na yɛbɛboa wo de wo afa kwan a wobɛfa so na wo nsa aka w'asɛdeɛ.

Wo san wɔ ho kwan sɛ wode wo soboɔ bi kɔ Information Commissioner's Office fa ɔhaw a ɛfa wo ho nsɛm a ɛbɛma obi ahunu wo wɔ tetefon yi so: 0303 123 1113.

Akyirikwan ho nsɛm

Sɛ wowɔ nsɛmmisa biara fa adesua no anaa w'ani gye ho sɛ wode wo ho hyɛ mu a, yɛpa wo kyɛw, kɔhunu nhwehwɛmu adwumayɛni BRIGHT OCANSEY (bright.ocansey@postgrad.manchester.ac.uk, +233 54 279 0540).

Study 2 and 3

Yareɛ nhwehwɛmu a ɛfa *chronic pulmonary aspergillosis* ho (yareɛ nyaa a ɛnyini yɛ kɛseɛ nkakrankakra sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) wɔ nnipa bi a wɔwɔ Ghana na yɛsusu sɛ wɔbɔ Nasamanwa (TB)

Krataasini a yɛtwerɛ nnipa a wɔde wɔn ho hyɛ nhwehwɛmu bi mu ho nsɛm agu so

Yɛreto nsa afrɛ wo sɛ bɛka adesua nhwehwɛmu bi a ɛbɛma yahunu sɛdeɛ *chronic pulmonary aspergillosis* (yareɛ nyaa a ɛnyini yɛ kɛseɛ nkakrankakra sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) saa nhwehwɛmu yi bɛtumi ayɛ yareɛ bi nkyerɛkyerɛmu a ɛnyɛ nokware anaa TB a ano ayɛ den. Yɛde *tuuls* nketenkete na ɛbɛyɛ nhwehwɛmu no afa adesua bi a yɛreyɛ de akɔgye PhD abɔdin bi. Ansa na wobɛyɛ w'adwene sɛ wode wo de wo ho bɛhyɛ mu no, ɛho hia sɛ wote deɛ enti a wɔreyɛ saa nhwehwɛmu yi ne deɛ ɛbɛka ho. Mepa wokyɛw sɛ nya berɛ kenkan nsɛm a ɛdidi soɔ yi yie ansa na wayɛ w'adwene sɛ wode wo ho bɛhyɛ mu. Sɛ wopɛ a, wobɛtumi ne nnipa afoforɔ adi ho nkɔmmɔ. Mepa wo kyɛw, sɛ biribi wɔ hɔ a wonte aseɛ a, anaa wohia nkyerɛkyerɛmu bio a, bisa. Meda wo ase sɛ woanyɛ berɛ rekenkean yei.

Deɛ ɛfa nhwehwɛmu no ho

- **Hwan na ɔrebɛyɛ nhwehwɛmu no?**

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University of Manchester, UK

- **Nhwehwɛmu no botaeɛ ne sɛn?**

Nhwehwɛmu no botaeɛ ne sɛ ɛbɛma yahunu sɛdeɛ *chronic pulmonary aspergillosis* tebea teɛ (yareɛ nyaa a ɛnyini yɛ kɛseɛ nkakrankakra sɛi hurututu (lungs), farebae ne *Aspergillus mmoawa,* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) a ɛbɛtumi ayɛ yareɛ bi nkyerɛkyerɛmu a ɛnyɛ nokware anaa TB a ano ayɛ den. Yɛde *tuul*s nketenkete na ɛbɛyɛ.

Yaba wo nkyɛn ɛfiri sɛ wo dɔkota susu sɛ sɛ ɔhwɛ nsɛnkyerɛnee ne wo nnipadua mu nsakraeɛ a, ebia, wowɔ TB, anaa ɛfiri sɛ wofata na wopɛ sɛ wofiri wo pɛ mu de wo mogya ma. Adesua a wayɛ dada kyerɛ sɛ chronic pulmonary aspergillosis (yɛ yareɛ nyaa a ɛyɛ kɛseɛ nkakrankakra sɛi hurututu (lungs), deɛ ɛde ba ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no ntumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) bɛtumi ayɛ nkyerɛkyerɛmu a ɛfa TB ho a ɛnyɛ nokware anaa TB a ano ayɛ den. Adesua yi bɛhwehwɛ ayarefoɔ 180 a wɔsusu sɛ wɔwɔ TB na wɔde wɔn mogya ne ahorɔ nhwɛsoɔ ama na yahwehwɛ *Aspergillus mmoawa* (mmoawa a wɔde *chronic pulmonary aspergillosis* (yareɛ nyaa a ɛyɛ kɛseɛ nkakrankakra sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no ntumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) ba), yɛbɛyɛ antibodi tɛst, mikroskop ne *culture* (sɛ yɛrehyɛ da ayɛn abɔdeɛ nketewa a yɛmfa aniwa nhunu) Yɛbɛtwa wo koko r-ray nso de ahwɛ sɛdeɛ wo hurututu (lungs) no teɛ. Sɛ wɔrehwɛ wo afa TB ho na sɛ ɛdi abosome 6 ne 12 a, wɔbɛyɛ laboretri tɛst nhwehwɛmu no bio. Wɔbɛsan ahwɛ wo a ɛbɛgyina sɛdeɛ wo laboretri tɛst ne x-ray nsunsuanesoɔ no teɛ; wɔbɛtumi ayɛ CT scan ama wo. Adesua no bɛsan nso ahwehwɛ nnipa 90 a wɔnni abakɔsɛm wɔ yareɛ a ɛka nnipadua mu home ne mframa akwantu nhyehyɛe a wɔbɛma mogya na wɔde wɔn atoto nhwehwɛmu no tɛst no ho.

Wode deɛ ɛbɛfiri nhwehwɛmu no aba no bɛto dwa?

Yɛrehwɛ kwan sɛ adesua no bɛkyerɛ sɛdeɛ *chronic pulmonary aspergillosis* (yareɛ nyaa a ɛnyini yɛ kɛseɛ nkakrankakra, sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) taa yɛ ayarefoɔ a yɛsusu sɛ wɔwɔ TB ne wɔn a yɛrema wɔn TB nnuro. Yei bɛkyerɛ adɔkotafoɔ kwan berɛ a wɔrehwɛ TB ayarefoɔ afoforɔ ne adada nyinaa. Yɛde labɔretri ne *imaging* (sɛ yɛrefa ayaresa kwan so de x-ray ne mfidie atwa biribi mfoni) nsunsuanesoɔ no bɛkyerɛ wo. Yɛbɛkyekyɛ nhwehwɛmu no nsɛmboano (data) no mu ayɛ bi adesua tiisiisi a wɔde to dwa wɔ adesuade nwoma (journal) mu ne nhyiamu anaa badwa sɛmina ase.

Hwan na ahwɛ nhwehwɛmu adwuma no mu agye atom?

The University Manchester Kɔmitii a ɛhwɛ Nneyɛe Pa a ɛfa Nhwehwɛmu adwuma ho so (Research Ethics Committee), UK ne Institutional Review Board a ɛwɔ Korle-Bu Teaching Hospital, Ghana ahwɛ adesua yi agye atom.

Hwan na wafa nhwehwɛma adwuma no ho ka?

CARIGEST SA ne The University of Manchester na afa saa adesua yi ho ka.

Me ho a mede bɛhyɛ adesua no mu ne sɛn?

Sɛ mede me ho hyɛ mu a ɛdeɛn nsɛm na wɔbɛbisa me?

Yɛbɛsrɛ ama wayi nsɛm a ɛgu nsɛmmisa a yahyehyɛ a ɛfa wo ho nsɛm nketenkete, adwuma ne apɔmmuden ka ho. Yɛbɛgye wo mogya nhwɛsoɔ kakra na yayɛ wo koko x-ray. Yɛde siringye bɛtwe wo mogya no, na ebia, ɛbɛyɛ wo ya kakra, awɔtre wo honam, anaa mogya kakra bɛba nanso ɛrenkɔba sɛ ɛbɛha w'apɔmuden. Ɔhaw nsunsuanesoɔ bɔne a ɛwɔ x-ray no mu no wɔ fam pa ara. Wo dɔkota bɛhwɛ asɔ ɔhaw biara ano. Sɛ wobɔ wa yi ahorɔ a, yɛde ahorɔ nhwɛsoɔ no fa bi a wode bɛma yɛn wɔ daa daa TB tɛst mu no bɛyɛ nhwenwɛmu no tɛsting. Worentua laboretri ne x-ray tɛst no ho ka. Sɛ yɛfiri wo na yɛhunu sɛ wowɔ *chronic pulmonary aspergillosis* (yareɛ nyaa a ɛnyini yɛ kɛseɛ nkakrankakra, sɛi hurututu (lungs), farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) a anka ɛbɛtumi ahunta a, nanso ayaresa a ɛfata a wobɛnya no bɛyɛ mfasoɔ ama wo.

Wo ho a wode bɛhyɛ mu no mmoro sima 75. Sima 15 na wode bɛyiyi nsɛmmisa no ano na wode sima 60 ama yɛn mogya ne ahorɔ nhwɛsoɔ no, atwa koko x-ray no. Yɛbɛyɛ laboretri nhwehwɛmu no akuo akuo, dapɛn biara baako. Sɛ laboretri nsunsuanesoɔ ne x-ray ho amaneɛbɔ no yɛ krado a, ntɛm pa ara no yɛde bɛma wo ne wo dɔkota. Berɛ a adesua no bɛdi ansa na aba awieyɛ yɛ abosome 12. Sɛ yɛrema wo TB nnuro (na sɛ wobɔ wa yi ahorɔ a), wobɛma mogya ne ahorɔ na watwa koko so x-ray wɔ wo daa daa nsrahwɛ a ɛdi akyire no mu, wɔ abosome 6 ne 12 mu. Berɛ a yɛbɛhia ama nsrahwɛ a ɛdi akyire yɛ dɔnhwere baako. Yɛbɛtumi ayɛ koko so *CT scan* ama wo, na ɛbɛgyina wo laboretri ne x-ray ho amanebɔ no so, ɛne sɛ, x-ray a ɛnyɛ na ɛkyerɛ *chronic pulmonary aspergillosis* (yareɛ nyaa a ɛnyini yɛ kɛseɛ nkakrankakra sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa,* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) ne *Aspergillosis* antibodi tɛst wɔ nsrahwɛ a ɛdi akyire mu (sɛ adi abosome 6 anaa 12). Yɛbɛtumi de nhwɛsoɔ no asie na nhwehwɛmukuo no anaa wɔn a wɔne wɔn yɛ adwuma bom bɛtumi aba wo nkyɛn abɛyɛ adesua foforɔ.

Sɛ woka ntotoho kuo no ho a, yɛde wo mogya 3ml bɛsie de ayɛ nhwehwɛmu tɛst, afei biribiara nni hɔ a ɛka ho bio.

Mɛnya akatua bi afiri me ho a mede bɛhyɛ mu no?

Yɛbɛma wo GHS 30.00 wɔ nsrahwɛ a ɛdi akyire biara de atua wo kaasika ne berɛ a wobɛsɛe no wɔ ayaresabea hɔ no.

Sɛ mempɛ sɛ mede ho hyɛ mu anaa mesesa m'adwene a, ɛdeɛn na ɛbɛsi?

Ɛgyina wo ankasa wo so sɛ wode wo ho bɛhyɛ mu anaasɛ womfa wo ho nhyɛ mu. Sɛ wo kenkan saa nsɛmfua krataa yi wie na wonya wo nsɛmmisa no ho anoyie a yɛbɛma wo kwan na waka sɛ wode wo ho bɛhyɛ mu anaasɛ womfa wo ho nhyɛ mu. Sɛ woyɛ w'adwene sɛ wode wo ho bɛhyɛ mu a, yɛde saa nsɛmfua krataa yi bɛma wo asie na yasrɛ ama wode wo nsa ahyɛ penee krataa bi ase. Sɛ woyɛ w'adwene sɛ wode wo ho bɛhyɛ mu a woda so wɔ ho kwan sɛ wotwe wo ho firi mu berɛ biara a womma nkyɛrɛkyerɛmu biara na ɛremfa ɔhaw mmrɛ wo. Nanso sɛ wɔnya yi din nsɛm firi ho a wɔntumi nyi wo nsɛmboano mfiri adwuma no mu esiane sɛ yɛntumi nhunu wo nsɛmboano potee. Yei nha asɛdeɛ a wowɔ fa wo nsɛmbuano bambɔ ho. Sa woyɛ w'adewene sɛ womfa wo ho nhyɛ mu a, ɛho nhia sɛ woyɛ biribi foforɔ biara bio.

<u>Nsɛmbano ho bambɔ ne kokoam mu nsɛm</u>

Nsɛm bɛn na wobɛgye afa me ho?

Sɛ ɛbɛboa na wode wo ho ahyɛ nhwehwɛmu aduwuma yi mu nti, ɛsɛ sɛ yɛgye nsɛm a ɛbɛma yahyɛ wo nso, a yato din "**nsɛm a ɛhyɛ obi nso**" (personal identifiable information). Ne titire no, yɛbɛhia sɛ yɛbɛgye:

- Din
- Awoda
- Bɔbea
- Adwuma
- Baabi a wote

Mmara bɛn so na woregyina agye saa nsɛm yi?

Yɛgyina nsɛmboa bambɔ mmara a ɛbɔ w'asɛdeɛ ho ban so na yɛregye nsɛm a ɛhyɛ obi nso. Yeinom ka sɛ, ɛsɛ sɛ yɛgina mmara so (botaeɛ pɔtee) a yɛde regye wo nsɛmboa no. Wɔ saa adesua yi ho no, botaeɛ pɔtee ne sɛ "ɛyɛ ɔmanfoɔ yiedie adwuma" ne "kwan bi a ɛho hia na yɛfa so yɛ nhwehwɛmu botaeɛ enti"

Deɛn ne m'asɛdeɛ wɔ nsɛm a wobɛgye afa me ho no?

Nsɛmboa Bambɔ Mmara a ɛfa wo ho nsɛm ho no, ma wo asɛdeɛ pii. ɛbi ne sɛ, wobɛtumi abisa nsɛm a yagye afa wo ho no nhwɛsoɔ (copy).

Sɛ wo pɛ sɛ wohunu biribi bio fa w'asɛdeɛ ahodoɔ ho anaa kwan a yɛfa so de wo ho nsɛm yɛ adwuma sɛdeɛ ɛbɛma yɛadi mmara no so a, yɛpa wo kyɛw, kenkan firi yɛn Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ a ɛka yei ho no.

Me ho a mede bɛhyɛ adesua no mu bɛyɛ kokoam adeɛ na mobɛbɔ me nsɛm a ɛhyɛ me nso no (personal identifiable information) ho ban?

Sɛdeɛ nsɛmboa bambɔ mmara teɛ no, ɛyɛ The University of Manchester na ɛwɔ Nsɛmbano no so Tumi fa saa adwuma yi ho. Yei kyerɛ sɛ ɛyɛ yɛn asɛdeɛ sɛ yɛhwɛ yie sɛ yɛde wo ho nsɛm no bɛsie kokoam na yɛde yɛ adwuma sɛdeɛ yaka akyerɛ wo sɛ yɛde bɛyɛ adwuma no. Yatete nhwehwɛmu adwumayɛfoɔ no nyinaa de wei ato wɔn adwene mu, na kwan a ɛdi soɔ yi so na yɛbɛfa ahwɛ wo nsɛmboa no so:

Yɛde nsɛm a yɛbɛgye afa wo ho nyinaa bɛyɛ adwuma afa saa adesua yi ho. Yɛde nsɛmboano biara bɛsie wɔ pendrive a internet password da so a ɛhyɛ The University of Manchester nsa. Kokoa mu nsɛm biara wɔn a wɔde wɔn ho bɛhyɛ mu no de bɛma no, yɛbɛhwɛ so yie na yabɔ ho ban.

Yɛbɛyi biribiara a ɛbɛma obi ahu wo afiri nsɛmboano no ho, ɛbi ne sɛ, yɛbɛyi wo din ne nsɛm foforɔ biara obi bɛfa so ahunu wo afiri hɔ de ID nɔma a obiara nnim deɛ ɛbɛfiri aba ahyɛ ananmu, na yɛde safoa a yɛde bue ano no asie wɔ Suapɔn no server a bambɔ da ho na nhwehwɛmu adwumayɛfoɔ Yɛde nhwehwɛmu a ɛfa nsɛmboano no bɛsie mfie num. Yɛde 'nsemboano kuo' (data set) no bɛtwo dwa na yafa The University of Manchester ahyehyɛdeɛ adekorabea so akyekyɛ mu.

Sɛ wogye tom sɛ wode wo ho bɛhyɛ nhwehwɛmu adesua bi mu a, yɛbɛtumi de wo ho nsɛm no ama nhwehwɛmufoɔ a wɔreyɛ nhwehwɛnu adesua foforɔ wɔ saa dwumabea ha. Daakye nhwehwɛmu no tebea ne saa nhwehwɛmu adwuma yi bɛsɛ na deɛ ɛbɛyɛ ne sɛ, ɛde nsɛmboano dada no nko ara na ɛbɛyɛ adwuma. Saa dwumabea yi ne nhwehwɛmufoɔ bɛfa The University of Manchester Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ so (Research Privacy Notice) na wɔde wo ho nsɛm bɛyɛ adwuma. Saa nsɛm yi nna wo adi na yɛmfa nka nsɛm foforɔ ho wɔ kwan biara so a, ɛbɛma obi ahunu sɛ ɛyɛ wo. Wɔde nsɛm no bɛyɛ adwuma nkutoo a ne botaeɛ sɛ ɛbɛboa asi adesua dada bi so dua, na wɔntumi nnyina so mfrɛ wo wɔ asɛm biara ho. Wɔntumi nnyina so mfa adwene wɔ daakye som bi a ɛbɛwɔ hɔ ama wo ho.

Berɛ a yɛbɛtumi ada kokoa mu nsɛm adi

Sɛ ɛkɔba sɛ adesua no mu no, yɛhyia ɔhaw fa wo bambɔ ho anaa afoforɔ bambɔ ho a, yɛbɛbɔ wo dɔkota, ahwɛfokuo anaa abusuafoɔ amaneɛ. Bio, sɛ ɛho hia a, yɛbɛma wo ne wo dɔkota ahunu laboretri nhwehwɛmu nsunsuanesoɔ afa kwan pa a wɔbɛfa so ahwɛ so.

Yɛpa wo kyɛw, hyɛ no nso sɛ ankorɛankorɛ bi firi The University of Manchester anaa nhyehyɛe sodifoɔ bɛtumi ahia sɛ wɔhwɛ nsɛmboano a yagyegye wɔ saa adesua yi ho ama yayɛ adwuma no sɛdeɛ yahyehyɛ ato hɔ no. Yei bɛtumi afa ho sɛ wɔrehwɛ nsɛmboano a ɛda nso. Ankorɛankorɛ biara a wɔka adesua no nhwehwɛmu a ɛfa nokwaredie ne ahwɛfoɔ bɛyɛ wɔn adwuma pɛpɛɛpɛ afa kokoa mu nsɛm a ɛfa wo ho sɛ obi a wode wo ho abɛhyɛ nhwehwɛmu mu.

Na sɛ mewɔ ɔhaw bi ɛ?

Nkyerɛkyerɛmu a ɛfa baabi a wode wo haw bɛkɔ.

Sɛ wonya ɔhaw bi na wopɛ sɛ nhwehwɛmu adwumakuo no aso te a, yɛpa wo kyɛw, kɔhu **Dr Jane Afriyie-Mensah (**jafriyiemensah@yahoo.com**, +233 20 630 1108)**

Sɛ wopɛ sɛ wofa mmara kwan so de wo haw no kɔma obi a ɔnka nhwehwɛmu adwumakuo no ho anaasɛ w'ani nnye mmuaeɛ a wanya afiri nhwenhwɛmufoɔ ho berɛ a ɛdi kan no deɛ a, yɛpa wokyɛw, twerɛ kɔ:

The Research Ethics Manager, Research Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL, fa email so: research.complaints@manchester.ac.uk ana frɛ tetefon yi so: 0161 275 2674.

Anaa

Research and Development Unit, Medical Directorate, Central Admin Block, Korle-Bu Teaching Hospital, P. O. Box KB 77, Accra, Ghana, e-mail: rdo@kbth.gov.gh or Phone: + 233-302739510.

Sɛ wopɛ sɛ wo ne yɛn kasa fa asɛdeɛ a wowɔ fa wo nsɛmboano bambɔ ho a, yɛpa wo kyɛw fafa email yi so: dataprotection@manchester.ac.uk anaa twerɛ kɔ The Information Governance Office, Christie Building, The University of Manchester, Oxford Road, M13 9PL wɔ Suapɔn no mu, na yɛbɛboa wo de wo afa kwan a wobɛfa so na wo nsa aka w'asɛdeɛ.

Wo san wɔ ho kwan sɛ wode wo soboɔ bi kɔ Information Commissioner's Office fa ɔhaw a ɛfa wo ho nsɛm a ɛbɛma obi ahunu wo wɔ tetefon yi so: 0303 123 1113.

Akyirikwan ho nsɛm

Sɛ wowɔ nsɛmmisa biara fa adesua no anaa w'ani gye ho sɛ wode wo ho hyɛ mu a, yɛpa wo kyɛw, kɔhunu nhwehwɛmu adwumayɛni BRIGHT OCANSEY (bright.ocansey@postgrad.manchester.ac.uk, +233 54 279 0540).

Study 4

Yareɛ nhwehwɛmu a ɛfa *invasive aspergillosis* ho (yareɛ nyaa a sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) wɔ nnipa bi a wowɔ mogya cancer wɔ Korle-bu Teaching Hospital wɔ Ghana

Krataasini a yɛtwerɛ nnipa a wɔde wɔn ho hyɛ nhwehwɛmu bi mu ho nsɛm agu so

Yɛreto nsa afrɛ wo sɛ bɛka adesua nhwehwɛmu bi a ɛbɛma yahunu sɛdeɛ *invasive aspergillosis* (yareɛ nyaa a sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ). Yɛde *tuuls* nketenkete na ɛbɛyɛ nhwehwɛmu no afa adesua bi a yɛreyɛ de akɔgye PhD abɔdin bi. Ansa na wobɛyɛ w'adwene sɛ wode wo de wo ho bɛhyɛ mu no, ɛho hia sɛ wote deɛ enti a wɔreyɛ saa nhwehwɛmu yi ne deɛ ɛbɛka ho. Mepa wokyɛw sɛ nya berɛ kenkan nsɛm a ɛdidi soɔ yi yie ansa na wayɛ w'adwene sɛ wode wo ho bɛhyɛ mu. Sɛ wopɛ a, wobɛtumi ne nnipa afoforɔ adi ho nkɔmmɔ. Mepa wo kyɛw, sɛ biribi wɔ hɔ a wonte aseɛ a, anaa wohia nkyerɛkyerɛmu bio a, bisa. Meda wo ase sɛ woanyɛ berɛ rekenkean yei.

Deɛ ɛfa nhwehwɛmu no ho

- **Hwan na ɔrebɛyɛ nhwehwɛmu no?**

Bright Ocansey (bright.ocansey@postgrad.manchester.ac.uk)

Division of Evolution, Infection and Genomics

School of Biological Sciences

Faculty of Biology, Medicine and Health

University of Manchester, UK

- **Nhwehwɛmu no botaeɛ ne sɛn?**

Nhwehwɛmu no botaeɛ ne sɛ ɛbɛma yahunu sɛdeɛ *invasive aspergillosis* tebea teɛ (yareɛ nyaa a sɛi hurututu (lungs), farebae ne *Aspergillus mmoawa,* ewɔ omɔ wɔwɔ mogya cancer wɔ Ghana. Yɛde *tuuls* nketenkete na ɛbɛyɛ.

Yaba wo nkyɛn ɛfiri sɛ wowɔ mogya cancer, anaa ɛfiri sɛ wofata na wopɛ sɛ wofiri wo pɛ mu de wo mogya ma. Adesua a wayɛ dada kyerɛ sɛ invasive aspergillosis, deɛ ɛde ba ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no ntumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) bɛtumi ayɛ nkyerɛkyerɛmu a ɛfa mogya cancer ho, yɛbɛyɛ antigen tɛst, mikroskop ne *culture* (sɛ yɛrehyɛ da ayɛn abɔdeɛ nketewa a yɛmfa aniwa nhunu) Yɛbɛtwa wo koko CT scan nso de ahwɛ sɛdeɛ wo hurututu (lungs) no teɛ.

Wode deɛ ɛbɛfiri nhwehwɛmu no aba no bɛto dwa?

Yɛrehwɛ kwan sɛ adesua no bɛkyerɛ sɛdeɛ *invasive aspergillosis* (yareɛ nyaa a ɛnyini yɛ kɛseɛ nkakrankakra, sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne *wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) taa yɛ ayarefoɔ* wohɔ mogya cancer. Yɛde labɔretri ne *imaging* (sɛ yɛrefa ayaresa kwan so de CT scan ne mfidie atwa biribi mfoni) nsunsuanesoɔ no bɛkyerɛ wo. Yɛbɛkyekyɛ nhwehwɛmu no nsɛmboano (data) no mu ayɛ bi adesua tiisiisi a wɔde to dwa wɔ adesuade nwoma (journal) mu ne nhyiamu anaa badwa sɛmina ase.

Hwan na ahwɛ nhwehwɛmu adwuma no mu agye atom?

The University of Manchester Kɔmitii a ɛhwɛ Nneyɛe Pa a ɛfa Nhwehwɛmu adwuma ho so (Research Ethics Committee), UK ne Institutional Review Board a ɛwɔ Korle-Bu Teaching Hospital, Ghana ahwɛ adesua yi agye atom.

Hwan na wafa nhwehwɛma adwuma no ho ka?

CARIGEST SA ne The University of Manchester na afa saa adesua yi ho ka.

Me ho a mede bɛhyɛ adesua no mu ne sɛn? Sɛ mede me ho hyɛ mu a ɛdeɛn nsɛm na wɔbɛbisa me?

Yɛbɛsrɛ ama wayi nsɛm a ɛgu nsɛmmisa a yahyehyɛ a ɛfa wo ho nsɛm nketenkete, adwuma ne apɔmmuden ka ho. Yɛbɛgye wo mogya nhwɛsoɔ kakra na yayɛ wo koko CT scan. Yɛde siringye bɛtwe wo mogya no, na ebia, ɛbɛyɛ wo ya kakra, awɔtre wo honam, anaa mogya kakra bɛba nanso ɛrenkɔba sɛ ɛbɛha w'apɔmuden. Ɔhaw nsunsuanesoɔ bɔne a ɛwɔ CT scan no mu no wɔ fam pa ara. Wo dɔkota bɛhwɛ asɔ ɔhaw biara ano. Worentua laboretri ne CT scan tɛst no ho ka. Sɛ yɛfiri wo na yɛhunu sɛ wowɔ *invasive aspergillosis* a anka ɛbɛtumi ahunta a, nanso ayaresa a ɛfata a wobɛnya no bɛyɛ mfasoɔ ama wo.

Wo ho a wode bɛhyɛ mu no mmoro sima 60 minutes. Sima 15 na wode bɛyiyi nsɛmmisa no ano na wode sima 60 ama yɛn mogya ne ahorɔ nhwɛsoɔ no, atwa koko CT scan no. Yɛbɛyɛ laboretri nhwehwɛmu no akuo akuo, dapɛn biara baako. Sɛ laboretri nsunsuanesoɔ ne CT scan ho amaneɛbɔ no yɛ krado a, ntɛm pa ara no yɛde bɛma wo ne wo dɔkota. **Yɛbɛtumi de nhwɛsoɔ no asie na nhwehwɛmukuo no anaa wɔn a wɔne wɔn yɛ adwuma bom bɛtumi aba wo nkyɛn abɛyɛ adesua foforɔ.**

Sɛ woka ntotoho kuo no ho a, yɛde wo mogya 3ml bɛsie de ayɛ nhwehwɛmu tɛst, afei biribiara nni hɔ a ɛka ho bio.

Mɛnya akatua bi afiri me ho a mede bɛhyɛ mu no?

Yɛbɛma wo GHS 45.00 (£5.00) wɔ nsrahwɛ a ɛdi akyire biara de atua wo kaasika ne berɛ a wobɛsɛe no wɔ ayaresabea hɔ no.

Sɛ mempɛ sɛ mede ho hyɛ mu anaa mesesa m'adwene a, ɛdeɛn na ɛbɛsi?

Ɛgyina wo ankasa wo so sɛ wode wo ho bɛhyɛ mu anaasɛ womfa wo ho nhyɛ mu. Sɛ wo kenkan saa nsɛmfua krataa yi wie na wonya wo nsɛmmisa no ho anoyie a yɛbɛma wo kwan na waka sɛ wode wo ho bɛhyɛ mu anaasɛ womfa wo ho nhyɛ mu. Sɛ woyɛ w'adwene sɛ wode wo ho bɛhyɛ mu a, yɛde saa nsɛmfua krataa yi bɛma wo asie na yasrɛ ama wode wo nsa ahyɛ penee krataa bi ase. Sɛ woyɛ w'adwene sɛ wode wo ho bɛhyɛ mu a woda so wɔ ho kwan sɛ wotwe wo ho firi mu berɛ biara a womma nkyɛrɛkyerɛmu biara na ɛremfa ɔhaw mmrɛ wo. Nanso sɛ wɔnya yi din nsɛm firi ho a wɔntumi nyi wo nsɛmboano mfiri adwuma no mu esiane sɛ yɛntumi nhunu wo nsɛmboano potee. Yei nha asɛdeɛ a wowɔ fa wo nsɛmbuano bambɔ ho. Sa woyɛ w'adewene sɛ womfa wo ho nhyɛ mu a, ɛho nhia sɛ woyɛ biribi foforɔ biara bio.

Nsɛmbano ho bambɔ ne kokoam mu nsɛm. Nsɛm bɛn na wobɛgye afa me ho?

Sɛ ɛbɛboa na wode wo ho ahyɛ nhwehwɛmu aduwuma yi mu nti, ɛsɛ sɛ yɛgye nsɛm a ɛbɛma yahyɛ wo nso, a yato din "nsɛm a ɛhyɛ obi nso" (personal identifiable information). Ne titire no, yɛbɛhia sɛ yɛbɛgye:

- Din
- Awoda
- Bɔbea
- Adwuma
- Baabi a wote

Mmara bɛn so na woregyina agye saa nsɛm yi?

Yɛgyina nsɛmboa bambɔ mmara a ɛbɔ w'asɛdeɛ ho ban so na yɛregye nsɛm a ɛhyɛ obi nso. Yeinom ka sɛ, ɛsɛ sɛ yɛgina mmara so (botaeɛ pɔtee) a yɛde regye wo nsɛmboa no. Wɔ saa adesua yi ho no, botaeɛ pɔtee ne sɛ "ɛyɛ ɔmanfoɔ yiedie adwuma" ne "kwan bi a ɛho hia na yɛfa so yɛ nhwehwɛmu botaeɛ enti"

Deɛn ne m'asɛdeɛ wɔ nsɛm a wobɛgye afa me ho no?

Nsɛmboa Bambɔ Mmara a ɛfa wo ho nsɛm ho no, ma wo asɛdeɛ pii. ɛbi ne sɛ, wobɛtumi abisa nsɛm a yagye afa wo ho no nhwɛsoɔ (copy).

Sɛ wo pɛ sɛ wohunu biribi bio fa w'asɛdeɛ ahodoɔ ho anaa kwan a yɛfa so de wo wo ho nsɛm yɛ adwuma sɛdeɛ ɛbɛma yɛadi mmara no so a, yɛpa wo kyɛw, kenkan firi yɛn Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ a ɛka yei ho no.

Me ho a mede bɛhyɛ adesua no mu bɛyɛ kokoam adeɛ na mobɛbɔ me nsɛm a ɛhyɛ me nso no (personal identSFIable information) ho ban?

Sɛdeɛ nsɛmboa bambɔ mmara teɛ no, ɛyɛ The University of Manchester na ɛwɔ Nsɛmbano no so Tumi fa saa adwuma yi ho. Yei kyerɛ sɛ ɛyɛ yɛn asɛdeɛ sɛ yɛhwɛ yie sɛ yɛde wo ho nsɛm no bɛsie kokoam na yɛde yɛ adwuma sɛdeɛ yaka akyerɛ wo sɛ yɛde bɛyɛ adwuma no. Yatete nhwehwɛmu adwumayɛfoɔ no nyinaa de wei ato wɔn adwene mu, na kwan a ɛdi soɔ yi so na yɛbɛfa ahwɛ wo nsɛmboa no so:

Yɛde nsɛm a yɛbɛgye afa wo ho nyinaa bɛyɛ adwuma afa saa adesua yi ho. Yɛde nsɛmboano biara bɛsie wɔ pendrive a internet password da so a ɛhyɛ The University of Manchester nsa. Kokoa mu nsɛm biara wɔn a wɔde wɔn ho bɛhyɛ mu no de bɛma no, yɛbɛhwɛ so yie na yabɔ ho ban.

Yɛbɛyi biribiara a ɛbɛma obi ahu wo afiri nsɛmboano no ho, ɛbi ne sɛ, yɛbɛyi wo din ne nsɛm foforɔ biara obi bɛfa so ahunu wo afiri hɔ de ID nɔma a obiara nnim deɛ ɛbɛfiri aba ahyɛ ananmu, na yɛde safoa a yɛde bue ano no asie wɔ Suapɔn no server a bambɔ da ho na nhwehwɛmu adwumayɛfoɔ Yɛde nhwehwɛmu a ɛfa nsɛmboano no bɛsie mfie num. Yɛde 'nsemboano kuo' (data set) no bɛtwo dwa na yafa The University of Manchester ahyehyɛdeɛ adekorabea so akyekyɛ mu.

Sɛ wogye tom sɛ wode wo ho bɛhyɛ nhwehwɛmu adesua bi mu a, yɛbɛtumi de wo ho nsɛm no ama nhwehwɛmufoɔ a wɔreyɛ nhwehwɛnu adesua foforɔ wɔ saa dwumabea ha. Daakye nhwehwɛmu no tebea ne saa nhwehwɛmu adwuma yi bɛsɛ na deɛ ɛbɛyɛ ne sɛ, ɛde nsɛmboano dada no nko ara na ɛbɛyɛ adwuma. Saa dwumabea yi ne nhwehwɛmufoɔ bɛfa The University of Manchester Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ so (Research Privacy Notice) na wɔde wo ho nsɛm bɛyɛ adwuma. Saa nsɛm yi nna wo adi na yɛmfa nka nsɛm foforɔ ho wɔ kwan biara so a, ɛbɛma obi

ahunu sɛ ɛyɛ wo. Wɔde nsɛm no bɛyɛ adwuma nkutoo a ne botaeɛ sɛ ɛbɛboa asi adesua dada bi so dua, na wɔntumi nnyina so mfrɛ wo wɔ asɛm biara ho. Wɔntumi nnyina so mfa adwene wɔ daakye som bi a ɛbɛwɔ hɔ ama wo ho.

Berɛ a yɛbɛtumi ada kokoa mu nsɛm adi

Sɛ ɛkɔba sɛ adesua no mu no, yɛhyia ɔhaw fa wo bambɔ ho anaa afoforɔ bambɔ ho a, yɛbɛbɔ wo dɔkota, ahwɛfokuo anaa abusuafoɔ amaneɛ. Bio, sɛ ɛho hia a, yɛbɛma wo ne wo dɔkota ahunu laboretri nhwehwɛmu nsunsuanesoɔ afa kwan pa a wɔbɛfa so ahwɛ so.

Yɛpa wo kyɛw, hyɛ no nso sɛ ankorɛankorɛ bi firi The University of Manchester anaa nhyehyɛe sodifoɔ bɛtumi ahia sɛ wɔhwɛ nsɛmboano a yagyegye wɔ saa adesua yi ho ama yayɛ adwuma no sɛdeɛ yahyehyɛ ato hɔ no. Yei bɛtumi afa ho sɛ wɔrehwɛ nsɛmboano a ɛda nso. Ankorɛankorɛ biara a wɔka adesua no nhwehwɛmu a ɛfa nokwaredie ne ahwɛfoɔ bɛyɛ wɔn adwuma pɛpɛɛpɛ afa kokoa mu nsɛm a ɛfa wo ho sɛ obi a wode wo ho abɛhyɛ nhwehwɛmu mu.

<u>Na sɛ mewɔ ɔhaw bi ɛ?</u>

Nkyerɛkyerɛmu a ɛfa baabi a wode wo haw bɛkɔ.

Sɛ wonya ɔhaw bi na wopɛ sɛ nhwehwɛmu adwumakuo no aso te a, yɛpa wo kyɛw, kɔhu: Opanyin ewɔ Department of Haematology-Dr. Yvonne Dei-Adomako (yadei-adomakoh.ug.edu.gh, +233 243550980)

Sɛ wopɛ sɛ wofa mmara kwan so de wo haw no kɔma obi a ɔnka nhwehwɛmu adwumakuo no ho anaasɛ w'ani nnye mmuaeɛ a wanya afiri nhwenhwɛmufoɔ ho berɛ a ɛdi kan no deɛ a, yɛpa wokyɛw, twerɛ kɔ:

The Research Ethics Manager, Research Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL, fa email so: research.complaints@manchester.ac.uk ana frɛ tetefon yi so: 0161 275 2674.

Anaa

Research and Development Unit, Medical Directorate, Central Admin Block, Korle-Bu Teaching Hospital, P. O. Box KB 77, Accra, Ghana, e-mail: rdo@kbth.gov.gh or Phone: + 233-302739510.

Sɛ wopɛ sɛ wo ne yɛn kasa fa asɛdeɛ a wowɔ fa wo nsɛmboano bambɔ ho a, yɛpa wo kyɛw fafa email yi so: dataprotection@manchester.ac.uk anaa twerɛ kɔ The Information Governance Office,

Christie Building, The University of Manchester, Oxford Road, M13 9PL wɔ Suapɔn no mu, na yɛbɛboa wo de wo afa kwan a wobɛfa so na wo nsa aka w'asɛdeɛ.

Wo san wɔ ho kwan sɛ wode wo soboɔ bi kɔ Information Commissioner's Office fa ɔhaw a ɛfa wo ho nsɛm a ɛbɛma obi ahunu wo wɔ tetefon yi so: 0303 123 1113.

Akyirikwan ho nsɛm

Sɛ wowɔ nsɛmmisa biara fa adesua no anaa w'ani gye ho sɛ wode wo ho hyɛ mu a, yɛpa wo kyɛw, kɔhunu nhwehwɛmu adwumayɛni BRIGHT OCANSEY (bright.ocansey@postgrad.manchester.ac.uk, +233 54 279 0540).

Appendix 7: Consent forms - Local dialect (Twi)

Study 1

Nhwehwɛmu a yɛde non-culture-based assays yɛ fa *invasive fungal diseases* (nyarɛwa a ano yɛ den a yeast na ɛde ba) ho wɔ nnipa a wɔwɔ HIV yareɛ na wɔrenom nnuro a ɛbɛbrɛ HIV mmoawa no ase (ART)

Penee Krataa (Consent Form)

Sɛ w'ani gye sɛ wode wo ho hyɛ mu a yɛpa wo kyɛw fa nsɛm hyehyɛ pene krataa ɛwɔ aseɛ hɔ no mu na fa wo nsa hyɛ aseɛ.

	Nnwumadie	Nsaanodin
1	Meka si so dua sɛ makenkan krataa sini a nsɛm a yatwerɛ agu so no fa adesua a yaka ho asɛm dada no ka ho, na manya kwan ahwehwɛ nsɛm no mu, abisabisa nsɛm anya ho anoyie a ɛtɔ m'asom.	
2	Mete aseɛ sɛ me ho a mede hyɛ adesua no mu no firi me pɛ mu, na mewɔ ho kwan sɛ metwe me ho firi mu a memma nkyerɛkyerɛmu na ɛnha me nso. Mete aseɛ sɛ, sɛ wɔnya yi me din ne nsɛm a ɛbɛma obi ahunu me firi me nsɛmboano no ho na ɛkɔka nsɛmboano akuo no ho pɛ a, wɔntumi nyi mfiri adwuma no mu bio Yei nti mepene so sɛ mede me ho hyɛ mu.	
3	Mepene so sɛ wɔka me ho a mede bɛhyɛ adesua yi mu no kyerɛ me dɔkota.	
4	Mepene so sɛ wɔbɛka akyerɛ me dɔkota sɛ wɔntwe me mogya ne me dwonsɔ nhwɛsoɔ a ne botaeɛ ne sɛ wɔde bɛyɛ nwhehwɛmu no sɛdeɛ wakyerɛkyerɛ mu akyerɛ me no. Mete aseɛ sɛ nhwehwɛmu no wɔde me nhwɛsoɔ bɛyɛ tɛst apɛ antigyen (aboa bɔne bi a ɛkɔ mogya mu kɔdi bɔne	
5	Mete aseɛ sɛ wɔn a wɔde wɔn sika asɔ saa aedusa yi asene bɛtumi de me mogya ne me dwonsɔ nhwɛsoɔ no ama nhwehwɛmufoɔ afoforɔ ayɛ adeusa daakye na nhwhwɛmufoɔ a wɔwɔ amanɔne bɛtumi aka ho. Mema ho kwan sɛ saa nnipa	

	ankorɛankorɛ yi nsa bɛtumi aka me nhwɛsoɔ no, (na ɛnyɛ nsɛm a ɛda me adi biara). Mede me nhwɛsoɔ ma sɛ akyɛdeɛ.	
6	Mete aseɛ sɛ nsɛmboano a wɔgye no adesua berɛ mu no ankorɛankorɛ bi firi The University of Manchester anaa nhyehyɛe sodifoɔ, bɛtumi ahwehwɛ mu anaa, berɛ a me ho hia sɛ mede hyɛ saa nhwehwɛmu yi mu no. Mema saa ankorɛankorɛ yi ho kwan sɛ wɔtumi fa me nsɛmboano no.	
7	Mepene so sɛ nsɛmboano biara wayi din ne adeɛ a ɛbɛma obi ho ada adi afiri ho no, wɔbɛtumi ne nwhwhwɛmufoɔ a wɔwɔ adwumakuo afoforɔ mu akyɛ.	
8	Mepene so sɛ nsɛmboano biara a wɔbɛgye no wɔbɛtumi de ato dwa a wɔmmɔ din biara wɔ adesua tiisisi, adesua nwoma anaa adesuade nwoma mu.	
9	Mepene so sɛ nhwehwvmufoɔ a wɔwɔ adwumakuo afoforɔ biara mu bɛtumi afrɛ me daakye wɔ nhwehwɛmu adwuma afoforɔ ho.	
10	Mepene so sɛ nhwehwɛmufoɔ no bɛtumi de me m'akyiri kwan ho nsɛm asie sɛdeɛ wɔbɛtumi de nsunsuanesoɔ a ɛbɛfiri saa adesua yi mu abɔ no tɔfa ama me.	
11	Mepene so sɛ nhwehwɛmufoɔ no bɛtumi aka tɛst no nsunsuanesoɔ akyerɛ me dɔkota.	
12	Mepene so de me ho hyɛ saa adesua yi mu.	

Nsɛmboano ho bambɔ

Yɛbɛfa nsɛmboano ho bambɔ mmara a yakyerɛkyerɛ mu wɔ krataasini a yɛtwerɛ nnipa a wɔde wɔn ho bɛhyɛ nhwehwɛmu ho nsɛm agu so no mu no ne Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ so ayɛ adwuma

_____ _____ _____

Deɛ ɔde ne ho ahyɛ mu no din Nsaanodin Deeti

_____	_____	_____
Nnipa a ɔregye penee no din	Nsaanodin	Deeti

(Yɛde penee krataa no nhwɛsoɔ baako bɛma deɛ ɔde ne ho hyɛ adesua no mu no, nhwehwɛmukuo no (deɛ ɛdi kan) ne ahwɛfokuo)

Study 2 and 3

Ayaresa nhwehwɛmu a ɛfa *chronic pulmonary aspergillosis* (yareɛ nyaa a ɛnyini yɛ kɛseɛ nkakrankakra sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) wɔ nnipa bi a wɔwɔ Ghana na yɛsusu sɛ wɔbɔ Nasamanwa (TB)

Penee Krataa (Consent Form)

Sɛ w'ani gye sɛ wode wo ho hyɛ mu a yɛpa wo kyɛw fa nsɛm hyehyɛ pene krataa ɛwɔ aseɛ hɔ no mu na fa wo nsa hyɛ aseɛ.

	Nnwumadie	Nsaanodin
1	Meka si so dua sɛ makenkan krataa sini a nsɛm a yatwerɛ agu so no fa adesua a yaka ho asɛm dada no ka ho, na manya kwan ahwehwɛ nsɛm no mu, abisabisa nsɛm anya ho anoyie a ɛtɔ m'asom.	
2	Mete aseɛ sɛ me ho a mede hyɛ adesua no mu no firi me pɛ mu, na mewɔ ho kwan sɛ metwe me ho firi mu a memma nkyerɛkyerɛmu na ɛnha me nso. Mete aseɛ sɛ, sɛ wɔnya yi me din ne nsɛm a ɛbɛma obi ahunu me firi me nsɛmboano no ho na ɛkɔka nsɛmboano akuo no ho pɛ a, wɔntumi nyi mfiri adwuma no mu bio Yei nti mepene so sɛ mede me ho hyɛ mu.	
3	Mepene so sɛ wɔka me ho a mede bɛhyɛ adesua yi mu no kyerɛ me dɔkota.	
4	Mepene so sɛ mede me mogya ne me ahorɔ nwhesoɔ a ne botaeɛ ne sɛ wɔde bɛyɛ nwhehwɛmu no sɛdeɛ wakyerɛkyerɛ mu akyerɛ me no. Mete aseɛ sɛ wɔbɛyɛ mpɛnsɛnmpɛsɛnmu wɔ me nhwɛsoɔ no ho ahwehwɛ fungi mmoawa. Mesan pene so sɛ mɛyɛ koko-x-ray na sɛ ɛho hia, *CT scan*.	
5	Mete aseɛ sɛ wɔn a wɔde wɔn sika asɔ saa aedusa yi asene bɛtumi de me mogya ne me ahorɔ nhwɛsoɔ no ama nhwehwɛmufoɔ afoforɔ ayɛ adeusa daakye na nhwhwɛmufoɔ a wɔwɔ amanɔne bɛtumi aka ho. Mema ho kwan sɛ saa nnipa	

	ankorɛankorɛ yi nsa bɛtumi aka me nhwɛsoɔ no, (na ɛnyɛ nsɛm a ɛda me adi biara). Mede me nhwɛsoɔ ma sɛ akyɛdeɛ.	
6	Mete aseɛ sɛ nsɛmboano a wɔgye no adesua berɛ mu no ankorɛankorɛ bi firi The University of Manchester anaa nhyehyɛe sodifoɔ, bɛtumi ahwehwɛ mu anaa, berɛ a me ho hia sɛ mede hyɛ saa nhwehwɛmu yi mu no. Mema saa ankorɛankorɛ yi ho kwan sɛ wɔtumi fa me nsɛmboano no.	
7	Mepene so sɛ nsɛmboano biara wayi din ne adeɛ a ɛbɛma obi ho ada adi afiri ho no, wɔbɛtumi ne nwhwhwɛmufoɔ a wɔwɔ adwumakuo afoforɔ mu akyɛ.	
8	Mepene so sɛ nsɛmboano biara a wɔbɛgye no wɔbɛtumi de ato dwa a wɔmmɔ din biara wɔ adesua tiisisi, adesua nwoma anaa adesuade nwoma mu.	
9	Mepene so sɛ nhwehwvmufoɔ a wɔwɔ adwumakuo afoforɔ biara mu bɛtumi afrɛ me daakye wɔ nhwehwɛmu adwuma afoforɔ ho.	
10	Mepene so sɛ nhwehwɛmufoɔ no bɛtumi de me m'akyiri kwan ho nsɛm asie sɛdeɛ wɔbɛtumi de nsunsuanesoɔ a ɛbɛfiri saa adesua yi mu abɔ no tɔfa ama me.	
11	Mepene so sɛ nhwehwɛmufoɔ no bɛtumi aka tɛst no nsunsuanesoɔ akyerɛ me dɔkota.	
12	Mepene so de me ho hyɛ saa adesua yi mu.	

Nsɛmboano ho bambɔ

Yɛbɛfa nsɛmboano ho bambɔ mmara a yakyerɛkyerɛ mu wɔ krataasini a yɛtwerɛ nnipa a wɔde wɔn ho bɛhyɛ nhwehwɛmu ho nsɛm agu so no mu no ne Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ so ayɛ adwuma

_____ _____ _____

Deɛ ɔde ne ho ahyɛ mu no din Nsaanodin Deeti

_____	_____	_____
Nnipa a ɔregye penee no din	Nsaanodin	Deeti

(Yɛde penee krataa no nhwɛsoɔ baako bɛma deɛ ɔde ne ho hyɛ adesua no mu no, nhwehwɛmukuo no (deɛ ɛdi kan) ne ahwɛfokuo)

Study 4

Yareɛ nhwehwɛmu a ɛfa *invasive aspergillosis* ho (yareɛ nyaa a sɛi hurututu (lungs), ne farebae ne *Aspergillus mmoawa* na ɛyɛ ayarefoɔ a wɔn nnipadua no tumi wo nkwaadɔm bi a ɛko tia nyarewa ne wɔn a wɔn nkwaadɔm no ayɛ mmerɛ) wɔ nnipa bi a wowɔ mogya cancer wɔ Korle-bu Teaching Hospital wɔ Ghana

Penee Krataa (Consent Form)

Sɛ w'ani gye sɛ wode wo ho hyɛ mu a yɛpa wo kyɛw fa nsɛm hyehyɛ pene krataa ɛwɔ aseɛ hɔ no mu na fa wo nsa hyɛ aseɛ.

	Nnwumadie	Nsaanodin
1	Meka si so dua sɛ makenkan krataa sini a nsɛm a yatwerɛ agu so no fa adesua a yaka ho asɛm dada no ka ho, na manya kwan ahwehwɛ nsɛm no mu, abisabisa nsɛm anya ho anoyie a ɛtɔ m'asom.	
2	Mete aseɛ sɛ me ho a mede hyɛ adesua no mu no firi me pɛ mu, na mewɔ ho kwan sɛ metwe me ho firi mu a memma nkyerɛkyerɛmu na ɛnha me nso. Mete aseɛ sɛ, sɛ wɔnya yi me din ne nsɛm a ɛbɛma obi ahunu me firi me nsɛmboano no ho na ɛkɔka nsɛmboano akuo no ho pɛ a, wɔntumi nyi mfiri adwuma no mu bio Yei nti mepene so sɛ mede me ho hyɛ mu.	
3	Mepene so sɛ wɔka me ho a mede bɛhyɛ adesua yi mu no kyerɛ me dɔkota.	
4	Mepene so sɛ mede me mogya ne me ahorɔ nwhesoɔ a ne botaeɛ ne sɛ wɔde bɛyɛ nwhehwɛmu no sɛdeɛ wakyerɛkyerɛ mu akyerɛ me no. Mete aseɛ sɛ wɔbɛyɛ mpɛnsɛnmpɛsɛnmu wɔ me nhwɛsoɔ no ho ahwehwɛ fungi mmoawa. Mesan pene so sɛ mɛyɛ koko-*CT scan*.	
5	Mete aseɛ sɛ wɔn a wɔde wɔn sika asɔ saa aedusa yi asene bɛtumi de me mogya ne me ahorɔ nhwɛsoɔ no ama nhwehwɛmufoɔ afoforɔ ayɛ adeusa daakye na nhwhwɛmufoɔ a wɔwɔ amanɔne bɛtumi aka ho. Mema ho kwan sɛ saa nnipa ankorɛankorɛ yi nsa bɛtumi aka me nhwɛsoɔ no, (na ɛnyɛ nsɛm a ɛda me adi biara). Mede me nhwɛsoɔ ma sɛ akyɛdeɛ.	
6	Mete aseɛ sɛ nsɛmboano a wɔgye no adesua berɛ mu no ankorɛankorɛ bi firi The University of Manchester anaa nhyehyɛe sodifoɔ, bɛtumi ahwehwɛ mu anaa, berɛ a me ho hia sɛ mede hyɛ saa nhwehwɛmu yi mu no. Mema saa ankorɛankorɛ yi ho kwan sɛ wɔtumi fa me nsɛmboano no.	
7	Mepene so sɛ nsɛmboano biara wayi din ne adeɛ a ɛbɛma obi ho ada adi afiri ho no, wɔbɛtumi ne nwhwhwɛmufoɔ a wɔwɔ adwumakuo afoforɔ mu akyɛ.	
8	Mepene so sɛ nsɛmboano biara a wɔbɛgye no wɔbɛtumi de ato dwa a wɔmmɔ din biara wɔ adesua tiisisi, adesua nwoma anaa adesuade nwoma mu.	

9	Mepene so sɛ nhwehwvmufoɔ a wɔwɔ adwumakuo afoforɔ biara mu bɛtumi afrɛ me daakye wɔ nhwehwɛmu adwuma afoforɔ ho.	
10	Mepene so sɛ nhwehwɛmufoɔ no bɛtumi de me m'akyiri kwan ho nsɛm asie sɛdeɛ wɔbɛtumi de nsunsuanesoɔ a ɛbɛfiri saa adesua yi mu abɔ no tɔfa ama me.	
11	Mepene so sɛ nhwehwɛmufoɔ no bɛtumi aka tɛst no nsunsuanesoɔ akyerɛ me dɔkota.	
12	Mepene so de me ho hyɛ saa adesua yi mu.	

Nsɛmboano ho bambɔ

Yɛbɛfa nsɛmboano ho bambɔ mmara a yakyerɛkyerɛ mu wɔ krataasini a yɛtwerɛ nnipa a wɔde wɔn ho bɛhyɛ nhwehwɛmu ho nsɛm agu so no mu no ne Kokoa mu Kɔkɔbɔ a yɛde ma Nwehwɛmufoɔ so ayɛ adwuma

Deɛ ɔde ne ho ahyɛ mu no din	Nsaanodin	Deeti
Nnipa a ɔregye penee no din	Nsaanodin	Deeti

(Yɛde penee krataa no nhwɛsoɔ baako bɛma deɛ ɔde ne ho hyɛ adesua no mu no, nhwehwɛmukuo no (deɛ ɛdi kan) ne ahwɛfokuo)

Appendix 8: Questionnaires

Study 1

Screening for invasive fungal diseases among HIV patients using non-culture-based assays

PATIENT QUESTIONNAIRE

Study number: _____

Data collector's initials: _____ Date of data collection: _____/_____/_____

I. Sociodemographic Information

1) Coded ID _____/_____ 2) Gender ☐ Male ☐ Female

3) Date of birth ____/____/_____ (dd/mm/yyyyy) or Age_____ years

4) Weight: _____ kg 5) Height: _____ cm

6) Occupation: Current _____

Previous: _____

7) Residence: Current_____

Previous: _____

8) Travel history: _____

II. Medical and Laboratory History

9) Date of HIV diagnosis: ____/____/_____ (dd/mm/yyyy) 10) HIV type ☐HIV1 ☐HIV2 ☐HIV 1 & 2

11) Mode of HIV acquisition: ☐Heterosexual ☐Homosexual ☐Injection drug use ☐Transfusion ☐Unknown

12) What is the current HIV staging? ☐Stage I ☐Stage II ☐Stage III ☐Stage IV

13) Hospitalized within the last 6 months? ☐ Yes ☐ No

14) Previous or current underlying condition (s): _____

15) Record of CD4 count beginning with most recent test

a. _____ mm/µl Date: _____/_____/_____

b. _____ mm/µl Date: _____/_____/_____

c. _____ mm/µl Date: _____/_____/_____

16) Record of HIV viral load beginning with most recent test

a. _____ RNA copies/ml Date: _____/_____/_____

b. _____ RNA copies/ml Date: _____/_____/_____

c. _____ RNA copies/ml Date: _____/_____/_____

17) Please indicate below chronic condition(s) the patient have:

Asthma..... ☐

COPD ..... ☐

Diabetes ..... ☐

Cancer (other than lung)..... ☐

Lung Cancer..... ☐

High blood pressure ..... ☐

Arthritis or other rheumatic disease ..... ☐

Other chronic condition (specify) ..... ☐

III. Drug History

18) Is the patient currently on ART therapy? ☐Yes ☐No

19) Record of ART regimen, beginning with current regimen

a. _____ Start date: _____/_____/_____ Stop date: _____/_____/_____

b. _____ Start date: _____/_____/_____ Stop date: _____/_____/_____

c. _____ Start date: _____/_____/_____ Stop date: _____/_____/_____

20) Has patient had any ARV resistance? ☐Yes ☐No ☐Unknown

If 'Yes' name regimen (s):_____

21) Is patient complying with ART during the last two appointments? ☐Yes ☐No ☐Unknown

22) Has participant interrupted ART and duration? ☐Yes ☐No ☐Unknown_____

23) History of ART in a different facility: _____

24) Total duration on ART: _____

25) Record of antifungal treatment, beginning with current treatment

a. _____ Start date: _____/_____/_____ Stop date: _____/_____/_____

b. _____ Start date: _____/_____/_____ Stop date: _____/_____/_____

c. _____ Start date: _____/_____/_____ Stop date: _____/_____/_____

IV. Current Clinical Assessment Information

26) Clinical symptoms and signs (Select all that apply please)

☐Headache ☐Fever ☐Nausea/vomiting

☐Seizures ☐Stiff neck ☐Confusion/neurological symptoms

☐Weight loss ☐Dyspnoea ☐Chills

☐Cough ☐Skin lesions

Others (please state): _____

27) Duration of symptoms: _____

V. Risk Exposures

28) Has your house experienced any flood, leaks or serious damp issues in the last 12 months?
Yes ☐ No ☐

If yes, Is it currently wet or damp? Yes ☐ No ☐

If yes, How many days has moisture been present?

☐1week ☐2 weeks ☐3 weeks or more

29) How often does the rain come into your house?

Almost all the time ☐

Frequently, for examples on most rainy days ☐

Occasionally, for example only heavy rains ☐

Rarely or not at all ☐

30) Do you have frequent contact with the following?

Soil (gardening or farming) Yes ☐ No☐

Cave (Visits) Yes ☐ No☐

Animal faeces or urine Yes ☐ No☐

If 'Yes' which animal.....

31) Do you smoke? Yes ☐ No☐

32) Does anyone else smoke in your house? Yes ☐ No☐

33) Have you ever smoked? Yes ☐ No☐

34) House or workplace infested by cellar bats? ☐ Yes ☐ No ☐ Unknown

Study 2 and 3

Screening for chronic pulmonary aspergillosis in a cohort of suspected TB in Ghana

QUESTIONNAIRE

Study number: _____

Data collector's initials: _____ Date of data collection: _____/_____/_____

I. Sociodemographic Information

1) Coded ID _____/_____ 2) Sex ☐ Male ☐ Female

3) Date of birth ____/____/_____ (dd/mm/yyyyy) or Age_____ years

4) Weight: _____ kg 5) Height: _____ cm

6) Previous and current occupation(s): _____

7) Previous and current residence details: _____

II. Medical and Drug History

8) Previous diagnosis of TB: ☐ Yes ☐ No

9) History of other chronic lung disease: _____

10) HIV status: ☐ Positive ☐ Negative ☐ Unknown If positive recent CD4 count: _____

11) Drug History: _____

III. Clinical Assessment Information

12) Clinical symptoms and signs (Select all that apply please)

☐Cough ☐Fever ☐Haemoptysis

☐Fatigue ☐ Night sweats ☐Chest pain

☐Weight loss ☐Dyspnoea ☐Chills

Others (please state): _____

13) Duration of symptoms: _____

IV. Risk factors and Exposures

14) Dampness in house: ☐ Yes ☐ No ☐ Unknown

15) Cooking with charcoal: ☐ Yes ☐ No ☐ Unknown

16) Tobacco smoker ☐ Non-smoker ☐ Past smoker ☐ Current smoker

Study 4

MANCHESTER 1824
The University of Manchester

Screening for invasive aspergillosis among haematological malignancy patients at the Korle-bu Teaching Hospital

PARTICIPANT QUESTIONNAIRE

Study ID: _____ Date of data collection: _____/_____/_____

Section A: To be completed by Research Assistant

I. Sociodemographic Information

1) Gender: ☐ Male ☐ Female

2) Date of birth: ____/____/_____ (dd/mm/yyyy) or Age_____ years

4) Occupation: Current _____

Previous: _____

5) Residence: Current_____

Previous: _____

II. Medical and Treatment History

6) Haematological malignancy: _____

7) Date of diagnosis: ____/____/_____ (dd/mm/yyyy) or duration_____ years

7) Patient category: ☐newly diagnosed ☐progression ☐relapse

8) Type of chemotherapy: ☐induction ☐consolidation ☐Maintenance ☐salvage ☐none

9) Hospitalized within the last 6 months? ☐ Yes ☐ No

10) Please indicate any chronic condition(s) the patient have: _____

11) Has the patient ever received chemotherapy? ☐Yes ☐No

12) Record of chemotherapy, beginning with current one

a. Name of drug(s): _____ Start date: _____/_____/_____ Duration: _____

b. Name of drug(s): _____ Start date: _____/_____/_____ Duration: _____

c. Name of drug(s): _____ Start date: _____/_____/_____ Duration: _____

13) Total cycles/duration on chemotherapy: _____

14) Is the patient currently on chemotherapy? ☐Yes ☐No

15) Record of antifungal prophylaxis/treatment, beginning with current treatment

a. _____ Start date: _____/_____/_____ Duration: _____

b. _____ Start date: _____/_____/_____ Duration: _____

c. _____ Start date: _____/_____/_____ Duration: _____

III. Risk Exposures

16) Has your house experienced any flood, leaks or serious damp issues in the last 6 months? Yes ☐ No ☐

17) Are you involved gardening or farming Yes ☐ No ☐

18) Have you been exposed to construction, renovation or demolition areas Yes ☐ No ☐

19) Do you smoke or regularly exposed to smoke? Yes ☐ No ☐

Section B: To be completed by Clinician/Principal Investigator

IV. Clinical Assessment Information

20) Symptom(s) and sign(s) prior to research investigation (Select all that apply please)

Persistent fever ☐

Respiratory: cough ☐ chest pain ☐ shortness of breath ☐ haemoptysis ☐

Neurologic: headache ☐ stroke-like features ☐, convulsion ☐

Nasal: congestion ☐ facial swelling ☐ orbital swelling ☐ nasal discharge ☐ palate abnormalities ☐

Cutaneous lesions: nodular ☐ ulcerative ☐ papular ☐ suppurative ☐ cold abscess ☐

Others (please state): _____

21) Duration of symptoms: _____

22) Current Neutrophil count: _____

23) History of neutropenia: ☐ Yes ☐ No Duration of neutropenia: _____

24) Corticosteroid use: ☐ Yes ☐ No

Name of drug: _____ Dose: _____ Duration: _____

25) Antibiotic use: ☐ Yes ☐ No

Name of drug: _____ Dose: _____ Duration: _____

Appendix 9: Chapter 3 paper/ Paper 2

DOI: 10.1093/ofid/ofac277

Open Forum Infectious Diseases
MAJOR ARTICLE

Cryptococcal and *Histoplasma* Antigen Screening Among People With Human Immunodeficiency Virus in Ghana and Comparative Analysis of OIDx *Histoplasma* Lateral Flow Assay and IMMY *Histoplasma* Enzyme Immunoassay

Bright K. Ocansey,[1] Benjamin Otoo,[2] Isabella Asamoah,[3] Vincent Ganu,[3] Kofi P. Berko,[3] Oluwakemi Oladele,[3] Emmanuella A. Amankwa,[3] Bismark Opoku-Asare,[3] Martin Agyei,[4,5] Lawrence George,[6] Fleischer C. N. Kotey,[7,8] Chris Kosmidis,[1,9] Peter Puplampu,[3,10] Japheth A. Opintan,[6] and David W. Denning[1]

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Background. Cryptococcal meningitis (CM) and disseminated histoplasmosis (DH) are common in people with human immunodeficiency virus (PWH) and diagnosed by detecting cryptococcal antigen (CrAg) and *Histoplasma* antigen (HistoAg), respectively. In Ghana, CM and DH are rarely suspected by clinicians due to limited epidemiological data.

Methods. This study was conducted among PWH in Ghana who are unwell. Sociodemographic and clinical data were collected by questionnaire. Serum and/or urine were screened for CrAg and HistoAg, using IMMY CrAg lateral flow assay (LFA) and IMMY *Histoplasma* enzyme immunoassay (EIA) kits, respectively, regardless of symptoms. Samples run with IMMY *Histoplasma* EIA were simultaneously run with Optimum Imaging Diagnostics (OIDx) *Histoplasma* LFA. Laboratory investigations were conducted by the research team, and diagnosis incorporating clinical assessment, screening, and confirmatory testing results and treatment decisions were made by the clinical team. Treatment and outcome information on CM and DH patients were evaluated.

Results. Overall, 150 participants were recruited. There were 73% ($n = 109$) females, and the age range was 18–62 years. The prevalence rates of CrAg and HistoAg were 2.7% (4 of 150) and 4.7% (5 of 107), respectively. The OIDx *Histoplasma* LFA showed a high concordance (98.4%) with the IMMY *Histoplasma* EIA. All antigen-positive cases by standard tests were diagnosed with CM and DH. Antifungal treatment was given in 5 patients and follow-up revealed 2 deaths and 3 recoveries.

Conclusions. Histoplasmosis among PWH may be more common than previously anticipated and may be more frequent than cryptococcosis in Ghana. The performance of the OIDx *Histoplasma* LFA should be further explored.

Keywords. antigen tests; cryptococcosis; Ghana; histoplasmosis; people with HIV.

Invasive fungal infections (IFIs) are an important cause of ill health and deaths among people with human immunodeficiency virus (PWH). Despite the global rollout of highly active antiretroviral therapy (ART), IFIs continue to affect PWH particularly in sub-Saharan Africa (SSA). This has largely been attributed to delayed human immunodeficiency virus (HIV) diagnosis, interruption of ART care, and high burden of advanced HIV disease (AHD) [1]. Globally, IFIs are collectively estimated to cause approximately 47% of all acquired immune deficiency syndrome (AIDS)-related deaths [2]. The IFIs associated with the highest morbidity and mortality in PWH are cryptococcal meningitis (CM), disseminated histoplasmosis (DH), and *Pneumocystis jirovecii* pneumonia [3].

Annually, over 200 000 CM cases occur globally, with 73% in SSA and responsible for 15% of AIDS-related deaths [4]. At this time, the World Health Organization (WHO) recommends testing for cryptococcal antigen (CrAg) in PWH with a CD4 count less than 100 cells/μL [5]. This recommendation has been evaluated to be cost-effective even at a low CrAg prevalence rate of 1.4% [6]. Earlier studies on CrAg screening and CM had focused on ART-naive patients, but recent studies among ART-experienced patients report similar rates [7–9].

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Appendix 10: Chapter 4 paper/ Paper 3

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Original Article
Advance access publication date: 11 August 2022

Chronic pulmonary aspergillosis is common among patients with presumed tuberculosis relapse in Ghana

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Abstract

Chronic pulmonary aspergillosis (CPA) may mimic pulmonary tuberculosis (PTB). The two diseases are clinically indistinguishable and may result in CPA misdiagnosed as PTB or vice versa. Although PTB is largely recognised as a differential diagnosis of CPA and often ruled out prior to CPA diagnosis, the reverse is uncommon. The aim of this study was to determine the proportion of CPA cases among patients being assessed for PTB. A cross-sectional survey was conducted among consecutive patients referred for GeneXpert *Mycobacterium tuberculosis* test for the diagnosis of PTB at the Korle-Bu Teaching Hospital, Accra, Ghana. Patients' demographics, clinical and socioeconomic details were obtained using a structured questionnaire. Blood was collected for *Aspergillus* and HIV serology, and sputum samples obtained for *Aspergillus* culture. Chest radiograph was obtained, and computed tomography scan was also done for patients with positive *Aspergillus* serology or cavitation. CPA was defined using an algorithm developed by the Global Action for Fungal Infections (GAFFI) international expert panel. A total of 154 patients were included in the analysis, of whom 134 (87%) did not have a prior PTB diagnosis. There were 41 (26.6%) GeneXpert positive cases. CPA prevalence was 9.7% overall, but 50% in patients with a prior history of PTB and 3.7% in those without previous PTB. Although CPA is rarely considered as a differential diagnosis of PTB in Ghana, our findings show that CPA may affect half of patients being assessed for PTB relapse. Efforts to diagnose CPA should be prioritised in this patient group.

Lay Summary

Chronic pulmonary aspergillosis (CPA) may be misdiagnosed as pulmonary tuberculosis (PTB), or vice versa due to clinical similarities. Screening for CPA among patients undergoing investigation for relapsed PTB and new PTB revealed that half and about four in 100 patients, respectively, had CPA.

Keywords: Aspergillus serology, chronic pulmonary aspergillosis, Ghana, relapse, tuberculosis

Introduction

Pulmonary fungal infections have increased in clinical significance in recent times, and although many of them mimic pulmonary tuberculosis (PTB), chronic pulmonary aspergillosis (CPA) is one of the most common.[1] CPA is a slow, progressive, and destructive lung disease associated with both respiratory and systemic symptoms. Globally, approximately 3 million people suffer from CPA, with 1.2 million occurring as a sequel of PTB.[2] In Ghana, CPA among PTB patients is estimated at 2600 cases annually.[3] PTB is a common differential diagnosis of CPA, and could occur before, after, or infrequently, together with CPA.[4] There are many similarities between PTB and CPA in terms of risk factors, clinical presentation, and radiological features, making the two diseases clinically indistinguishable.[5] This may result in misdiagnosis of CPA as PTB, or vice versa. As PTB is more common and largely recognised globally, the index of suspicion for PTB is likely higher compared to CPA, particularly in settings with a high PTB burden. Being mostly diagnosed as a post-PTB complication, CPA may be misdiagnosed as relapsed PTB infection and managed as such.[5] CPA may also be occasionally misdiagnosed as primary TB infection.[5] Some studies have reported CPA misdiagnosed as acid-fast bacilli (AFB) smear-negative or GeneXpert *Mycobacterium tuberculosis* (MTB)-negative PTB and resulting in worsening symptoms and anti-TB treatment failure.[6,7] Previous and present guidelines for CPA diagnosis have recommended a necessary exclusion of PTB.[4,8] However, with emerging concerns of primary CPA and CPA co-existing with PTB, it may be equally important to rule out CPA when making a diagnosis of PTB to avoid inappropriate exposure of patients to anti-TB medications.

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Appendix 11: Chapter 5 paper/Paper 4

DOI: 10.3390/jof9010026

Journal of *Fungi*

MDPI

Article

Importance of *Aspergillus*-Specific Antibody Screening for Diagnosis of Chronic Pulmonary Aspergillosis after Tuberculosis Treatment: A Prospective Follow-Up Study in Ghana

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Abstract: Chronic pulmonary aspergillosis (CPA) often occurs in patients that have been previously treated for pulmonary tuberculosis (PTB). A limited number of studies have looked at the development of CPA at different times following the completion of a PTB treatment course. This prospective longitudinal study aimed to determine the incidence of CPA at two timepoints, at the end of the PTB treatment (T_1) and six months post-treatment (T_2). Patients with confirmed PTB from a previous study who were placed on anti-TB medication were followed up and screened for CPA at T_1 and T_2 by assessing their symptoms, evaluating their quality of life, and screening them for *Aspergillus* infection by performing antibody testing and cultures. CPA was defined by the Global Action for Fungal Infections (GAFFI) diagnostic algorithm. Forty-one patients were enrolled, of whom thirty-three patients (80%) and twenty-eight patients (68%) were resurveyed at T_1 and T_2, respectively. The rate of new CPA was 3.3% (1/33) and 7.4% (2/27) at T_1 and T_2, respectively, with an overall incidence of 10.7% (3/28) among the patients at both T_1 and T_2. A positive *Aspergillus*-specific antibody test was an indicator for CPA in all three patients. *Aspergillus*-specific antibody screening during and after the end of an anti-TB treatment regimen may be important for early detection of CPA in high-PTB-burden settings.

Keywords: *Aspergillus* antibody; chronic pulmonary aspergillosis; Ghana; tuberculosis

check for updates

Citation: Ocansey, B.K.; Otoo, B.; Gbadamosi, H.; Afriyie-Mensah, J.S.; Opintan, J.A.; Kosmidis, C.; Denning, D.W. Importance of *Aspergillus*-Specific Antibody Screening for Diagnosis of Chronic Pulmonary Aspergillosis after Tuberculosis Treatment: A Prospective Follow-Up Study in Ghana. *J. Fungi* 2023, 9, 26. https://doi.org/10.3390/jof9010026

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1. Introduction

Pulmonary tuberculosis (PTB) remains a major global health problem, with a high burden in low- and middle-income countries (LMICs). In 2020, there were 4.8 million people diagnosed with PTB globally, and 59% of these cases had been bacteriologically confirmed [1]. About 85% of the people diagnosed with PTB are generally successfully treated with a 6 month drug regimen [1]. Unfortunately, patients with PTB after a successful treatment become more exposed to secondary respiratory infections that are uncommon in patients without prior PTB [2]. These infections can be chronic, and they are associated with high morbidity and mortality rates. Chronic pulmonary aspergillosis (CPA) is one of the common infections, and the high-risk group are patients with PTB with overt lung cavities [3]. The prevalence of CPA as a sequel of PTB worldwide was estimated at

Appendix 12: Chapter 6 paper/Paper 5

PMID: 37390225/

WEST AFRICAN JOURNAL OF MEDICINE

ORIGINAL ARTICLE

Invasive Aspergillosis among Haematological Malignancy Patients in Ghana: A Pilot Study at the National Referral Hospital

Aspergillose Invasive chez les Patients Atteints d'Hémopathie Maligne au Ghana : Une Etude Pilote a l'Hôpital National de Référence

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ABSTRACT

BACKGROUND: Invasive aspergillosis (IA) among haematological malignancy patients is rarely diagnosed or studied in many African countries. *Aspergillus* galactomannan (GM) enzyme immunoassay (EIA) utilized in aiding diagnosis is not readily accessible in Ghana. Previous studies have evaluated the IMMY sōna® *Aspergillus* GM lateral flow assay (LFA) and suggested it as a potential alternative to the GM EIA.

OBJECTIVES: We aimed to use the LFA in international (EORTC/MSGERC) definitions to obtain preliminary data on IA among patients with haematological malignancies in Ghana with a focus on the prevalence and antifungal prophylaxis.

METHODS: We conducted a pilot study among patients with haematological malignancies at the Korle-Bu Teaching Hospital, Ghana using the LFA, culture and computed tomography scan to screen for and classify IA cases according to international definitions.

RESULTS: A total of 56 adult patients were recruited including acute leukaemia 14 (25.0%), chronic leukaemia 38 (67.9%), and lymphoma 4 (7.1%). Nine (16.1%) patients had a history of severe neutropenic episodes. All patients were on at least one chemotherapy drug. Three (5.4%) patients met the criteria for IA, comprising two probable IA in acute myeloid leukaemia and one possible IA in non-Hodgkin's lymphoma and constitutes one of five (20%) patients with ongoing severe neutropenia. The LFA was diagnostic in two IA patients. The IA cases were among 49 (87.5%) patients who did not receive antifungal prophylaxis.

CONCLUSION: Proactive diagnostic approaches to IA and effective antifungal prophylaxis may be significant in the management of haematological malignancy patients with severe neutropenia in Ghana. **WAJM 2023; 40(6): 613–618.**

Keywords: Invasive aspergillosis, Haematological malignancy, Ghana, *Aspergillus* galactomannan, Neutropenia, Antifungal prophylaxis.

RÉSUMÉ

CONTEXTE: L'aspergillose invasive (AI) parmi les hémopathies malignes est rarement diagnostiquée ou étudiée dans de nombreux pays africains et le dosage immunoenzymatique (EIA) d'*Aspergillus* galactomannane (GM) utilisé pour faciliter le diagnostic n'est pas facilement accessible. Le test à flux latéral (TFL) IMMY sōna® *Aspergillus* GM récemment introduit est évalué et suggéré comme alternative au GM EIA.

OBJECTIFS: Nous avons cherché à utiliser les définitions TFA et les définitions internationales (EORTC/MSGERC) pour obtenir des données préliminaires sur l'AI dans les hémopathies malignes au Ghana en mettant l'accent sur la prévalence et la prophylaxie antifongique.

Méthodes: Nous avons mené une étude pilote auprès de patients atteints d'hémopathie maligne à l'hôpital universitaire de Korle-Bu, au Ghana, en utilisant le TFL, la culture et la tomodensitométrie pour dépister et classer les cas d'AI selon les définitions internationales.

RÉSULTATS: Au total, 56 patients adultes ont été recrutés, dont une leucémie aiguë (25 %), une leucémie chronique (67,9 %) et un lymphome (7,1 %), neuf (16,1 %) ayant des antécédents d'épisodes neutropéniques. La plupart des patients (70 %) avaient une maladie évolutive. Trois patients répondaient aux critères d'AI, comprenant deux AI probables et une AI possible, uniquement chez des patients atteints de leucémie aiguë et un sur cinq (20 %) avec une neutropénie en cours. Le TFL était utilisé comme méthode de diagnostique chez deux patients d'AI. Les cas d'AI concernaient tous les 49 (87,5 %) des patients n'ayant pas reçu de prophylaxie antifongique.

CONCLUSION: L'AI a probablement une incidence de 5,4 % dans les leucémies, mais de 20 % chez les patients neutropéniques et chez aucun patient recevant une prophylaxie antifongique. Des approches diagnostiques proactives de l'AI et une prophylaxie antifongique efficace peuvent être importantes dans la prise en charge des hémopathies malignes au Ghana. **WAJM 2023; 40(6): 613–618.**

Mots clés: Aspergillose invasive, Hémopathie maligne, Ghana, *Aspergillus* galactomannan, Neutropénie, Prophylaxie antifongique.

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Appendix 13: Demographic, clinical and laboratory details of the 107 cases -

(Chapter 7/Study 5)

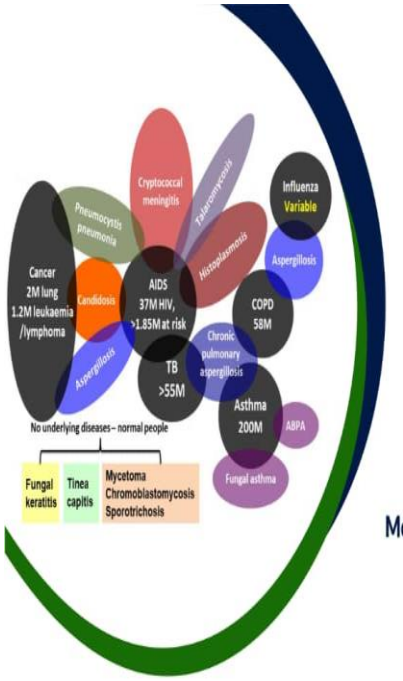
Study ID	Sex	Age	Clinical data	Sample site	Stain	Diagnosis
FHM001	F	55	Not retrieved	Nasal biopsy	H&E	Aspergillosis
FHM002	F	39	Not retrieved	Maxillary sinuses	H&E	Mucormycosis
FHM003	M	28	Not retrieved	Skin	H&E	Dermatophytosis
FHM004	M	50	Not retrieved	Elbow	H&E	Aspergillosis
FHM005	M	49	Not retrieved	Neck	H&E, PAS	-
FHM006	M	53	Not retrieved	Naso-labium	H&E, PAS	Sporotrichosis
FHM007	M	42	Not retrieved	Back and face	H&E	Malassezia
FHM008	M	30	Not retrieved	Larynx	H&E	-
FHM009	M	73	Not retrieved	Sinus	H&E	Mucormycosis
FHM010	M	56	Not retrieved	Stomach	H&E	Aspergillosis
FHM011	F	74	Not retrieved	Vagina	H&E	Candidiasis
FHM012	M	69	Not retrieved	Nose	H&E	Aspergillosis
FHM013	F	39	Not retrieved	Nose	H&E	Aspergillosis
FHM014	M	38	Not retrieved	Larynx	H&E, special stain	Candidiasis
FHM015	F	23	Not retrieved	Nose	H&E, PAS	Aspergillosis
FHM016	F	28	Not retrieved	Nose	H&E	Aspergillosis
FHM017	F	77	Not retrieved	Right elbow	H&E	Aspergillosis
FHM018	M	28	Not retrieved	Periauricular aspirate & right femur	H&E	-
FHM019	F	51	Not retrieved	Endometrium	H&E	-
FHM020	M	61	Not retrieved	Nose	H&E, PAS	Aspergillosis
FHM021	F	41	Not retrieved	Right maxillary mass	H&E	Aspergillosis
FHM022	M	35	Not retrieved	Nose	H&E	-
FHM023	M	41	Not retrieved	Skin	H&E	Dermatophytosis
FHM024	F	10	Not retrieved	Oesophagus	H&E	Candidiasis
FHM025	F	65	Not retrieved	Skin	H&E	Candidiasis
FHM026	F	70	Not retrieved	Duodenum	H&E	Candidiasis
FHM027	F	50	Not retrieved	Biliary tract	H&E	-
FHM028	F	56	Not retrieved	Naso-orbital mass	H&E	Aspergillosis
FHM029	F	56	Not retrieved	Oesophagus	H&E	-
FHM030	F	26	Not retrieved	Left anterior chest wall	H&E	Histoplasmosis
FHM031	M	24	Not retrieved	Skin	H&E	Dermatophytosis
FHM032	F	30	Not retrieved	Tongue	H&E	-
FHM033	F	35	Not retrieved	Right nasal cavity	H&E	-
FHM034	F	58	Not retrieved	Uterus	H&E	Candidiasis
FHM035	F	55	Not retrieved	Nose	H&E	Aspergillosis
FHM036	M	48	Not retrieved	Skin	H&E	-
FHM037	F	48	Not retrieved	Right elbow	H&E	Candidiasis
FHM038	M	38	Not retrieved	Nose	H&E	-

FHM039	M	34	Not retrieved	Skin	H&E	Tinea incognito/ Dermatophytosis
FHM040	M	58	Not retrieved	Oesophagus	H&E	Candidiasis
FHM041	F	52	Not retrieved	Right nostril	H&E	Aspergillosis
FHM042	F	6	Not retrieved	Lip/mouth	H&E	Dermatophytosis
FHM043	M	31	Not retrieved	Oesophagus	H&E	Candidiasis
FHM044	M	50	Not retrieved	Nose	H&E, PAS	-
FHM045	M	61	Not retrieved	Paranasal	H&E	Aspergillosis
FHM046	M	7	Not retrieved	Scalp	H&E, PAS	Mycetoma
FHM047	F	55	Not retrieved	Skin /eye	H&E	Tinea cutis/ Dermatophytosis
FHM048	F	48	Not retrieved	Uterus	H&E	Aspergillosis
FHM049	F	32	Not retrieved	Right toe	H&E	-
FHM050	M	4	Facial swelling	Face	H&E, GMS	-
FHM051	M	47	Persistent with nasal blockage and pain	Nasal	H&E, Special stain	Aspergillosis
FHM052	M	33	Chronic left ankle ulcer (5yrs +)	Left ankle/leg	H&E	Histoplasmosis
FHM053	F	35	Recurrent epistaxis frontal headache	Sphenoid sinus		-
FHM054	M	43	Not retrieved	L&R sole + left shin	H&E	-
FHM055	F	24	Recurrent nasal mass	Nasal mass	H&E	Mucormycosis
FHM056	F	41	Pus with a fibrofatty mass on the right skin	Mass on right shin, excision biopsy following drainage	H&E, PAS	Candidiasis
FHM057	F	84	Ingrowth of toenail	Toenail	H&E	-
FHM058	F	55	Epistaxis, chronic headache	Left antral mass	H&E, Special stain	Aspergillosis
FHM059	M	33	10 year right nasal obstruction	Right maxillary ethmoid sphenoid	H&E	-
FHM060	M	49	Infected ganglion	Left foot	H&E	Chromomycosis/ Chromoblastomycosis
FHM061	F	31	Fungal rhinosinusitis	Left frontal and ethmoidal sinuses	H&E	-
FHM062	M	45	Sinonasal tumour ethmoid? fungal infection	Right sinonasal	H&E, GMS	-
FHM063	F	38	Recurrent left epistasis	Left paranasal sinuses	H&E	-

FHM064	F	60	Chronic leg ulcer with multinodular floor	Leg	H&E	-
FHM065	M	24	Chronic granuloma	Left orbital mass	H&E, PAS	Aspergillosis
FHM066	F	7	Not retrieved	Cervical incisional biopsy	H&E	Phycomycosis/ Entomophthoromycosis
FHM067	F	41	Gestational trophoblastic	Uterus	H&E	-
FHM068	M	28	Chronic cough, weight loss	Lung, pleura	H&E, PAS	-
FHM069	M	6	Basidiomycosis, filariasis	Upper limb	H&E, PAS	Phycomycosis/ Entomophthoromycosis
FHM070	F	24	Bilateral nasal congestion	Nasal mass	H&E	-
FHM071	F	7	TB, pleural effusion	Mesenteric lymph node/lung	H&E, PAS	-
FHM072	F	24	Bilateral nasal congestion	Nasal mass	H&E	-
FHM073	M	40	Hyperpigmented	Palm	H&E, PAS	Pityriasis versicolor
FHM074	F	55	Post coital bleeding	Cervix	H&E	-
FHM075	F	86	Epigastric pain, jaundice	Gastric mucosa	H&E	Candidiasis
FHM076	F	78	Stomach cancer	Gastric biopsy	H&E	-
FHM077	M	43	Not retrieved	Right eye	H&E	-
FHM078	F	26	Tinea versicolor, urticaria vasculitis	Shoulder	H&E	-
FHM079	F	60	*Candida* oesophagitis	Oesophagus	H&E	Candidiasis
FHM080	M	67	Dyspepsia	Oesophagus	H&E	-
FHM081	M	76	Recurrent epigastric pain	Gastric ulcer	H&E	-
FHM082	F	5	Previous CIP, now diarrhoea	Retroperitonal space?	H&E	-
FHM083	M	42	Right paraspinal lesion with lytic bone destruction	Spinal tumour/Spine	H&E, PAS, GMS	Cryptococcosis
FHM084	M	79	History of dysphagia,	Oesophagus	H&E	-

FHM085	M	30	Gastric tumour	Gastric biopsy	H&E	-
FHM086	F	58	Weight loss	Stomach	H&E	-
FHM087	M	32	Fungal foot lesions	Foot	H&E	-
FHM088	F	40	Not retrieved	Nasal antral mass	H&E	-
FHM089	F	58	Upper GI bleed	Polyploid lesion	H&E	-
FHM090	M	38	Left axillary mass	Left axillary mass	H&E	Chromoblastomycosis
FHM091	M	18	Swelling of buccal sulcus	Periapical cyst	H&E	-
FHM092	M	9	Right parietal scalp lesion	Scalp	H&E	-
FHM093	M	50	Upper GI bleed	Gastric biopsy	H&E	-
FHM094	F	36	Not retrieved	Endometrium curetting	H&E	-
FHM095	M	18	Abnormal growth of toe	Left second toe	PAS	Onychomycosis
FHM096	F	60	Not retrieved	Gastric tract	H&E	Candidiasis
FHM097	M	18	Not retrieved	Nasal	H&E	Sporotrichosis
FHM098	F	38	Not retrieved	Antrum	H&E, PAS	Aspergillosis
FHM099	M	46	Not retrieved	Ankle	H&E, PAS	-
FHM100	M	7	Not retrieved	Larynx	H&E	-
FHM101	M	46	Not retrieved	Pharynx	H&E	Candidiasis
FHM102	F	41	Not retrieved	Nasal cavity	H&E	Aspergillosis
FHM103	F	40	Not retrieved	Nasal, maxilla	H&E	Aspergillosis
FHM104	F	34	Not retrieved	Nose	H&E	-
FHM105	F	41	Not retrieved	Nose	H&E	Aspergillosis
FHM106	F	22	Not retrieved	Scalp	H&E	Mycetoma
FHM107	F	4	Oesophageal stricture, secondary caustic ingestion	GIT	H&E	-

Appendix 14: Flyers for sensitization and capacity building meetings



GHANA MEDICAL MYCOLOGY GROUP

Commemorates

FUNGAL DISEASE AWARENESS WEEK

THEME

IMPROVING DETECTION AND MANAGEMENT OF SERIOUS FUNGAL INFECTIONS IN GHANA: BASIC APPROACHES

Date:
Monday, 20th - Friday, 24th September 2021

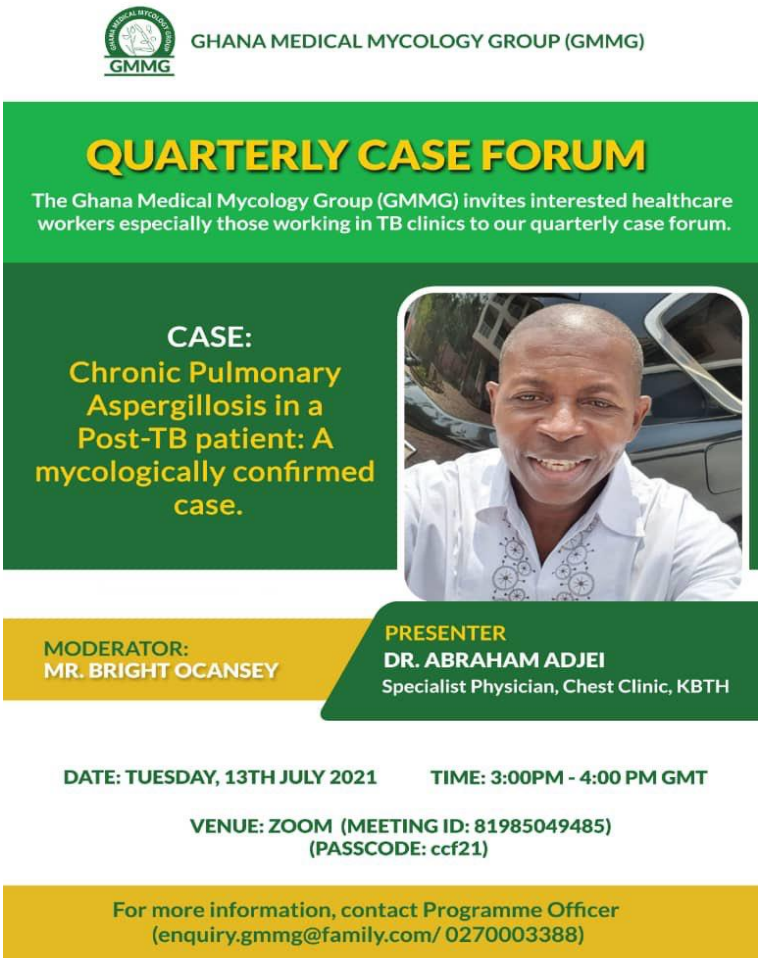
ACTIVITIES

- Virtual Symposium
- Public engagement

RSVP: 0542646303/0270003388

GMMG IMMY GAFFI MANCHESTER 1824 The University of Manchester FIKI FUNGAL INFECTIONS KARE INITIATIVE GHANA


WORLD ASPERGILLOSIS DAY 2022-#WAD22
GHANA MEDICAL MYCOLOGY GROUP (GMMG)
PRESENTS
2ND ASPERGILLOSIS LECTURES
DATE: 2ND FEBRUARY, 2022
TIME: 2:30-4:00 PM (GMT)
VENUE: ZOOM
1ST TOPIC:
INVASIVE ASPERGILLOSIS IN COVID-19: RISK STRATIFICATION, PROPHYLAXIS, DIAGNOSIS AND MANAGEMENT
1ST SPEAKER
Prof. Martin Hoenigl
Medical University of Graz, Austria
University of California San Diego, USA
2ND TOPIC:
APPROACHES TO MANAGEMENT OF CHRONIC PULMONARY ASPERGILLOSIS
2ND SPEAKER
Dr. Chris Kosmidis
National Aspergillosis Centre, UK
University of Manchester, UK
CHAIRS
Dr. Joseph Oliver-Commey
Ghana Infectious Disease Centre, Ghana
Dr. Jane Afriyie-Mensah
University of Ghana Medical School, Ghana
Korle-bu Teaching Hospital, Ghana
MODERATOR
Mr. Bright Ocanse
Korle-bu Teaching Hospital, Ghana
University of Manchester, UK
Registration link:
https://us02web.zoom.us/webinar/register/WN_xjbuSg_DTamOw1cyJZlaGQ
Webinar ID: 81504794339
Password: AL2022
For more information; enquiry.gmmg@gmail.com or 0270003388/0542646303
Supported by:
FIKI, Ghana


GHANA MEDICAL MYCOLOGY GROUP (GMMG)
QUARTERLY CASE FORUM
The Ghana Medical Mycology Group (GMMG) invites interested healthcare workers especially those working in TB clinics to our quarterly case forum.
CASE:
Chronic Pulmonary Aspergillosis in a Post-TB patient: A mycologically confirmed case.
MODERATOR:
MR. BRIGHT OCANSEY
PRESENTER
DR. ABRAHAM ADJEI
Specialist Physician, Chest Clinic, KBTH
DATE: TUESDAY, 13TH JULY 2021
TIME: 3:00PM - 4:00 PM GMT
VENUE: ZOOM (MEETING ID: 81985049485)
(PASSCODE: ccf21)
For more information, contact Programme Officer
(enquiry.gmmg@family.com/ 0270003388)

Appendix 15: Peer-reviewed and published papers inspired by PhD thesis

Date	Paper	Description	Role
2022	Mycologically confirmed chronic pulmonary aspergillosis in a post-pulmonary tuberculosis patient in Ghana	This is a report of the first CPA case classified in the Study 2 of the PhD thesis. To the best of the authors knowledge this is the first mycologically confirmed case in Ghana. This paper has been published in the *Ghana Medical Journal*. DOI: 10.4314/gmj.v56i4.13	-Undertook all mycology laboratory testing -Retrieved data from medical records -Wrote the manuscript
2022	Disseminated Histoplasmosis in a Ghanaian HIV Patient: Role of Urine *Histoplasma* Antigen Testing in Rapid Diagnosis	This is a report of the only fatal case of disseminated histoplasmosis in Study 1 of the PhD thesis, which was detected by a *Histoplasma* antigen testing and confirmed by histopathology. To the best of the authors knowledge this is the first application of the *Histoplasma* antigen testing to diagnose histoplasmosis in Ghana. This case report was presented as a poster at the 21st Congress of the International Society of Human and Animal Mycology and poster published in the journal, *Medical Mycology*. DOI: 10.1093/mmy/myac072.P251	-Undertook all mycology laboratory testing -Co-wrote the abstract for poster with BO*
2022	Improving Awareness, Diagnosis and Management of Invasive Fungal Infections in Ghana: Establishment of the Ghana Medical Mycology Society	This paper discusses the present status of serious fungal infections and medical mycology in Ghana and the vision and efforts of the Ghana Medical Mycology Society to improve the status quo.	-Gathered all relevant data from literature and personal engagements -Wrote the manuscript

		This paper has been published in the journal, *Medical Mycology*. DOI: 10.1093/mmy/myac069	

*BO = Benjamin Otoo was an external research assistant associated with the PhD project