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## CHAPTER 8

# SUMMARIZING DISCUSSION

The work presented in this thesis was conducted in Mwanza, Tanzania, and was inspired by the tremendous burden of disease in the Tanzanian population. Neglected tropical diseases such as schistosomiasis, which may be seen once or twice in a lifetime by a physician practicing in a high-income country, are weekly if not daily diagnoses in health centers and hospitals, and disproportionately affect younger patients of lower socioeconomic status. HIV, though a worldwide epidemic, has also particularly afflicted sub-Saharan Africa. Over 1 in 20 Tanzanian adults is HIV-infected, and nearly 60% of these are young to middle-aged women (1). Moreover, like many other illnesses in Tanzania, HIV is often not diagnosed until patients have developed advanced disease, at which point it may be too late.

The goal of the thesis has been to conduct studies that, ultimately, will lessen the disproportionate burden of infectious diseases in sub-Saharan Africa, whether by preventing them altogether, by diagnosing them earlier, or by working to alleviate complications attributable to co-infections. The work has focused primarily on schistosomiasis and HIV in Tanzania, building on prior work in the field from Zimbabwe, Kenya, and several other countries in sub-Saharan Africa.

The discussion will be divided into three sections: Schistosomiasis and HIV Risk; Managing Schistosomiasis-HIV Co-Infection; and an Implementation Science Study to Improve Early Infant HIV Diagnosis. Each of these topics will be first discussed with reference to the contribution made by the findings presented in the preceding chapters, and then will turn towards an examination of the needed next research steps.

## I. Schistosomiasis and HIV Risk

### ***Schistosoma haematobium* Infection as a Risk Factor for HIV Acquisition**

*S. haematobium* infection was postulated to be a risk factor for incident HIV infection at least as early as 1994, long before Kjetland and colleagues published their clinical findings from Zimbabwe (2). A causal relationship between *S. haematobium* infection, usually acquired in childhood well before sexual debut, and HIV infection is biologically plausible for a number of reasons, as outlined in the introduction. Kjetland and colleagues documented for the first time a clinical association between *S. haematobium* infection and HIV in Zimbabwean women (3). Because of the biologic plausibility and multiple other lines of evidence suggesting that schistosomiasis could be a risk factor for HIV acquisition, the Zimbabwe findings were compelling. However, that work was conducted at a single site that may have had uniquely high rates of both *S. haematobium* (39%) and HIV (25%) infections. In addition, due to the study's cross-sectional design,

it was not able to demonstrate that *S. haematobium* infection preceded HIV infection, but merely that the two were significantly associated with one another.

Our work presented in **Chapter 2** provides strong new, independent evidence in support of the HIV-*S. haematobium* association. We enrolled 457 women living in eight rural villages in Tanzania; the prevalence of *S. haematobium* infection varied by village from 0 to 11% and the overall prevalence of HIV infection was 5%. In this moderately-endemic setting, *S. haematobium* infection was again shown to be strongly associated with HIV, with an OR of 4.0 [IQR, 1.2-13.5]. Our OR was comparable to the OR of 2.9 reported by Kjetland and colleagues, despite the markedly higher prevalence of both *S. haematobium* infection (40-50%) and HIV infection (28%) that were observed in Zimbabwe (3). Thus our study provided important corroboration of the Zimbabwe study, because there now exist two separate studies, conducted in different countries with varying intensities of *S. haematobium* and HIV infections, both demonstrating the disturbing finding that women with *S. haematobium* infection have a 3-4 -fold higher odds of being HIV-infected than women without *S. haematobium* infection.

Worry about the potential HIV risk caused by *S. haematobium* infection is further increased by results reported in our longitudinal study in **Chapter 4**. We documented that praziquantel treatment of urogenital schistosomiasis resolved neither the gynecologic abnormalities associated with *S. haematobium* infection, nor the presence of the parasite as demonstrated by detectable schistosome DNA, 6 months after treatment. Our work extended others' findings that the clinical signs of schistosomiasis did not resolve up to 12 months after treatment (4,5) by documenting that, additionally, schistosome DNA could be identified in both urine and cervical lavage. Women in our study had a median age of 22 [IQR, 20-28], and one-fourth reported never being previously treated for schistosomiasis. Many of them may therefore have resembled the older Zimbabwean women, described by Kjetland and colleagues, in whom praziquantel treatment after age 20 was not effective in reversing cervical pathology (6). If *S. haematobium* infection is indeed a risk factor for HIV acquisition, and if the effects that lead to increased HIV susceptibility are incompletely reversed when treated according to the current standard of care, this is an area for urgent future research.

Moreover, if *S. haematobium* is a risk factor for HIV acquisition and it is at least partially irreversible, then this must prompt a redoubling of efforts to eradicate the disease. Vaccines against *S. mansoni* are currently in Phase I clinical trials in the United States and Brazil, while a Phase III clinical trial for a vaccine for *S. haematobium* is expected to be completed shortly in Senegal and Nigeria (7). The vaccine development process for schistosomiasis and other neglected

tropical diseases has been fraught with challenges including the complexity of the eukaryotic parasite genome, the difficulty measuring effectiveness in infections that cause more disability than death, and the dearth of fully-translatable animal models (7). In the interim until vaccines become available, early and regular provision of anti-schistosome medications to prevent disease morbidity must be urgently prioritized, particularly since only approximately 15% of people who needed treatment actually received it in 2013 (6,8,9). As the current gold-standard medication praziquantel is made more widely available to populations in need, its administration must be accompanied by robust studies to determine optimum dose and frequency of administration, as well as to quantify clinical effectiveness. This is particularly important given the findings of a recent Cochrane review that treating soil-transmitted helminth infections among children did not improve mean nutritional status, cognition, hemoglobin level, or survival (10). The ultimate aim, listed as one of the 2015-2030 United Nations Sustainable Development Goals is to eliminate schistosomiasis and other neglected tropical diseases altogether (11).

Other investigators have also explored additional implications of the association between *S. haematobium* and HIV infection. Men with *S. haematobium* infection have been shown to have bloody ejaculate containing higher levels of lymphocytes and inflammatory cytokines than men without schistosomiasis, which may facilitate HIV transmission in men with both *S. haematobium* and HIV infections (12). For women living in endemic settings, *S. haematobium* infection may render them doubly susceptible to HIV acquisition, due to both their own increased susceptibility and to the increased HIV transmissibility of their *S. haematobium*-infected male partners. It has been recently suggested that the failure to account for *S. haematobium* as a potential HIV risk factor may have been a major confounding factor in 8 randomized controlled trials of treatment of STIs to prevent HIV acquisition in sub-Saharan Africa (13). In fact, in several of the studies, “control” treatment included albendazole or provision of improved sanitation—both of which would effectively decrease *S. haematobium* infections and bias the study towards a null result. If *S. haematobium* infection does indeed increase the odds of HIV acquisition by 3-4-fold, as in these two clinical studies, then the effect size of *S. haematobium* infection is comparable to the increased odds of HIV acquisition attributable to STIs (14) and *S. haematobium* infection could certainly have obscured the effects of STI treatment in these randomized trials.

### ***Schistosoma mansoni* Infection as a Risk Factor for HIV Acquisition**

The findings described in **Chapter 3** were surprising. Our hypothesis had been that *S. haematobium* increased HIV susceptibility predominantly by causing

lesions in the genital mucosa. However, we observed that women with *S. mansoni* infection also had an approximately four-fold increased odds of HIV infection, and that increasing intensity of *S. mansoni* infection, as measured by CAA level, was strongly and significantly correlated with the prevalence of HIV infection. One other study, performed in fishing villages in Uganda, also suggested that *S. mansoni* could be associated with HIV acquisition. In that study, HIV-infected individuals living in an area endemic for *S. mansoni* were found to more frequently have antibodies to schistosome antigens than did HIV-uninfected individuals (15). As with our study, the Uganda study was cross-sectional and unable to demonstrate which infection preceded the other. The Uganda study also relied on an antibody test that can be positive both during and after infection, in contrast to the CAA assay that we used, which is positive during active infection and falls rapidly after treatment (16,17).

In contrast to our findings, two recent clinical studies have reported no association between *S. mansoni* and HIV infection, but they have been limited by methodological issues. Mazigo and colleagues screened 1,785 adults in Tanzanian fishing villages for HIV and *S. mansoni* infections, and found no association between these infections (adjusted OR 1.01 [IQR 0.84-1.21]) (18). This study relied on a single Kato-Katz smear and therefore may have missed lighter infections, which is notable given several reports that HIV-infected individuals may excrete fewer schistosome eggs (19,20). Impaired egg excretion by HIV-infected individuals would result in misclassification of some HIV-infected patients as being negative for *S. mansoni* infection, thereby biasing the study towards a negative finding. Sanya and colleagues in Uganda tested 1,412 adults using both a single Kato-Katz smear and a rapid Circulating Cathodic Antigen (CCA) point-of-care test (21). They found no significant relationship between *S. mansoni* and HIV infection by stool microscopy (OR 1.04 [IQR 0.74-1.47],  $p=0.81$ ). CCA testing was performed in only 650 individuals, yielding an OR for association with HIV infection that trended towards significance (OR 1.53 [0.78-3.00],  $p=0.19$ ). Importantly, this sub-analysis only had the power to detect a difference of more than 10% in the prevalence of *S. mansoni* infection using CCA as the diagnostic tool, so the authors' conclusion that their study demonstrates no association between *S. mansoni* and HIV infection raises questions.

Strong animal data from rhesus macaques clearly demonstrates that macaques with *S. mansoni* infection are more susceptible to simian HIV (SHIV) infection than uninfected macaques. Macaques have been previously demonstrated to be a robust model for HIV infection in humans, with macaques exhibiting parallel differential transmission risks across various mucosae and similarly slow progression of SHIV after infection (22,23). Macaques were inoculated rectally with increasing doses

of SHIV until they ultimately developed systemic SHIV infection. Macaques chronically infected with *S. mansoni* (n=8) developed SHIV at a median inoculum that was 17 times lower than macaques without *S. mansoni* (n=9) ( $p < 0.001$ ) (24). Median peak SHIV viral load was also  $>1$  log copies/ml higher in *S. mansoni*-infected macaques than the macaques without *S. mansoni* ( $p = 0.004$ ). Of note, no significant difference in inoculum size or viral load was found when the experiment was repeated using intravenous inoculation instead of rectal mucosal inoculation (23). These findings directly implicate changes in the rectal mucosa, rather than in the systemic circulation, as the major contributors to increased HIV susceptibility in macaques with *S. mansoni* infection.

Our findings in women with *S. mansoni*, together with these elegant macaque studies, have led us to reformulate our hypotheses about the mechanisms of HIV susceptibility in the setting of schistosome infection. It seems very likely that the epithelial breaches incited by *S. haematobium* ova migrating through the cervix are an important contributor to HIV risk. In addition, because *S. mansoni* eggs do not typically damage the genital mucosa in humans, we further postulate that schistosome infection triggers a generalized mucosal immune response, which may involve both rectal and genital mucosa. We hypothesize that this mucosal inflammation could lead to recruitment of HIV-susceptible cells to the mucosal surface and could thereby foster an immune environment in the mucosal tissue, at the initial site of HIV exposure, which is permissive to HIV infection.

#### ***Next Steps: Schistosomiasis and HIV Risk***

Therefore, a variety of evidence suggests that both *S. haematobium* and *S. mansoni* infections increase the odds of HIV acquisition. Mathematical modeling predicts that routine praziquantel administration to adults living in schistosome-endemic areas would be highly cost-effective at \$295 per HIV infection averted (25,26). However, until a robust prospective study quantitates the importance of schistosomiasis in HIV acquisition and definitively documents the causal relationship between schistosomiasis and HIV infection, schistosomiasis treatment will not be a public health priority for HIV prevention.

Given the ethical complexity of conducting such a study prospectively, our work in **Chapter 5** lays important groundwork that will make a retrospective longitudinal study possible. In Tanzania, serum samples have been stored as DBS from a cohort of 30,000 adults in rural villages who have been followed for over 20 years for HIV-seroconversion. In the proof-of-concept study described in Chapter 5, we documented that CAA can be reliably, accurately quantified in DBS as compared to serum, even in DBS that had been stored for up to 8 years. Our optimization of a technique to elute and quantify CAA from Whatman 903

paper, the most commonly-used DBS paper worldwide, opens new possibilities for other research studies on interactions between schistosomiasis and a variety of other communicable and noncommunicable diseases. We are currently using the technique that we have described to quantify CAA in banked DBS collected from adults prior to their HIV-seroconversion, and to compare this to the quantity of CAA in DBS from adults who did not HIV-seroconvert. We are additionally determining HIV-1 RNA viral load set-points as copies per milliliter of blood in new HIV-seroconverters who had and did not have schistosome infection at the time of HIV-seroconversion. This will provide human prospective data on the relationships between HIV susceptibility, schistosomiasis, and early HIV virologic control, with the potential to impact health policy and HIV prevention strategies throughout sub-Saharan Africa.

To investigate further the mucosal immunity hypothesis, studies are needed to identify and quantify differences in frequency, function, and types of immune cells in the cervical mucosal tissue of women with and without *S. haematobium* infection. This can ultimately be expanded to men and to individuals with and without *S. mansoni*. In addition, it will be important to determine whether, as reported with interleukin-17 production in mouse models, abnormal cytokine levels in tissue are reflected in peripheral blood (27).

## II. Managing Schistosomiasis-HIV Co-infection

### *Association between Schistosome Infection and Immunological Failure in HIV-Infected Patients Receiving Antiretroviral Therapy*

Growing evidence suggests that schistosomiasis may be not only a risk factor for HIV acquisition, but that it also may play an important role in HIV disease progression in individuals with HIV-schistosome co-infection. The single randomized trial in HIV-schistosome co-infected patients documented that patients treated with praziquantel had lower viral load increases than those who received delayed praziquantel treatment (28). A large randomized trial that treated helminth infections more generally and did not observe improvements in viral load was conducted in a setting with a low prevalence of schistosome infections (29). Neither of these trials enrolled HIV-infected patients on ART, which is a growing proportion of the HIV-infected population in sub-Saharan Africa. We hypothesized that schistosome infection could affect clinical outcomes and/or response to ART in HIV-infected patients and therefore performed the study in **Chapter 6** to explore this question.

This project was conducted among 351 HIV-infected adult outpatients who had been taking ART for at least six months and resided in an area in which



*S. mansoni* is highly endemic. We reported that 28% of patients had concurrent schistosome co-infection, as documented by a positive CCA rapid test. This finding alone suggests the importance of screening and treating HIV-infected patients in our setting for schistosome infection in order to prevent schistosome-associated morbidity and mortality. More troubling, HIV-infected patients with schistosome co-infection had a four-fold higher odds of having immunological failure than HIV-infected patients without schistosome co-infection. Patients with schistosome co-infection also had significantly lower CD4 count increases than those without schistosome co-infection after controlling for level of education, baseline CD4 count, and body mass index. Limitations of this study include our inability to study patients prospectively to determine the impact of untreated schistosome infection on immunological response, lack of information about other potential confounders including STIs, and the unavailability of HIV-1 RNA viral load level quantification at Bugando Medical Centre.

A study of CD4 counts in South African girls and young women demonstrated no significant differences in CD4 counts of girls with versus those without *S. haematobium* infection, as defined either by ova in urine or by gynecological abnormalities (30). In contrast to our study, in which all HIV-infected patients had been taking ART for at least 6 months, only approximately 15% of the HIV-infected South African women were on ART. Another key distinction is different species of parasites (*S. haematobium* is endemic in South Africa and stool was not tested, while the vast majority in our study had *S. mansoni*). We also used both microbiological and antigen testing for diagnosis, which may have higher sensitivity for diagnosis of schistosome infection particularly in HIV-infected individuals who have been suggested to excrete fewer eggs (19). Therefore it is possible that the effects on CD4 counts are limited to *S. mansoni* infection, which has been associated with increased density of HIV-co-receptors on the surfaces of peripheral blood CD4 cells (31). Another possibility is that the plethora of systemic immune alterations caused by chronic schistosomiasis, including increased T-regulatory cells, increased Th2 immune response, and impaired innate Th1 immunity, impair the body's ability to control viremia and in this way may precipitate true virological failure (32–34).

Gaining a clearer understanding of these phenomena is urgent. Clinicians caring for most HIV-infected patients in sub-Saharan Africa still do not have access to gold-standard viral load monitoring for patients on ART, and are therefore dependent on CD4 counts to determine whether patients' antiretroviral therapy is succeeding or failing, in accordance with the WHO's clinical and immunological criteria for treatment failure (35). If schistosomiasis affects CD4 count measurements, whether or not it actually impairs patients' response to

antiretroviral therapy, then this will strongly impact treatment decisions for HIV-schistosomiasis co-infected patients in sub-Saharan Africa.

***Next Steps: Managing Schistosomiasis-HIV Co-infection***

A major limitation of the work presented in Chapter 6 was our inability to measure viral loads. We were therefore unable to determine whether the immunological failure that was associated with schistosome co-infection was reflective of true virological failure in patients on ART. It is certainly possible that schistosome infection could increase the risk of virological failure, perhaps through its induction of a Th2-type immune environment that is permissive to viral replication (32,36). Conversely, it is also possible that schistosome co-infection in HIV-infected patients could cause an immunological phenomenon that is independent of viral load suppression. Elliott and colleagues reported a difference in CD4:CD8 T-cell ratios between HIV-infected patients with versus without schistosome co-infection who were not on ART (37), and others have described a mechanism by which schistosome infection may induce the mild neutropenia observed clinically in patients (38). It is therefore possible that schistosome infection affects blood lymphocytes, and particularly that it could impair the ability of HIV-infected patients to mount high CD4 counts, even though they are successfully virologically suppressed. Impaired CD4 count recovery, even in patients with virological suppression, has been associated with increased AIDS-related clinical outcomes, AIDS-related mortality, and non-AIDS related mortality (39).

Therefore, a clear next step is to assess whether schistosomiasis is associated with virological failure and/or impaired CD4 count recovery in patients on ART. This could be examined using CAA testing in a cohort of patients for whom serum or dried blood spots were stored at the time of ART initiation. Because the majority of patients in sub-Saharan Africa are still managed based on CD4 counts, it is essential to ascertain whether immunological failure in the setting of schistosome co-infection represents true treatment failure, legitimizing a switch from first- to second-line ART. If it does not represent true treatment failure, millions of patients with HIV-schistosomiasis co-infection are at risk of unnecessarily being started on costlier, more toxic second-line ART.

Other studies in this field should further characterize the interactions between HIV and CD4 counts in patients with schistosome co-infection, particularly with regard to the rate of decrease following HIV acquisition and to the functionality of CD4 cells in preventing opportunistic infections. Moreover, regardless of whether schistosomiasis is associated with only immunological or both immunological and virological failure, future studies should assess whether patients with HIV-

schistosomiasis co-infection have poorer outcomes.

### **III. Implementation Science: Improving Efficiency of Early Infant Diagnosis of HIV by Dried Blood Spot Testing**

Finally, our work in **Chapter 7** provides an example of a low-budget implementation science project that, with sequential interventions, led to sustained improvement in early diagnosis for HIV-exposed infants. The local challenges that inspired this study typify the indispensable nature of quality improvement work to make scientific advancements in HIV care accessible in resource-limited settings. Importantly, the genesis of the project came from a local health centre nurse, who had experienced first-hand the frustrations of an ineffective system for HIV diagnosis. She proposed innovative, inexpensive ideas that decreased the turn-around time from dried blood spot collection to result availability at the rural clinic from 55 to 38 days. While there remains room for additional improvement (38 days is still far from optimal), this project has invigorated local staff to devise new solutions that will both continue to shorten the turn-around time for DBS and will solve other local healthcare delivery problems. Moreover, this work can serve as a model for others seeking to address programmatic challenges in resource-poor settings.

#### ***Next Steps: Implementation Science Studies for Further Systems Improvement and to Maintain Impact***

Implementation science is an essential aspect of providing HIV care and treatment in sub-Saharan Africa. As the WHO advises a shift toward viral load monitoring for management of HIV-infected patients on ART (40), the availability of viral load testing will continue to increase. In addition, point-of-care viral load tests, which could provide same-day results in HIV-exposed newborns, are also on the horizon (41,42). As these technologies and others like them are scaled up in sub-Saharan Africa, it will be vital that the scale-up is implemented in ways that ensure reliability, quality, and sustainability. Operational projects to assess the effectiveness and longevity of new programs must be integrated into the normal workflow of overburdened clinics in ways that empower, rather than add to the work burden of, local staff (43).

Implementation science needs also to be a cornerstone of public health prevention measures. As public awareness grows regarding the relationship between schistosomiasis and HIV infection, it will be essential to ensure that individuals with schistosomiasis do not become stigmatized (44), whether due to public perceptions of their poverty or uncleanness or to incorrectly-perceived sexual promiscuity. Uptake of routine anti-schistosome treatment, vaccination, or other

novel interventions to treat schistosomiasis and decrease HIV risk will likely not be maximized if public perceptions are not managed appropriately.

## Concluding Remarks

This thesis began by describing the persistent burden of infectious diseases in sub-Saharan Africa at a time when many wealthier parts of the world, having gained relative control of many infectious diseases, are now focusing on non-communicable diseases. It also touched on the morbidity and mortality caused by schistosomiasis, which, in and of itself, merits dedicated efforts at treatment. The bulk of the work presented in this thesis, displaying additional overlap between HIV infection and schistosomiasis, only further strengthens the imperative to treat patients suffering from this neglected tropical disease. Operational studies on other topics related to HIV prevention and diagnosis can serve as models for implementation science work that will improve upon the recent estimates that only 13% of those needing schistosomiasis treatment were treated in 2013 (9). Not only would treatment optimization decrease suffering from schistosomiasis itself, but its impact may be far broader if indeed schistosomiasis is a risk factor for HIV infection.

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