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Pathology-based research in Africa

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INTRODUCTION

The combination of the climate, the socioeconomic and geopolitical landscape, and history of colonialism has created many challenges for the continent of Africa, including a pronounced burden of disease. The African HIV epidemic is paramount with South Africa leading the world with the largest number of people living with HIV/AIDS.¹ There are increasing efforts to provide access to prevention and treatment, yet there is no cure and almost a third of new infections are seen in young people ages 15–24.²

In addition to HIV, older disease like tuberculosis and malaria have also had severe impacts on families, communities, and the economic development of countries in Africa. In the case of malaria, public health efforts including insecticide-treated mosquito nets, indoor residual spraying, and access to diagnostic testing and treatment have led to a decrease in infection and mortality.³ While malaria is no longer the leading cause of death in children in sub-Saharan Africa (SSA), chronic malarial infections, infections with other viruses and parasites, and malnutrition can lead to long term health effects.

As mortality attributable to communicable diseases continues to fall in resource-limited settings, cancer has become responsible for an increasing burden of morbidity and mortality, with an estimated 17.5 million cases diagnosed in 2015 and nearly 9 million deaths. Indeed, cancer is now likely responsible for a greater death toll than HIV, malaria, and tuberculosis combined and responsible for approximately 15% of all deaths globally.^{4–7} Given population growth, aging, and lifestyle factors (e.g. tobacco use) the cumulative burden and mortality rates attributed to cancer are expected to increase in the next 20 years globally and resource-limited regions will be disproportionately affected.^{4,8}

In light of the disease burden present in Africa, it makes sense to conduct pathology research efforts there. However, pathology research in Africa provides also insight into the following:

1. TB, malaria, HIV, and cancer are important globally, not just in Africa. They account for significant mortality/morbidity in developing countries too.
2. It is an ideal place to understand pathological features of diseases which have unique co-morbidities, TB-HIV infection being a classic inter-relationship.
3. To characterize and define treatments for disease states that are diagnosed later than in other parts of the world.
4. Because research is an important and successful method to create economic growth, private and government partnerships, build capacity and infrastructure.

5. In our globalized world, a high burden of infectious disease anywhere increases transmission everywhere.

This paper presents three research efforts that have used different aspects of pathology to dissect a facet of disease. From them, we can see the challenges but also the opportunities as we move forward in our global efforts.

CHALLENGES TO THE USE OF PATHOLOGY FOR THE STUDY OF FATAL MALARIA IN MALAWI, 1996–2010

Studies in the pathogenesis of severe malaria

For first 20 years after Independence in 1964, the Life President of Malawi forbade medical research; ‘I don’t want my people being treated as guinea-pigs,’ he said. With the early recognitions of HIV-AIDS in Malawi in 1984, and the acceleration of the epidemic during subsequent years, the need for research was increasingly appreciated. Malawi’s first medical school, the College of Medicine, graduated its first doctors in 1992, and research became an accepted function of this new institution. A National Health Sciences Research Committee (NHSRC) was formed to assess national research proposals, and the College of Medicine Research and Ethics Committee (COMREC) was then developed to attend to proposals emanating from the College.

The Ministry of Health (MOH) recognized the importance of malaria as a leading cause of morbidity in children, and in 1982 the new National Malaria Control Programme introduced country-wide first-line therapy with oral chloroquine for febrile illness presumed to be malaria. The MOH also recognized the burden of mortality from complicated malaria, especially the encephalopathic form ‘cerebral malaria’ (CM), with its fatality rate of 20–30% despite treatment, and highlighted it as a top research priority.

The NHSRC approved a proposal in 1987 for a clinical study of children admitted to hospital with coma attributed to malaria. At the time, very little was known regarding the clinical features and pathogenesis of this clinical syndrome. Several findings, including the importance of hypoglycemia and acidosis, emerged and routines for the precise recording of clinical and laboratory data in unconscious children were developed^{8–9}. It soon became clear that, without access to brain tissue, many conclusions about intracerebral pathophysiology remained speculative. This was particularly the case in malaria, a disease in which parasites are known to sequester in deep tissues to complete their maturation and replication.

Forensic autopsies had been allowed within the constitution of the Republic, which mandated hospital autopsies for ‘unexplained deaths’, although few practitioners knew of this law, and it was rarely carried out owing to the scarcity of pathologists. It was in this context that an additional proposal was approved in 1995, for a study to perform autopsies on children dying with malarial coma. Several requirements were stipulated by COMREC:

- The appropriate parent/s or guardian/s must give consent
- The explanation of the procedure, and the request for consent, must be delivered by a Malawian member of staff in the local language of the guardian/s

- After sampling, organs must be replaced in the body
- Autopsy must be performed with minimum delay

Obtaining consent

Before embarking on the study, the first of its kind for the study of pediatric cerebral malaria, the researchers were advised against using focus groups to assess the attitudes of parents and guardians in the general pediatric wards to the concept of autopsy. If the scenario set before a focus group actually came to pass, the organizers of the discussion, would be culpable. Instead, discussions were broached with the clinical team and responses were diverse. Many stated that they would refuse autopsy under any circumstances, while others indicated that they could approve if certain reasons prevailed. Common objections to autopsy included:

- distaste for the idea
- beliefs/suspicions about the misuse or selling of body parts
- concern about delay - the compelling tradition being that funeral and burial should take place quickly after death
- the need to view the body (at least the face) unmutilated
- 'it's not going to help – it's too late'.

Reasons put forward for possible acceptance of autopsy were:

- a wish to know the cause of death (which might dispel suspicions of witchcraft)
- a hope to prevent further deaths in the family/community
- willingness to contribute to medical knowledge

Guided by understandings about sensitivities gained in the preliminary discussions, consent forms in were prepared both English and Chichewa, and these were approved by COMREC.

Conducting the study: operational aspects

The aim of the study was to examine in detail the pathology of fatal pediatric malaria, in order to address several hypotheses concerning its pathogenesis. The procedure required collecting samples from numerous tissues, including many specified sites within the brain (Figure 1). Because the brain had to be returned to the skull before returning the body to the family, the brain had to be dissected and sampled in the mortuary, as part of the autopsy procedure.

To study microvasculature, smears of most tissues on slides were obtained. Samples were fixed in neutral buffered formalin for routine hematoxylin and eosin staining, in glutaraldehyde for electron microscopy, in OCT™ followed by liquid nitrogen for immunohistochemistry (IHC), and in RNA-later™, followed by liquid nitrogen for expression analyses. Samples flash frozen in liquid nitrogen in the mortuary were later transferred to a minus-20°C freezer for long-term storage. One coronal brain section was preserved, intact, in neutral buffered formalin. This section, as well as the dorsal and ventral

surfaces of the intact brain were photographed, using a photo stand, in the mortuary. In the early years, a film camera was used; eventually digital methods were implemented. The need for different sectioning and staining methods called for a variety of containers and preservatives. It was necessary to have trays prepared and labeled in advance, sufficient to provide for the next few autopsies.

In the event of the death of a study patient, the first constraint to autopsy was obtaining the consent of the right guardian/s, without unseemly haste. Often the guardian who was present felt s/he could not take responsibility for giving approval. Ascertaining and then finding the 'decision maker' in each family was the critical first step; occasionally this took too long, or proved to be impossible.

If consent was achieved, the next hurdle was assembling the team (mortuary technicians, clinical assistants, investigators and at least one pathologist). Because autopsies were infrequent and because the timing was unpredictable, the composition of the team varied considerably over the course of the study. Figure 2 shows an example of a body prior to autopsy.

Upon completion of the autopsy, the body had to be made as presentable as possible, with a shroud and coffin, and transport home was made available for the body and for closest relatives and guardians.

The correct documentation and appropriate storage of samples were crucially important. The precarious electricity supply necessitated reliable back-up generators, and liquid nitrogen or dry shippers had to be dependably replenished and maintained.

Conducting the study – ethical aspects

Researchers had concerned that some guardians might feel compelled to give their consent for autopsy. For this reason, a Malawian on the team was responsible for pursuing consent. The request was best given by a nurse or clinician who had been involved with the detailed management of the patient during life, and who was thus known to and trusted by the guardian. Sometimes provision of a coffin and/or transport home might afford an undue inducement: to counter this post-autopsy provisions were not be mentioned until after the guardian/s had decided whether or not to give their consent.

The statistics of consenting to the autopsy of children in this study suggest that guardians exercised their freedom to decline the procedure. Over the period of the pathology study, 2,147 patients with CM were enrolled into the overarching clinical study; of these 368 died, autopsy was requested from guardians of 325; requests were made when performance of an autopsy was deemed to be manageable. Of these 325 requests for consent to autopsy, consent was given in 103, a consent rate of 31.7 percent. This compares favorably to other pediatric autopsy series, in which acceptance rates ranged from 3.6% to 19%.^{10–11}

Reasons given by guardians for consenting to autopsy were similar to those identified in initial discussion with the clinical team: a wish to know the cause of death, a hope that others in the family could be spared a similar fate, a wish that the death could yet benefit future generations. It became obvious that a factor contributing to agreement was the fact

that the clinical team was perceived to have clearly worked hard to try to save the child's life.

Reasons given for declining were also similar to the former findings, but operational reasons were in greater prominence – especially the need to proceed quickly with the funeral and burial, which may have to take place at a distance from the hospital.

Feed-back about the findings of the autopsy was provided as soon as possible, to the guardians or parents. Sometimes this could be done immediately; more often it was helpful to wait a few days during which initial laboratory results on sampled tissues could become available, and the results could inform the feed-back at an appointment arranged with the family. Guardians almost always seemed to appreciate being able to discuss the child's illness and sometimes to know that there was an explanation other than witchcraft.

Special considerations

As the study progressed, the presentation of retinopathy was both diagnostic and prognostic in cerebral malaria.¹³ To know how this abnormality might relate to events in the brain at a histopathological level, it became important to obtain eyes at autopsy. Replacement with glass eyes minimized disfigurement of the face. This extension to the autopsy was permitted by COMREC and added to the consent process. When the collaborating ophthalmologist sought ethical approval at one of her academic affiliates outside of Malawi, it was denied; this generated an interesting and illuminating discussion about the ethical jurisdictions of local (vs remote) research ethics committees.¹⁴

When many full autopsies had eventually been conducted, additional questions relating to some localized tissues could be addressed with limited post-mortem sampling; these did not require the autopsy team or the presence of a pathologist. For example, samples of cerebral frontal lobe tissue (supra-orbital route)¹⁵ and of subcutaneous tissue were readily obtained by needle aspiration¹⁶, involving minimal expenditure of time and causing little or no disfigurement.

An extensive and prolonged study of this kind provided an important opportunity for both local and international trainees to acquire skills in clinical assessment and management, documentation, storage and analysis of data, and histopathological and ultrastructural techniques. Such training was a necessary and valuable component of the whole program.

Value and limitations of autopsy

It was essential to have detailed prospective documentation of all possible clinical and laboratory information about each patient, so that pathological findings could be set in the fullest possible clinical context. Nevertheless the limitations of autopsy are clear, including: (1) samples represent a single point in time, the time of death; (2) some delay is inevitable between the moment of death and the obtaining of tissues, during which further changes may occur; (3) an association of features may be demonstrated, but not whether that association is causal; (4) the process of autopsy may itself alter the findings.

An example of the latter in this study was in the search for evidence that death in cerebral malaria may be due to raised intracranial pressure and cerebral herniation. While signs of raised pressure were common at autopsy, significant herniation was only occasionally found. When ante-mortem magnetic resonance imaging (MRI) became available, herniation was common and was associated with a fatal outcome, but that it was reversible in survivors.¹⁷ It became clear that removal of the calvarium at autopsy allowed the macroscopic features of herniation to resolve so that they could not be detected when the base of the brain was examined. These findings have provided a basis for imminent clinical studies to test the hypothesis that preventing cerebral swelling or its consequences may reduce the case fatality of cerebral malaria in children.

Findings in brief

Autopsies have led to several new insights into the pathogenesis of the most commonly fatal form of severe *P falciparum* malaria. They demonstrated that not all patients dying with coma and parasitemia died of malaria, and that retinopathy identified during life could distinguish malaria deaths from others.¹⁸ Autopsies showed that in cerebral malaria, parasites sequester in multiple tissues as well as the brain,¹⁹ and genetic analysis of parasites indicated a narrower range of parasite clonality – in both peripheral blood and all tissues – in fatal disease than in uncomplicated infections. At sequestration sites throughout the body there is commonly deposition of fibrin and accumulation of platelets within microvessels,²⁰ associated with a loss of endothelial protein C receptors.²¹ The study confirmed clinical evidence that severe hepatic, pulmonary and renal damage are not important features of fatal malaria in children.²² Autopsies proved the common presence of cerebral swelling in fatal malaria, although it took ante-mortem MRI to quantify intracranial pressure and to demonstrate cerebral herniation, features associated with a fatal outcome.²³

While histopathology has both benefits and limitations, in the context of clinical, laboratory and radiological assessments it may allow the development of hypotheses testable in life. Further studies may contribute to progress against this major cause of death in African children.

CONSIDERATIONS FOR PATHOLOGY ENDPOINTS IN HIV STUDIES IN SOUTH AFRICA

Human Immunodeficiency Virus (HIV) and HIV clinical research in South Africa

South Africa has the largest HIV epidemic in the world and the largest antiretroviral treatment program.^{1,24} Voluntary medical male circumcision (VMMC), provision of pre-exposure prophylaxis (PreP) for special populations, programs and widespread distribution of condoms have reduced new HIV infections, but young women remain at extreme risk of acquiring HIV.^{1,24}

Because South Africa continues to have high HIV incidence, and also has well developed infrastructure, it has also become a leading site for HIV vaccine research studies, with informed/mobilized communities who participate in disrupting the HIV epidemic, and strong support from international collaborations.^{25,26} Such HIV research efforts have begun

to incorporate pathology approaches in clinical research, and have most recently even incorporated the use of immunohistochemistry/Immunofluorescence (IHC/IF) microscopy endpoints into some clinical studies.

Usefulness of pathology approaches and IHC/IF microscopy in HIV clinical studies

As the majority of HIV infections in South Africa and the world occur via sexual transmission²⁷, it is important to determine how the mucosal environment can facilitate or prevent the establishment of HIV infection. This is highlighted by the fact that immune defenses in the genital/mucosal compartment are often compartmentalized.^{28–33} IHC/IF approaches are uniquely suited to characterize the localization and distribution of responses at sites of HIV exposure.

HIV prevention strategies may also need to balance priming of a potent immune response with minimizing inflammation at mucosal sites, as inflammation may contribute to increased HIV transmission.^{34,35} In this context, pathology tools are capable of assessing clinical and subclinical inflammation in these tissues, and can support safety assessments in vaccine trials.³³ This may be especially important in Africa, as inflammation at the mucosal/genital compartments can be greater in individuals at risk of HIV^{36,37} and some studies have indicated that local inflammation can affect the efficacy of interventions.^{38–40}

Pathology approaches are also important in informing public health decisions regarding HIV care. Studies demonstrating that treatable infections, such as tuberculosis (TB) and cryptococcal disease are common autopsy findings in HIV-associated mortality,^{41,42} have promoted screening and treatment programs for HIV infected individuals aiming to reduce mortality and to improve TB control.

However, pathology approaches, and especially IHC/IF do not have the throughput or sensitivity of other approaches, and thus are unlikely to become assays used in large and complex observational studies. They also are not included in HIV prevention efficacy studies, where intact mucosal surfaces are essential to estimate an intervention's activity. Therefore, most pathology approaches to date have been introduced in small phase I studies and cohort analysis.

Tissue Sample Collections in South Africa

In high HIV prevalence settings, VMMC provides men with a cost-effective intervention that achieves a 53–66% reduction in the risk of infection.^{43–47} In only 6 years, South Africa aims to circumcise 4.3 million, and reach 80% circumcision prevalence among men 15–49.¹ The scaling up of VMMC provides unique opportunities to access discarded tissue from circumcisions, which under normal circumstances would be incinerated. As most of the men circumcised are under 25 and have been tested for HIV, such tissue affords the opportunity to characterize HIV target cells in the foreskin, as well as the barriers that reduce infections in heterosexual men.

Various programs have planned pathology approaches to study discarded tissue from VMMC. For example, the Male Mucosal Study funded by the European & Developing Countries Clinical Trials Partnership (EDCTP) focused on collecting formalin-fixed foreskin

samples from young men 14–25 years of age obtained from VMMC at Kwazulu-Natal's Edendale Hospital and Whizzkids. A collaborative project among the University of Cape Town, North Western University, University College London, and the Karolinska Institute plans to characterize the immune targets of HIV infection, as well as increase understanding of the effect of sexually transmitted infections on HIV susceptibility. Using immunofluorescence microscopy, this group evaluated keratin thickness of inner and outer foreskin, as a measure of barrier functions in adolescents.⁴⁸

A collaboration between the Khula Ndoda circumcision clinic, managed by the Perinatal HIV Research Unit, at the Chris Hani-Baragwanath Academic Hospital in Soweto, Fred Hutchinson Cancer Research Center and the National Institute of Communicable Diseases (NICD) has operationalized the collection of fresh foreskin tissue for explant culture. With the support of the Collaboration for AIDS Vaccine Discovery (CAVD) from the Bill and Melinda Gates Foundation, they conducted studies of antibody transudation in foreskin explants of HIV negative men by immunofluorescence. They demonstrated that antibody first diffuses freely into dermal compartments of the foreskin (Figure 3), and it is later captured by cells that localize it into epithelial compartments.

In addition to tissue studies of the male foreskin, the gastrointestinal and female genital tract mucosae are also important sites of HIV transmission that need to be assessed in countries with high HIV incidence. Small biopsies of these mucosal tissues can be collected by specialized clinicians, but may require a collaborative effort among several specialists, presenting large operational challenges. The Cape Town Assay Controls (CTAC) mucosal protocol was established among the Desmond Tutu HIV Centre Groote Schuur Hospital Clinical Research Site (GSHCRS), the University of Cape Town Gastroenterology, Obstetrics and Gynaecology departments at the Groote Schuur Hospital, and the Cape Town HVTN Immunology Laboratory (CHIL). Since opening in 2014, the protocol has collected colonic (n=18), as well as vaginal and cervical tissue samples (n=12) from healthy volunteers with low HIV risk, as collections may increase HIV risk while healing.

It has been proposed that clinical trial volunteers might be less willing to donate biopsy samples, and that participant retention might be affected by this kind of invasive sampling. Although rectal biopsies are painless (as there are no pain receptors in the colon mucosa), anoscope insertion can be embarrassing and invasive. Cervical and vaginal biopsies can cause discomfort, although many female participants have experienced vaginal and cervical clinical procedures for cervical cancer prevention and management, such as speculum insertion and pap smears. To assess acceptability and tolerance of repeated biopsy collections in healthy, low risk volunteers from the rectum, vagina and cervix, the USA National Institute of Allergy and Infectious Disease (NIAID) funded the HIV Vaccine Trials Network HVTN116 ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02797171) identifier NCT02797171). The study has opened in February 2017, and plans to enroll 110 men and women with low HIV risk in four USA cities as well as Cape Town.

Fixation and Embedding

Formalin fixation provides great morphological preservation and long-term stability of the tissue after paraffin embedding. Such a technique was used in the Male Mucosal Study successfully, to measure the thickness of the foreskin epithelial barrier.⁴⁷

However, one limitation to processing of formalin fixed samples in South Africa, is securing a pure supply of ethanol without methanol contamination, which is essential for proper dehydration of formalin-fixed samples previous to embedding. Methanol contamination can impair many IHC/IF antigens, and most commercial pathology laboratories in the country perform ethanol/methanol or methanol based dehydration procedures for hematoxylin and eosin stains. One alcohol-free fixation/embedding procedure is presented in Figures 3 and 4, developed as part of the CAVD project in Johannesburg.

Freezing of fresh tissue samples in OCT compound is also possible, but requires -80°C storage, access to liquid nitrogen, methyl butane for freezing, with their associated logistical considerations. For example, HVTN116 will include embedding of rectal, vaginal and cervical biopsies in OCT compound in Cape Town. The goal is to study the localization of infused broadly neutralizing antibodies, VRC01 and VRC01-LS, in mucosal and genital surfaces.

IHC Staining and Image Analysis

For clinical trial purposes, sample sectioning, IHC staining, imaging and pathology analysis require more support. Many of the supplies for IHC need to be imported, and the supply chain can be slow. Several large public hospitals have their own pathology services, but they are understaffed and may not have both hematology and solid organ expertise, or capacity to evaluate clinical trial samples. There are at least two commercial pathology services certified by the South African National Accreditation System (SANAS) and ISO Standard 15189, with operational capacity in Cape Town, Johannesburg, Durban and Pretoria.

HVTN 116 involves sample collections, embedding of biopsies and ex-vivo explant assays in South Africa. However, to ensure comparability with US collected samples, IHC assays will be conducted at a single laboratory in the US, where anti-idiotypic staining will be used to identify the location of infused monoclonal antibodies VRC01 and VRC01-LS in tissues. If a successful validation of the preservation and embedding techniques in South Africa are demonstrated in this phase I study, further training of local staff might allow us to conduct the assay endpoint locally.

Conclusion

In South Africa, HIV clinical studies have recently introduced some pathology endpoints into cohort studies and phase I trials. Pathology approaches provide a unique ability to define immune response locations within the mucosa, and to characterize subclinical inflammatory responses potentially induced by an intervention. South Africa is developing strong collaborations to support the collection, processing, embedding, sectioning and staining of IHC samples. So far, pathology approaches have been instrumental for the study of HIV-TB co-morbidities, and the characterization of foreskin barrier functions and

antibody transudation. Results of these approaches in the study of preventive approaches within the female reproductive track and the colon will be available to the public in upcoming years.

ONCOLOGY IN LOW-RESOURCE SETTINGS IN AFRICA

Burden of cancers in adults and children in low and middle income countries

Among “less developed regions”, defined as all regions of Africa, Asia (excluding Japan), Latin America and the Caribbean, Melanesia, Micronesia and Polynesia, the leading causes of cancer among women are breast, cervical, and lung; breast cancer is the leading cause of cancer-related death among women in these regions, followed by lung cancer and cervical cancer. Among men, lung cancer, liver cancer, and stomach cancer are the leading causes of cancer and cancer-related death in developing nations. Unfortunately, the outcomes remain poor for most patients with cancer in resource-limited countries, with case fatality rates inversely correlated with economic development.^{4,49} This disparity is in large part due to a weak or nonexistent oncology health care infrastructure that results in both delays to diagnosis and subsequent treatment. As a result, most patients present with advanced disease with limited access to effective cancer directed therapy.^{50,51}

While cancer is comparably rare in children, making up about 2% of all worldwide cancer diagnoses, the majority of childhood cancers sufferers (80%) live in low-and middle-income countries (LMIC). Children with cancers living in resource rich countries have excellent outcomes with average five-year survival of about 80%.^{51–53} This contrasts to the overall survival of children with cancers in low-income countries where it is oftentimes as low as 10–30%.^{51,54,55}

The incidence of different childhood cancers appears to vary between more affluent countries and LMIC.^{5,8} For example, acute lymphoblastic leukemia (ALL) and brain tumors are the most common childhood cancers in the United States and Europe, however Burkitt Lymphoma, Wilms tumor and Kaposi sarcoma are more common in Sub-Saharan Africa (SSA).^{4,55} The increased incidences of Burkitt Lymphoma and Kaposi sarcoma in SSA are linked to higher incidences of or earlier infection with EBV and/or HIV.^{56,57} It is still unclear if there is a true difference in incidence for ALL and brain tumors, or if children with these cancers are misdiagnosed and/or never reach a cancer treatment centers.

There are many reasons why outcomes are inferior in LMIC: lack of access to cancer treatment centers, inability to give appropriate intensive treatment due to lack of supportive care and unavailable drugs, and treatment abandonment, just to name a few.^{52,54} How inaccuracies in pathology diagnosis impact survival is difficult to study in the setting of limited resources, due to inadequate data capturing and high rates of loss to follow up and treatment abandonment. However, the treatments for different childhood cancers vary significantly, therefore incorrect diagnoses would have an impact on the survival of children with cancers. For example, Burkitt Lymphoma, lymphoblastic lymphoma and rhabdomyosarcoma, can present in similar ways and appear as small round blue cells on hematoxylin and eosin staining (H&E). The treatment for each of these childhood cancers is very different in intensity and length of treatment, and treatment modalities.

Challenges to pathologic diagnosis in LMIC

Appropriate treatment of cancers requires correct diagnosis, resulting from the careful integration of the pathologist's findings in the tissue with the overall clinical setting. Because resources for appropriate tissue procurement, tissue processing, and pathologic interpretation are all limited in LMIC,^{58–63} there is an increased risk of incorrect diagnosis in such countries, compared to those in which these resources are available.

Challenges to tissue procurement include the frequent utilization of inexpensive fine needle aspiration (FNA), in which the tissue is disaggregated, in place of slightly more expensive core needle biopsies or significantly more expensive operating room-based excisions, in which the complex architecture of the tissue is retained. For solid tumors, in which the individual cancer cells usually look entirely different from their benign counterparts, the microscopic evaluation of FNAs can often provide strong support for a malignant diagnosis. In contrast, for tumors of hematopoietic origin (so-called “liquid tumors”), the individual benign and malignant cells often look very similar, such that evaluation of the growth pattern of these cells, in the context of the overall architecture of the intact tissue, is often critical for distinguishing benign from malignant processes.

Even when adequate starting tissue is obtained in LMIC for diagnosis, significant tissue-processing challenges often exist. In most pathology laboratories in both LMIC and more affluent settings, the substrate for the large majority of pathologic diagnoses is formalin-fixed, paraffin-embedded tissue (FFPE), which is microscopically evaluated by the pathologist on glass slides. The requirements for adequate tissue processing, routine sectioning, and staining are substantial and include specialized equipment, trained staff, and access to consumables and reagents. Unfortunately, compared to their counterparts in more affluent areas, pathology laboratories in LMIC experience higher rates of poor-quality fixation, embedding, and/or sectioning that limit the ability of the pathologist to visualize the cells of interest under the microscope.

In addition to suboptimal tissue procurement or processing, a third major challenge in LMIC pathology is the frequent reliance on routine, inexpensive, hematoxylin and eosin (H&E) staining, rather than more definitive techniques, to make cancer diagnoses. As our understanding of the biology of specific cancers has increased, so has our understanding of potential high-yield therapeutic targets that vary between the different cancer types; the ever-increasing cell type-specific nature of modern cancer therapy now requires exact knowledge of the tumor cell type to enable optimal therapy. While H&E histology is usually sufficient to distinguish benign from malignant cells in adequately fixed and processed tissue, it is often NOT sufficient to determine the exact tumor cell type among malignancies, particularly for metastatic cancers identified in secondary locations such as lymph node, liver, lung, and brain. For example, the so-called “small blue round cell tumors” are a family of very different tumor cell types requiring different therapies, which nonetheless look similar on H&E-stained sections. In such cases, more expensive and/or complex ancillary techniques, such as IHC, as shown in Figure 5, are usually required to facilitate the definitive identification of tumor cell type. Many pathology laboratories in LMIC lack the resources to perform IHC, and therefore run a much greater risk of misclassifying tumors compared to laboratories in which IHC is performed.

A fourth major challenge to accurate diagnosis and prognosis is the lack of standard genetic and/or molecular diagnostic techniques, including fluorescence in situ hybridization, chromogenic in situ hybridization, polymerase chain reaction, and cytogenetics. Like IHC, genetic and/or molecular techniques help establish diagnosis and/or prognosis, and are becoming more and more established in non-LMIC areas; in contrast, few LMIC pathology laboratories have access to these techniques.

Finally, for particularly aggressive hematopoietic cancers in which immediate diagnosis and initiation of therapy are critical for patient survival (e.g., acute leukemia and high-grade lymphoma), even good quality histology, IHC, and molecular diagnostics may not be adequate for optimal patient care. In such cases in which rapid and accurate diagnosis are required, multiparametric flow cytometry (MFC) on non-fixed tissue is currently without equal (Figure 6). Ironically, despite the fact that many LMIC already have MFC infrastructure to allow T cell subset monitoring in HIV-infected patients, this technology has not been adequately leveraged to aid in the diagnosis of aggressive hematopoietic cancers in LMIC.

The consequence of these challenges to the entire process of pathology in LMIC is an unacceptably high error rate when diagnostic accuracy is rigorously studied. The example of Burkitt lymphoma (BL) diagnosis will be used to propose a streamlined, cost-effective, manual IHC algorithm, plus a robust MFC assay, that both offer high diagnostic sensitivity and specificity, and should be relatively straightforward to implement in LMIC.

Streamlining of Burkitt Lymphoma diagnosis in Uganda

The Fred Hutchinson Cancer Research Center (FHCRC) and the Uganda Cancer Institute (UCI) have had a decade-long partnership with the goal of studying infectious disease-related cancers, like Kaposi sarcoma (HHV8), cervical cancer (HPV strains) and Burkitt Lymphoma (EBV). By studying the infectious agents and their role in oncogenesis, investigators hope to find new preventative and treatment modalities that can be applied to patients living in low-resource settings.

EBV was the first human virus known to cause cancer and is associated with Burkitt Lymphoma, nasopharyngeal carcinoma and certain stomach cancers. How EBV causes any of these cancers remains unclear and is the subject of ongoing research. The role EBV plays in the pathogenesis of endemic Burkitt Lymphoma is particularly interesting as it is found in 90–95% of all endemic Burkitt Lymphoma cases in Africa. There is also an overlap between areas where malaria is endemic and areas with high rates of BL.⁶⁴ This interplay of pathogens make BL the most common childhood cancer in Uganda and many other countries in sub-Saharan Africa.

There are more than 70 pediatric cases of suspected BL seen each year at the UCI, where it was first described by Denis Burkitt in 1958. These children are now followed through a clinical care project which assures that all patients receive a tumor biopsy and pathology diagnosis. For many of these patients a diagnosis from two pathology laboratories are available, both of which rely on just H&E staining. A discordance rate between the two pathology laboratories is as high as 40% (unpublished data) was noted.

To investigate inaccuracy in pediatric BL diagnosis at the UCI, the original FFPE blocks from the local pathology laboratories were obtained for roughly 60 purported BL cases after Institutional Review Board approval. These blocks were re-embed and sectioned in the new, state-of-the-art histopathology lab built on the UCI campus through a UCI-FHCRC collaboration. As part of this collaboration, a Ugandan histotechnologist was trained to by a highly skilled US histotechnologist. Because IHC is not yet on-line in this laboratory, the sections were sent to a commercial laboratory in the US (PhenoPath Laboratories, Seattle, WA), where the sections were immunostained and interpreted by a US-based pathologist, according to the LMIC-targeted algorithm in Figure 5A. The algorithm was designed to minimize both the number of immunostains required for definitive diagnosis of each individual case, and the total number of different immunostains required for the laboratory to effectively diagnose both hematopoietic and non-hematopoietic cancers. Underlying this emphasis on efficiency was the hope that the stains can be performed manually, without the need for expensive automated stainers or expensive proprietary reagents.

For the diagnosis of BL, the algorithm proved very successful, in that the use of only 3 stains - an H&E, plus CD20 and c-Myc IHC - enabled definitive recognition of all cases of BL (Figure 5B and C). Confirming the algorithm's utility for BL diagnosis were follow-up in situ hybridization studies showing the expected presence of EBV early RNA (EBER-1) in all IHC-identified BL cases. TdT IHC was included as a third initial immunostain, but did not provide significant information beyond the CD20 and c-Myc IHC, suggesting that TdT IHC can be omitted from the upfront diagnostic workup of suspected BL.

While the success of the algorithm as striking, it was disconcerting that about 20% of the 60 purported BL cases were misdiagnosed (unpublished data). Nevertheless, the algorithm did allow the large majority of non-BL cases to be classified, with the range of diagnoses including B cell non-Hodgkin lymphoma of non-BL type, myeloid sarcoma, rhabdomyosarcoma, and neuroendocrine neoplasms of apparent epithelial or non-epithelial origin.

In parallel with the development of the IHC algorithm for FFPE, a 4-tube, 5-color MFC assay capable of identifying the full range of hematopoietic neoplasms from a fresh specimen (blood, bone marrow, tissue, and/or body fluid) was designed and validated. MFC is particularly appropriate for settings in which urgent diagnosis is required, e.g., suspected acute leukemia or impending superior vena cava syndrome or spinal cord compression. The assay design appears in Figure 6A, and is based on new, dried-down antibody technology that obviates the need for cold chain for antibody transport, and is therefore ideal for LMIC in warm climates, such as sub-Saharan Africa. Although this assay has not yet been used on African patient material, it has been validated in a US flow cytometry laboratory (PhenoPath, Seattle, WA) on a large number of normal and abnormal specimens, with the abnormal specimens including a variety of different hematopoietic neoplasms. Figure 6B shows the ability of this assay to identify Ramos Burkitt lymphoma cells (colored black) in a background of normal lymphocytes.

Impact on Clinical Care of patients with cancers in LMIC

Diagnostic inaccuracies and uncertainties have a significant impact on the treatment of patients with cancers in LMIC. Physicians may not be able to rely solely on the histologic diagnosis and their clinical impressions become much more important which is problematic as many cancers can mimic each other. Uncertainty surrounding the pathology diagnosis leads to questions as to whether the patient is receiving the most appropriate tumor directed treatment, especially when patients do not respond to treatment. Misdiagnosis wasted valuable resources and can also financially strain families and fragile healthcare systems. Studies like these can help streamline diagnosis while delivering high quality and accurate results.

Breast cancer treatment in SSA

Breast cancer, the most common cancer in SSA with nearly 100,000 persons diagnosed in 2012 (and likely underestimated), is characterized by poor survival with less than half of women diagnosed with the disease alive at five years in most countries and remains the second leading cause of cancer death in SSA.^{8,65} There are a variety of factors responsible for the poor survival among women with breast cancer in SSA compared with resource-rich regions including late stage at presentation and inadequate diagnostics.

Among patients with breast cancer, an accurate determination of the receptor status (i.e. estrogen receptor [ER], progesterone receptor [PR]) and expression of the human epidermal growth factor receptor 2 [HER2]) is essential to guide therapeutic decision-making, yet this information is often unavailable in resource-limited regions. Even when available, data on receptor status have often been limited by poor specimen quality attributable to tissue degradation and improper storage, and may yield falsely negative test results.^{67–69} A recent systematic review which assessed 26 studies from SSA and incorporating nearly 5,000 women, found vast heterogeneity with the majority of estimates of ER positive disease ranging from 20% to 70%.⁷⁰ Oftentimes, receptor targeted agents (e.g. tamoxifen) are given empirically when available but not informed by pathologic characteristics. As such, the clinical efficacy of the medication may be limited and the potential for harm is greater.

There are plans to expand research at the UCI/Fred Hutch Ugandan clinic to:

1. Characterize the molecular portrait and subtype prevalence of women presenting with breast cancer
2. Evaluate the potential to use widely available molecular technology to improve the diagnosis of breast cancer in resource-limited settings
3. Determine the feasibility of an oral chemotherapy regimen for the treatment of locally advanced breast cancer.

CONCLUSIONS

While these three research efforts are diverse in subject and scope, they share commonalities. The first is that a great deal of knowledge can be gained by systematically studying patients and disease states in sufficient numbers to draw meaningful conclusions. In

the case of the malaria studies in Malawi, valuable insight was gained concerning the pathogenesis of cerebral malaria, including information about the importance of increased brain volume and variability in the sequestration of parasitized red cells. In the case of the HIV studies in South Africa, the researchers have been able to gain insights into TB associated mortality in HIV infected individuals, the barriers to HIV transmission, the composition and location genital mucosal cells and antibodies. Pathology approaches are planned to inform the mucosal pharmacokinetics of HIV prevention strategies, such as the passive transfer of broadly neutralizing monoclonal antibodies. Finally, in the case of cancer diagnostic testing in research Uganda, the researchers were able to capitalize on a large patient population and existing tissue collection system to establish pared down but valuable diagnostic approaches for Burkitt's lymphoma that make regional testing more feasible.

In every case, productive collaborations were established and sustained among multi-country investigators, physicians, healthcare providers, and technicians. In all of the cases, African partners were necessary for funding, study design, ethical review and informed consent of patients, collecting samples, and processing material for long-term study. All of these collaborations resulted in opportunities for partners to develop and hone new skills, and to address research questions relevant to their LMIC needs.

The foreign investigators benefited greatly from research. In the case of HIV prevention research, mucosal characterization is more relevant in individuals at risk of HIV, as inflammation at the mucosal/genital compartments can be greater^{37,38} and interventions less efficacious.³⁹⁻⁴¹ The malaria researchers in Malawi developed a more nuanced cultural perspective over the course of their study which enhanced trust between all members of the research team, the patients and their families. This sensitivity also ensured that vulnerable sectors of the community were protected from exploitation through thoughtful consenting so that factors such as poverty did not play a role in the decision to participate in studies.

Logistical challenges are more common in many countries in Africa. Depending on the location, the infrastructure may not include access to high quality water, liquid nitrogen, or ice. The power supply can be variable, which impacts air conditioning and refrigeration. All of these can damage instrumentation, impede specimen collection, or damage the integrity of tissue samples. Procuring the supplies and reagents required to perform assays or preserve tissue can be complicated. In many cases in SSA, there is little or no access to formalin, so tissue might be fixed in alcohol solutions or worse, left unfixed for long periods of time. In addition, there is often a lack of high quality ethanol and dehydrating solutions which can effect downstream assays. These variables can of course greatly affect the outcome of many assays and limit the usefulness of the samples for diagnosis and research. And, of course, there may be insufficient human resources to perform assays or assemble the necessary teams for activities like rapid autopsy, etc.

Investigators frequently find a dearth of individuals with technical knowledge in low resource areas, often resulting from a lack of pathology training and a lack of or superficial training in histopathology. Advances like digital pathology and organizations working to improve access to collaborating pathologists from other regions are helping to address this need. Further attention, however, needs to be placed on more and better trained histology

professionals. Basic skills from tissue fixation, adequate paraffin processing, correct frozen tissue preservation, sectioning, and H&E staining are paramount. South Africa has implemented training in basic histology and effort should then be placed on training technicians in other areas. Then there should be additional training in special stains and IHC followed by flow cytometry and molecular techniques. In addition, a culture of quality assurance and quality control needs to be instituted so that the value of samples being collected and used is guaranteed and maintained. A great example of this is the newly implemented histopathology lab at the UCI-FHCRC clinic. A very experienced histotechnician from the United States lived in Kampala for over a year and trained two Ugandan technicians in basic histology. Because of the excellence of their work, the Burkitt's lymphoma group was able to perform high quality IHC on FFPE samples collected at the UCI. HIV Prevention clinical studies in South Africa depend on locally trained technicians and pathologists, who could support training of other African nations with less capacity.

The outcome of sustained engagement in pathology research in Africa will continue to benefit scientists by producing meaningful and novel insight into the pathogenesis of disease while building relationships, capacity and infrastructure in low resource areas. Efforts directed toward building histology and pathology capacity should be complemented by efforts directed toward gaining a better understanding into cultural aspects of healthcare.

References

1. UNAIDS. The Gap Report. UNAIDS; Geneva: 2014.
2. UNAIDS. Global AIDS update 2016. UNAIDS; Geneva: 2016.
3. World Malaria report 2015. World Health Organization; 2015.
4. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010; 127(12):2893–2917. [PubMed: 21351269]
5. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*. 2006; 3(11):e442. [PubMed: 17132052]
6. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015; 65(2):87–108. [PubMed: 25651787]
7. Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and Trends—An Update. *Cancer Epidemiol Biomarkers Prev*. 2016; 25(1):16–27. [PubMed: 26667886]
8. Bray F, Jemal A, Grey N, Ferlay J, Forman D. Global cancer transitions according to the Human Development Index (2008–2030): a population-based study. *Lancet Oncol*. 2012; 13(8):790–801. [PubMed: 22658655]
9. Taylor TE, Molyneux ME, Wirima JJ, Fletcher KA, Morris K. Blood glucose levels in Malawian children before and during the administration of intravenous quinine for severe falciparum malaria. *N Engl J Med*. 1988; 319:1040–47. [PubMed: 3050516]
10. Ansari NA, Kombe AH, Kenyon TA, Mazhani L, Binkin N, Tappero JW, Gebrekristos T, Nyirenda S, Lucas SB. Pathology and causes of death in a series of human immunodeficiency virus-positive and -negative pediatric referral hospital admissions in Botswana. *Pediatr Infect Dis J*. 2003 Jan; 22(1):43–7. [PubMed: 12544408]
11. Oluwasola AO, Fawole OI, Otegbayo JA, Ayede IA, Ogun GO, Ukah CO, Bamigboye AE. Trends in clinical autopsy rate in a Nigerian tertiary hospital. *Afr J Med Med Sci*. 2007 Sep; 36(3):267–72. [PubMed: 18390067]
12. Molyneux ME, Taylor TE, Wirima JJ, Borgstein A. Clinical features and prognostic indicators in paediatric cerebral malaria: A study of 131 comatose Malawian children. *Q J Med*. 1989; 265:441–59.

13. Taylor TE. Caring for children with cerebral malaria: insights gleaned from 20 years on a research ward in Malawi. *Trans R Soc Trop Med Hyg.* 2009; 103(Suppl):S6–10. [PubMed: 19128813]
14. Lewallen S, Taylor TE, Molyneux ME, Wills BA, Courtright P. Ocular fundus findings in Malawian children with cerebral malaria. *Ophthalmology.* 1993; 100(6):857–61. [PubMed: 8510897]
15. Mfutso-Bengo JM, Taylor TE. Ethical jurisdictions in international biomedical research. *Trends in Parasitology.* 2002; 18(5):231–34. [PubMed: 11983605]
16. Milner DA, Dzamalala CP, Liomba NG, Molyneux ME, Taylor TE. Sampling of supraorbital brain tissue after death: improving on the clinical diagnosis of cerebral malaria. *J Infect Dis.* 2005; 191:805–808. [PubMed: 15688299]
17. Moxon CA, Wassmer SC, Milner DA Jr, Chisala NV, Taylor TE, Seydel KB, Molyneux ME, Faragher B, Esmon CT, Downey C, Toh CH, Craig AG, Heyderman RS. Loss of endothelial protein C receptors links coagulation and inflammation to parasite sequestration in cerebral malaria in African children. *Blood.* 2013; 122(5):842–51. [PubMed: 23741007]
18. Seydel KB, Kampondeni SD, Valim C, Potchen MJ, Milner DA, Muwalo FW, Birbeck GL, Bradley WG, Fox LL, Glover SJ, Hammond CA, Heyderman RS, Chilingulo CA, Molyneux ME, Taylor TE. Brain swelling and death in pediatric cerebral malaria. *N Engl J Med.* 2015; 72(12): 1126–37.
19. Taylor TE, Fu W, Carr R, Whitten R, Mueller J, Fosiko N, Lewallen S, Liomba N, George Molyneux M. Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nature Medicine.* 2004; 10(2):143–145.
20. Milner DA Jr, Lee JJ, Frantzreb C, Whitten RO, Kamiza S, Carr RA, Pradham A, Factor RE, Playforth K, Liomba G, Dzamalala C, Seydel KB, Molyneux ME, Taylor TE. Quantitative assessment of multiorgan sequestration of parasites in fatal pediatric cerebral malaria. *J Infect Dis.* 2015; 212(8):1317–21. [PubMed: 25852120]
21. Montgomery J, Milner DA, Tse MT, Njobvu A, Kayira K, Dzamalala F, Taylor TE, Rogerson SJ, Craig AG, Molyneux ME. Genetic analysis of circulating and sequestered populations of *Plasmodium falciparum* in fatal pediatric malaria. *J Infect Dis.* 2006; 194(1):115–122. [PubMed: 16741890]
22. Wassmer SC, Taylor T, MacLennan CA, Kanjala M, Mukaka M, Molyneux ME, Grau GE. Platelet-induced clumping of *Plasmodium falciparum*-infected erythrocytes from Malawian patients with cerebral malaria-possible modulation in vivo by thrombocytopenia. *J Infect Dis.* 2008; 197(1):72–8. [PubMed: 18171288]
23. Milner DA Jr, Whitten RO, Kamiza S, Carr R, Liomba G, Dzamalala C, Seydel KB, Molyneux ME, Taylor TE. The systemic pathology of cerebral malaria in African children. *Front Cell Infect Microbiol.* 2014; 4:104. [PubMed: 25191643]
24. SANAC SANAC. South Africa Global AIDS Progress Report. 2015:1–69.
25. Lan, GJ., Blom, A., Kamalski, J., Lau, G., Baas, J., Adil, MA. Decade of Development in Sub-Saharan African Science, Technology, Engineering & Mathematics Research. Elsevier. , editor. World Bank; Washington DC: 2014.
26. Pouris A. Science in South Africa: The dawn of a renaissance? *South African Journal of Science.* 2012; 108(7/8)
27. Shisana, O., R, T., Simbayi, LC., Zuma, K., Jooste, S., Zungu, N., Labadarios, D., Onoya, D., et al. Incidence and Behaviour Survey 2012. Cape Town: 2014. South African National HIV Prevalence.
28. Belyakov IM, et al. A novel functional CTL avidity/activity compartmentalization to the site of mucosal immunization contributes to protection of macaques against simian/human immunodeficiency viral depletion of mucosal CD4+ T cells. *J Immunol.* 2007; 178(11):7211–21. [PubMed: 17513770]
29. Perreau M, et al. DNA/NYVAC vaccine regimen induces HIV-specific CD4 and CD8 T-cell responses in intestinal mucosa. *J Virol.* 2011; 85(19):9854–62. [PubMed: 21775454]
30. Ferre AL, et al. Immunodominant HIV-specific CD8+ T-cell responses are common to blood and gastrointestinal mucosa, and Gag-specific responses dominate in rectal mucosa of HIV controllers. *J Virol.* 2010; 84(19):10354–65. [PubMed: 20668079]

31. Yang OO, et al. Differential blood and mucosal immune responses against an HIV-1 vaccine administered via inguinal or deltoid injection. *PLoS One*. 2014; 9(2):e88621. [PubMed: 24558403]
32. Lemos MP, et al. In men at risk of HIV infection, IgM, IgG1, IgG3, and IgA reach the human foreskin epidermis. *Mucosal Immunol*. 2015
33. Baden LR, et al. Induction of HIV-1-specific mucosal immune responses following intramuscular recombinant adenovirus serotype 26 HIV-1 vaccination of humans. *J Infect Dis*. 2015; 211(4):518–28. [PubMed: 25165165]
34. Haaland RE, et al. Inflammatory genital infections mitigate a severe genetic bottleneck in heterosexual transmission of subtype A and C HIV-1. *PLoS Pathog*. 2009; 5(1):e1000274. [PubMed: 19165325]
35. Lewis GK, DeVico AL, Gallo RC. Antibody persistence and T-cell balance: two key factors confronting HIV vaccine development. *Proc Natl Acad Sci U S A*. 2014; 111(44):15614–21. [PubMed: 25349379]
36. Taha TE, et al. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS*. 1998; 12(13):1699–706. [PubMed: 9764791]
37. Kaul R, et al. Inflammation and HIV Transmission in Sub-Saharan Africa. *Curr HIV/AIDS Rep*. 2015; 12(2):216–22. [PubMed: 25877253]
38. Naranbhai V, et al. Innate immune activation enhances hiv acquisition in women, diminishing the effectiveness of tenofovir microbicide gel. *J Infect Dis*. 2012; 206(7):993–1001. [PubMed: 22829639]
39. Moodie Z, et al. Continued Follow-Up of Phambili Phase 2b Randomized HIV-1 Vaccine Trial Participants Supports Increased HIV-1 Acquisition among Vaccinated Men. *PLoS One*. 2015; 10(9):e0137666. [PubMed: 26368824]
40. Benlahrech A, et al. Adenovirus vector vaccination induces expansion of memory CD4 T cells with a mucosal homing phenotype that are readily susceptible to HIV-1. *Proc Natl Acad Sci U S A*. 2009; 106(47):19940–5. [PubMed: 19918060]
41. Karat AS, et al. Measuring mortality due to HIV-associated tuberculosis among adults in South Africa: Comparing verbal autopsy, minimally-invasive autopsy, and research data. *PLoS One*. 2017; 12(3):e0174097. [PubMed: 28334030]
42. Karat AS, et al. Autopsy Prevalence of Tuberculosis and Other Potentially Treatable Infections among Adults with Advanced HIV Enrolled in Out-Patient Care in South Africa. *PLoS One*. 2016; 11(11):e0166158. [PubMed: 27829072]
43. Auvert B, et al. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. *PLoS Med*. 2005; 2(11):e298. [PubMed: 16231970]
44. Bailey RC, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. *Lancet*. 2007; 369(9562):643–56. [PubMed: 17321310]
45. Gray RH, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomized trial. *Lancet*. 2007; 369(9562):657–66. [PubMed: 17321311]
46. Impact, U.W.S.E.G.o.M.t. and H.I.V.P. Cost of Male Circumcision for, Male circumcision for HIV prevention in high HIV prevalence settings: what can mathematical modelling contribute to informed decision making? *PLoS Med*. 2009; 6(9):e1000109. [PubMed: 19901974]
47. Njeuhmeli E, et al. Voluntary medical male circumcision: modeling the impact and cost of expanding male circumcision for HIV prevention in eastern and southern Africa. *PLoS Med*. 2011; 8(11):e1001132. [PubMed: 22140367]
48. Chigorimbo-Tsikiwa, NGZ., Bekker, LG., Blackburn, J., Gray, C. R4P Research for Prevention. Chicago: 2016. Protein Correlates of HIV Prevention in the Male Genital Tract.
49. Farmer P, Frenk J, Knaul FM, et al. Expansion of cancer care and control in countries of low and middle income: a call to action. *Lancet*. 2010; 376(9747):1186–1193. [PubMed: 20709386]
50. Kingham TP, Alatisse OI, Vanderpuye V, et al. Treatment of cancer in sub-Saharan Africa. *Lancet Oncol*. 2013; 14(4):e158–167. [PubMed: 23561747]
51. Cazap E, Magrath I, Kingham TP, Elzawawy A. Structural Barriers to Diagnosis and Treatment of Cancer in Low- and Middle-Income Countries: The Urgent Need for Scaling Up. *J Clin Oncol*. 2016; 34(1):14–19. [PubMed: 26578618]

52. Gupta, SHS., Hunger, SP., et al. Treating Childhood Cancer in Low- and Middle-Income Countries. In: Gelband, HJP.Sankaranarayanan, R., et al., editors. Cancer: Disease Control Priorities. third. Vol. 3. Washington (DC): The International Bank for Reconstruction and Development/The World Bank; 2015 Nov 1. Chapter 7
53. Rodriguez-Galindo C, Friedrich P, Alcasabas P, et al. Toward the Cure of All Children With Cancer Through Collaborative Efforts: Pediatric Oncology As a Global Challenge. *J Clin Oncol*. 2015; 33(27):3065–3073. [PubMed: 26304881]
54. Hudson MM, Link MP, Simone JV. Milestones in the curability of pediatric cancers. *J Clin Oncol*. 2014; 32(23):2391–2397. [PubMed: 24733794]
55. Magrath I, Steliarova-Foucher E, Epelman S, et al. Paediatric cancer in low-income and middle-income countries. *Lancet Oncol*. 2013; 14(3):e104–116. [PubMed: 23434340]
56. Steliarova-Foucher, ECM.Ries, LAG.Hesseling, P.Moreno, F.Shin, HY., Stiller, CA., editors. International Incidence of Childhood Cancer. Vol. III. Lyon, France: International Agency for Research on Cancer; 2017. (electronic version)
57. Orem J, Mbidde EK, Lambert B, de Sanjose S, Weiderpass E. Burkitt's lymphoma in Africa, a review of the epidemiology and etiology. *Afr Health Sci*. 2007; 7(3):166–175. [PubMed: 18052871]
58. Tumwine LK, Orem J, Kerchan P, Byarugaba W, Pileri SA. EBV, HHV8 and HIV in B cell non Hodgkin lymphoma in Kampala, Uganda. *Infect Agent Cancer*. 2010; 5:12. [PubMed: 20591151]
59. Adesina A, Chumba D, Nelson AM, et al. Improvement of pathology in sub-Saharan Africa. *Lancet Oncol*. 2013; 14(4):e152–157. [PubMed: 23561746]
60. Barnabas M, Ngbea JA, Innocent E, Malu KN. Role of pathology in improving health care in poor resource environments. *Niger J Med*. 2014; 23(3):263–266. [PubMed: 25185385]
61. Naresh KN, Raphael M, Ayers L, et al. Lymphomas in sub-Saharan Africa—what can we learn and how can we help in improving diagnosis, managing patients and fostering translational research? *Br J Haematol*. 2011; 154(6):696–703. [PubMed: 21707579]
62. Ogwang MD, Zhao W, Ayers LW, Mbulaiteye SM. Accuracy of Burkitt lymphoma diagnosis in constrained pathology settings: importance to epidemiology. *Arch Pathol Lab Med*. 2011; 135(4): 445–450. [PubMed: 21466360]
63. Orem J, Sandin S, Weibull CE, et al. Agreement between diagnoses of childhood lymphoma assigned in Uganda and by an international reference laboratory. *Clin Epidemiol*. 2012; 4:339–347. [PubMed: 23277743]
64. Brady G, MacArthur GJ, PJ. Epstein–Barr virus and Burkitt lymphoma. *J Clin Pathol*. 2007 Dec; 60(12):1397–1402. [PubMed: 18042696]
65. Rambau PF. Pathology practice in a resource-poor setting: Mwanza, Tanzania. *Arch Pathol Lab Med*. 2011; 135(2):191–193. [PubMed: 21284436]
66. Sankaranarayanan R, Swaminathan R, Brenner H, et al. Cancer survival in Africa, Asia, and Central America: a population-based study. *Lancet Oncol*. 2010; 11(2):165–173. [PubMed: 20005175]
67. Bird PA, Hill AG, Houssami N. Poor hormone receptor expression in East African breast cancer: evidence of a biologically different disease? *Ann Surg Oncol*. 2008; 15(7):1983–1988. [PubMed: 18408976]
68. Kantelhardt EJ, Mathewos A, Aynalem A, et al. The prevalence of estrogen receptor-negative breast cancer in Ethiopia. *BMC Cancer*. 2014; 14:895. [PubMed: 25433805]
69. Nalwoga H, Arnes JB, Wabinga H, Akslen LA. Frequency of the basal-like phenotype in African breast cancer. *APMIS*. 2007; 115(12):1391–1399. [PubMed: 18184410]
70. Eng A, McCormack V, dos-Santos-Silva I. Receptor-defined subtypes of breast cancer in indigenous populations in Africa: a systematic review and meta-analysis. *PLoS Med*. 2014; 11(9):e1001720. [PubMed: 25202974]

SYNOPSIS

The process of conducting pathology research in Africa can be challenging. But the rewards in terms of knowledge gained, quality of collaborations, and impact to communities affected by infectious disease and cancer are great. This report reviews three different research efforts: fatal malaria in Malawi, mucosal immunity to HIV in South Africa, and cancer research in Uganda. What unifies them is the use of pathology based approaches to answer vital questions such as physiology, pathogenesis, predictors of clinical course, and diagnostic testing schemes.

KEY POINTS

- There is an increasing role for pathology data in understanding disease in low and middle income areas.
- Active collaboration with African partners is critical for the success of a study.
- There is a need to build pathology and histopathology infrastructure in low and middle income areas to support research.
- The capacity to perform complex techniques, such as multi-color immunofluorescence, flow cytometry, in situ hybridization, and digital pathology, will need to be added to research and clinical settings in Africa.

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A1	PITUITARY																							A1	PITUITARY
B1	FRONTAL																							B1	FRONTAL
B2	PARIETAL																							B2	PARIETAL
B3	TEMPORAL																							B3	TEMPORAL
B4	OCCIPITAL																							B4	OCCIPITAL
B5	HIPPOCAMPUS																							B5	HIPPOCAMPUS
B6	CAUDATE+CC																							B6	CAUDATE+CC
B7	THALAMUS																							B7	THALAMUS
B8	MIDBRAIN																							B8	MIDBRAIN
B9	PONS																							B9	PONS
B10	MEDULLA																							B10	MEDULLA
B11	CEREB TONSILS																							B11	CEREB TONSILS
B12	CER DENTATE																							B12	CER DENTATE
B13	SPINAL CORD																							B13	SPINAL CORD
B14	CHOROID PL																							B14	CHOROID PL
C1	OPTIC NERVE																							C1	OPTIC NERVE
D1	LUNG RUL											touch												D1	LUNG RUL
D2	LUNG RLL																							D2	LUNG RLL
D3	LUNG LUL																							D3	LUNG LUL
D4	LUNG LLL																							D4	LUNG LLL
D5	DIAPHRAGM																							D5	DIAPHRAGM
E2	R VENT+RA																							E2	R VENT+RA
E3	LEFT VENT											smear												E3	LEFT VENT
F1	SUBMANDIB + OESOPHAGUS																							F1	SUBMANDIB + OESOPHAGUS
F2	STOMACH + ILEUM																							F2	STOMACH + ILEUM
F3	JEJUNUM + RIGHT COLON																							F3	JEJUNUM + RIGHT COLON
F4	LIVER											touch												F4	LIVER
F5	PANCREAS + GALL BLADDER																							F5	PANCREAS + GALL BLADDER
G1	RIGHT KIDNEY																							G1	RIGHT KIDNEY
G2	LEFT KIDNEY																							G2	LEFT KIDNEY
G3	BLADDER + GONAD																							G3	BLADDER + GONAD
H1	THYROID + ADRENAL																							H1	THYROID + ADRENAL
J1	VERTEB BM																							J1	VERTEB BM
J2	SPLEEN + ABD O LN								spl spit				sp. only, touch				core							J2	SPLEEN + ABD O LN
J3	THYMUS CHEST LN																SP		SP		SP			J3	THYMUS CHEST LN
K1	PECTORALIS M. BRACHIAL PLEXUS WITH NECK M.																split							K1	PECTORALIS M. BRACHIAL PLEXUS WITH NECK M.
K2	CHEST SKIN + SUBCUTIS												fat only, smear											K2	CHEST SKIN + SUBCUTIS
N1	NEEDLE BX								fat only		fat only	fat only												N1	NEEDLE BX
N2	NEEDLE BX																							N2	NEEDLE BX

FIGURE 1.

Sampling proforma for autopsies performed for the study of clinico-pathological correlates of fatal cerebral malaria. Organs are listed by row. The first seven columns on the left represent samples destined for formalin fixation and paraffin embedding. Two samples (UBC vials and MAC) were preserved for electron microscopy. Tissue smears and touch preps were each prepared using 2 different approaches (air fixation, acetone fixation). Frozen samples (9 right most columns) were processed for expression analyses (in RNA later), IHC (in OCT), or were flash frozen.

FIGURE 2.

A body prior to autopsy, wrapped in the traditional shroud (a local chitenge), on the plinth of the mortuary at the Queen Elizabeth Central Hospital in Blantyre, Malawi.

FIGURE 3.

Within 2h of culture, injected IgG accumulates at the basal membrane of the epidermis in foreskin explants. Within 6h of collection, foreskin tissue explants from 3 men undergoing VMMC were sectioned, injected with antibodies (human IgG-Ax647), and cultured for 2h to observe the areas of antibody diffusion. Control samples received no antibody in their injection, or antibodies conjugated to another fluorochrome. Samples were fixed in formalin for 24h, and rinsed in water three times, rehydrated in two baths of 10% sucrose in 1X PBS, followed by three baths of 1X PBS, and embedded in OCT. After sectioning of the slides,

samples were stained with anti-CD138 stain and biotin-conjugated goat anti-mouse IgG1 F(ab')₂ and avidin-AlexaFluor568 (pseudocolored red). All slides were counterstained with DAPI.

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FIGURE 4.

Within 24h of foreskin explant culture, injected IgG can be identified in epithelial cells. Within 6h of collection, foreskin tissue explants from 3 men undergoing VMMC was sectioned, injected with antibodies (human IgG-Ax647), and cultured for 24h to observe the areas of antibody diffusion. Control samples received no antibody in their injection, or antibodies conjugated to another fluorochrome. Samples were stained as indicated in Figure 3. CD138 was lost from the explant's epithelium at long incubation times (24h), suggesting

some ex-vivo modifications. IgG deposited at epithelial basal membrane and dermis, with sparse cells.

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Figure 5.

Figure 5 A demonstrates the IHC algorithm tested on pediatric small blue round cell tumors from SSA. Because BL is the most common pediatric malignancy in SSA, the initial round of IHC was targeted to BL, and included antibodies to CD20, TdT, and c-MYC. When these three stains did not confirm BL, several second-tier IHC combinations were performed, according to the details of the algorithm. The application of this algorithm to roughly 65 pediatric SBRCT cases suggested that TdT IHC is expendable for reliable diagnosis of BL, allowing this diagnosis to be made with near certainty with only 3 slides: an H&E, and CD20 and c-MYC IHC. The lower 2 figures (Figure 5B) show low power (40 $\times$) and high power (400 $\times$) views of a representative, very small, 2 to 3 mm in oral biopsy from a pediatric BL patient in the patient series. The 40 $\times$ view shows an atypical diffuse lymphoid infiltrate, which at 400 $\times$ has the characteristic appearance of BL, including medium-sized tumor cells and a “starry sky” appearance. Importantly, using only CD20 and c-MYC IHC (Figure 5C), the diagnosis of BL can be rendered with nearly 100% sensitivity and specificity: the uniform CD20 supports a mature B cell process, while the uniform strong c-Myc protein expression strongly suggests the c-MYC gene-containing chromosome 8 translocation characteristic of eBL. Additional studies on this series confirmed the expected presence of EBV infection (positive EBER-1 in situ hybridization) and the expected absence of lymphoblastic differentiation (negative TdT IHC).

TUBE	FITC	PE	PE-TR	PE-Cy5	PE-Cy7
1	KAPPA / CD8	LAMBDA / CD4	CD45	CD3	CD20
2	CD5	CD10	CD45	CD34	CD19
3	CD38	CD7	CD45	CD56	CD33
4	CD15	CD117	CD45	CD14	HLA-DR

Figure 6.

Four-tube, five-color flow cytometric assay designed to detect all hematopoietic neoplasms. In this assay, a PE-Texas Red-conjugated CD45 antibody is included in each tube, to ensure comparability of cell identification (gating) across the tubes when used in conjunction with SSC and FSC. Tube 1 takes advantage of the mutually exclusive nature of immunoglobulin light chain (kappa/lambda) expression on B cells and CD4/CD8 expression on T cells, which allows this tube to contain FITC-conjugated kappa and CD8 antibodies, and PE-conjugated lambda and CD4 antibodies in the same tube. The CD3 and CD20 antibodies in this tube allow initial separation of T and B cells, respectively, among which kappa:lambda and CD4:CD8 ratios can be respectively determined. Figure 6B shows the proof of concept for BL diagnosis by the four-tube, five-color flow assay. The flow histograms (dot plots) in this figure show an experiment in which the BL cell line Ramos was added to benign pleural fluid at roughly 25% of the total cells. The flow assay easily identifies the tumor cells, which are colored black throughout the figure. As expected for BL, the tumor cells expressed the leukocyte-common antigen CD45, the pan-B cell antigens CD19, CD20, and HLA-DR, and the germinal center B cell antigens CD10 and CD38; the tumor cells lambda light chain-

restriction, confirming monoclonality, and increased forward scatter (FS INT) indicating increased cell size.

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