IMPROVING HIV DETECTION AT MALAWIAN SEXUALLY TRANSMITTED INFECTIONS CLINICS

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ABSTRACT

Jane S. Chen: Improving HIV Detection at Malawian Sexually Transmitted Infections Clinics
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HIV diagnosis is the essential first step for persons living with HIV (PLWH) to access HIV care, which can improve health outcomes and prevent onward transmission. Synergy between HIV and other sexually transmitted infections (STI) makes STI clinics effective settings to reach PLWH and to find persons unaware of their HIV infection. We assessed the efficacy of an intervention incorporating acute HIV infection (AHI) screening, contract partner notification, and social contact referral on HIV detection among the sociosexual networks of PLWH seeking STI care in Lilongwe, Malawi.

We conducted a randomized controlled trial to evaluate the combination intervention relative to the Malawian standard of care of rapid serological HIV tests and passive partner notification. Enrollment occurred in two STI clinics between 2015 and 2019. We also standardized the intervention and standard-of-care outcomes to six months of patient visits at Bwaila District Hospital's STI Clinic to assess potential real-world clinical impacts. We used the SEDIA LAg avidity assay to assess the stage of HIV infection among participants.

During study enrollment, 1230 HIV-seropositive persons received control arm procedures and 655 received intervention arm procedures, including 94 persons with AHI. The intervention was efficacious in increasing the total number of new HIV diagnoses made per index participant (ratio: 1.9 (95% confidence interval (CI): 1.2, 3.1)) versus the standard of care. When we standardized the trial outcomes to 4730 patient visits at Bwaila STI Clinic, we found estimated that the intervention would yield 6.7 times the number of new HIV diagnoses relative to the standard of care, including index participants diagnosed with AHI. Among STI patients

seeking care, we found that only 6% of patients newly diagnosed with HIV had a recent HIV infection, with a mean duration of infection of 130 days.

The combination intervention was efficacious in increasing HIV detection among the sociosexual networks of PLWH seeking STI care. Furthermore, when the acute HIV diagnoses among index participants were considered, the intervention greatly improved HIV detection beyond the standard of care. Integrating novel referral and HIV testing strategies can improve HIV detection, which is the first step to ending the HIV epidemic.

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When I started this program five years ago, I lived in constant dread that someone would ask me about my research interests because I simply had no idea what they were. I would say something vague and hope that there wouldn't be any follow-up questions. And while this accurately reflected my newness to the world of infectious disease epidemiology, it made the task of writing a dissertation incredibly daunting. And yet, I am now extremely grateful that this has been such a challenging and formative experience. In addition to improving my research skills, choosing these aims and writing this dissertation have helped me find purpose.

There are many people who have guided me throughout the process, for whom I am incredibly grateful. I thank Brian Pence for welcoming me into his advisee group and for being an irreplaceable voice of reason and encouragement. I thank Kim Powers for her thoughtfulness, poise, and invaluable feedback. Kathy Lancaster introduced me to the world of global health research, and her guidance gave me the necessary skillset to embark on this work. Bill Miller has shared innumerable hours of mentorship that have made me a better epidemiologist, a better writer, and maybe even a more thoughtful person. Finally, Irving Hoffman has shaped how I see the world and who I want to be as a researcher and human, simply by letting me witness him work. His guidance and example have been instrumental.

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LIST OF ABBREVIATIONS

AHI	Acute HIV Infection
ART	Antiretroviral Therapy
CI	Confidence Interval
EIA	Enzyme Immunoassay
EMR	Electronic Medical Record
HIV	Human Immunodeficiency Virus
HTS	HIV Testing Services
KCH	Kamuzu Central Hospital
NNI	Number Needed to Receive the Intervention
ODn	Normalized Optical Density
PCR	Polymerase Chain Reaction
PrEP	Pre-exposure Prophylaxis
PLWH	Person(s) Living with HIV
RCT	Randomized Controlled Trial
STI	Sexually Transmitted Infection

CHAPTER 1: SPECIFIC AIMS

The UNAIDS 90-90-90 goals outline three targets to end the HIV epidemic by 2020: 1) 90% of persons living with HIV (PLWH) will know their HIV-infected status, 2) 90% of diagnosed PLWH will receive sustained antiretroviral therapy (ART), and 3) 90% of PLWH taking ART will achieve viral suppression.[1] Meeting these targets will require novel approaches to disseminating HIV testing and care services to those who are not reliably reached through existing mechanisms and effectively linking them to sustainable HIV care.

To achieve the first 90, Malawi, a nation that has been deeply impacted by the HIV epidemic, has incorporated several HIV testing strategies into its clinical standard of care. Within sexually transmitted infections (STI) clinics, opt-out HIV testing protocols take advantage of the biological synergy between HIV and other STIs, as well as shared behavioral risk factors.[2] PLWH are then encouraged to refer their sexual partners for HIV testing, a strategy known as passive partner notification.[3,4] Despite these efforts, Malawi still falls short of the first 90 goal, and novel approaches are required.[5] One proposed strategy combined three additional mechanisms: 1) Introduce contract partner notification, enabling clinic staff to contact partners of PLWH for HIV testing if standard passive partner notification fails.[6] 2) Encourage PLWH to refer their social contacts for HIV testing in addition to sexual partners. 3) Detect acute HIV infection (AHI) among HIV-serodiscordant and -seronegative persons to promote early diagnosis and early ART initiation during the most infectious period of HIV infection.[7,8]

Between 2015 and 2019, these three strategies were evaluated as a single intervention to determine if HIV diagnostic outcomes could be improved beyond the standard of care in two STI clinics in Lilongwe, Malawi. All eligible HIV-seropositive patients were randomized to receive

either the intervention or the standard of care (control) and were counseled to refer the relevant persons for testing (sexual partners in the control arm; sexual partners and social contacts in the intervention arm). Additionally, eligible HIV-seronegative and -serodiscordant patients were screened for AHI; those diagnosed with AHI were offered enrollment into the intervention arm. All eligible referred persons were assigned the study procedures of the arm of their referring contact. All HIV-positive participants in the intervention arm provided blood samples for HIV recency testing. *The goal of the dissertation was to assess the impact of the intervention across three aims:*

Aim 1: To evaluate the efficacy of the intervention which includes contract partner notification, social contact referral, and AHI screening to find persons unaware of their HIV infection. Overview: We assessed the impact of the intervention at two STI clinics in Lilongwe, Malawi. We compared referral and HIV testing outcomes between the intervention and control arms with 'per index participant' metrics, focusing primarily on the number of new HIV cases diagnosed. We also examined these outcomes using the proportion of participants who referred at least one person of each outcome type, and by calculating the number needed to receive the intervention to refer an additional person of each outcome type.

Aim 2: To estimate the effect of the intervention and its component strategies had they been implemented as the standard of care at the Bwaila District Hospital STI Clinic. Overview: Using the Bwaila District Hospital STI Clinic's electronic medical record (EMR), we standardized the trial results to the clinic's patient population to estimate the number of expected new HIV cases identified had the intervention been implemented for six months, as compared to the standard of care. We additionally broke down the intervention into its discrete strategies (contract partner notification, social contact referral, and AHI screening), and estimated the number of new HIV diagnoses made had the three strategies been implemented independently as well.

Aim 3: To describe the distribution of HIV infection stage during STI clinical care encounters. Overview: Among participants receiving the intervention, we used rapid serological HIV tests, AHI screening, the SEDIA LAg Avidity assay,[9] and self-reported HIV testing history and ART use to categorize participants into HIV infection stages (HIV-negative, acute HIV infection, recent untreated HIV infection, long-term untreated HIV infection, and HIV-positive taking ART).[10-12] We used these categories to characterize index participants newly and previously diagnosed with HIV, as well as their referred sexual partners and social contacts.

This research proposal aimed to assess the population effects of the combination intervention and the implications of its implementation, as well as to provide actionable insight into achieving the UNAIDS 90-90-90 goals in Malawi.

CHAPTER 2: BACKGROUND

HIV has become a generalized epidemic in sub-Saharan Africa. While the HIV epidemic is recognized as a global concern, the burden of disease in sub-Saharan Africa far exceeds the rest of the world in scope and magnitude. An estimated 70% of PLWH around the world reside on the African continent, where the infection continues to claim more than 660,000 lives each year.[13] In Malawi, HIV prevalence is estimated to be around 9%, down from an estimated 25% at its peak in 1996, though the epidemic remains more concentrated in urban areas.[8,14] Furthermore, there are an estimated 33,000 incident cases of HIV in Malawi each year among adults ages 15-64, and household surveys show that 16% of rural households and 25% of urban households have at least one HIV-positive family member.[5,15]

The UNAIDS 90-90-90 goals are attainable in Malawi. To end the HIV epidemic, the UNAIDS 90-90-90 goals have been established as targets for 2020, and have set the following goals: 90% of PLWH know their HIV status, 90% of those who know their HIV-positive status have sustained access to ART, and 90% of those who have access to ART become virally suppressed in the blood.[1] A 2015-2016 survey of Malawi estimated that among adults ages 15-64, 77% of those who were HIV-positive were aware of their positive status, 91% of those who knew their status had sustained access to ART, and 91% of those on ART were virally suppressed.[5] Reaching the second and third goals is a remarkable achievement and illustrates the attainability of the UNAIDS goals. But the accomplishments of the second and third goals are limited by the success of the first, where there is still need for improvement. Moreover, percentages are heterogeneous across sub-populations, and are notably lower in the younger populations.[14] National surveys estimate that persons ages 15-24 years old have not met any of

the 90-90-90 goals, though they are a particularly vulnerable population (54%, 86%, 81%, respectively).[5]

Achieving '90% of PLWH know their HIV-infected status' requires novel strategies for outreach. While rapid serological HIV tests are widely available in clinical settings in Malawi and opt-out HIV testing is the standard of care at most medical encounters, universal and consistent HIV testing remains a challenge. An estimated 74% of Malawians report having ever had an HIV test, but only 36% of Malawians report having had an HIV test in the previous 12 months.[5] Several strategies to improve these statistics are being explored throughout the region. However, reaching persons who may not have direct or frequent access to health care settings must be further prioritized if the 90% goal is to be reached.

STI and HIV synergy makes STI clinics effective places to test for HIV.[16] The synergy between HIV and other bacterial and viral STIs was explored early in the epidemic and was termed 'synergy' by Wasserheit in 1992.[17,18] The phenomenon is explained as follows: STIs can increase the infectiousness of an HIV-infected person, or increase the susceptibility of an HIV-uninfected person, via a variety of mechanisms including increased viral shedding in the genital tract of an HIV-infected person and a hypothesized increase in target cells in the genital tract of an uninfected person.[2] Furthermore, STIs increase risk of transmission through increased physical contact with infectious bodily fluids through ulcers and other STI-related lesions. Finally, as HIV is transmitted sexually, it shares similar risk factors with other traditional STIs. Thus, testing for HIV in STI clinics is an effective strategy for identifying new cases because STI clinics bring together a concentrated group of people concerned about STI acquisition who may be at higher risk of HIV.

Assisted partner notification was officially added to the World Health Organization recommendations in 2016, as an effective strategy to be offered with HIV testing services (HTS).[19] And to date, at least 67 countries include it in their national HTS guidelines. Assisted partner notification includes strategies such as contract partner notification, which allows clinic

staff to reach out to partners who do not present to clinic for testing and counseling within a pre-specified time to improve referral rates above passive partner notification, where the patient is fully responsible for referring their partners to get tested.[20,21] These recommendations were based in part on work conducted by a University of North Carolina research team in two Malawian STI clinics in 2008-2009 who found contract partner notification and provider referral, another form of assisted partner notification, to be feasible and efficacious.[21,22] Results from that randomized controlled trial (RCT) showed that partners of participants who had either contract partner notification or provider referral were 2.1 times as likely to present to clinic as compared to those whose partners were responsible for passively referring them.[21] Furthermore, subsequent analyses have also shown that such strategies may also be cost-effective, as well as socially acceptable.[6,22] However, Malawian standard of care for partner notification remains passive partner notification for all STIs, including HIV.

Contract partner notification takes advantage of the clustering of HIV infection and sexual risk behaviors in partner networks. Like other infectious diseases, STIs are not spread uniformly across populations, and are instead often clustered.[23] By identifying sexual networks and testing sexual partners of PLWH, clusters of persons with HIV infection can be identified to prevent further transmission. Additionally, sexual risk behaviors, such as condom use and partner concurrency, can be clustered among sexual networks.[20,24] Therefore, using named sexual partner networks of PLWH to identify new HIV cases is an advantageous strategy.

Social networks of PLWH are also an effective target group for HIV screening due to clustering of social norms and sexual behaviors among social groups.[25] Sexual networks are the underpinning of STI transmission through populations, but broader social networks cannot not be ignored.[26] Patterns of similar sexual behaviors and social norms throughout social groups render the social contacts of PLWH a reachable and effective target for HIV screening.[27] Furthermore, social support around the acceptability of HIV testing and

HIV-related stigma are important tools for increasing the uptake of screening opportunities among the social networks of PLWH.[3,28]

Early and acute HIV infections contribute disproportionately to HIV transmission due to increased viral loads during the first several weeks of the infection.[29,30] Though there have been a range in estimates produced by mathematical models, likely due to the variety of setting and stage of epidemic, upper estimates place the contribution of AHI to new infections between 50% and 82%.[29,30] In one model parameterized with data from Lilongwe, Malawi, 38% of HIV transmissions were estimated to be from persons with an early infection.[8] Therefore, the identification of acute and early HIV infections has great potential to prevent onward HIV transmission through sexual risk education and early treatment. Importantly, the identification of an acute case of HIV is also the detection of a recent HIV transmission and identifying the transmitting partner of persons with AHI is an opportunity to prevent further spread of HIV.

Detection of AHI requires laboratory procedures beyond the standard point-of-care serological tests, but is feasible.[31] Rapid serological HIV tests, which are the standard of care for HIV diagnosis in Malawi, are unable to detect HIV infection prior to seroconversion.[32] The introduction of antibody/p24 antigen rapid HIV tests was intended to enable point-of-care acute HIV detection, however, the field evaluations of such tests have not been promising. One field evaluation in Malawi found that the antigen detection portion of the combination rapid tests identified zero of eight acute infections, and had comparable results with the standard rapid serological tests that do not detect acute infection.[33] While nucleic acid amplification tests are the gold standard for detecting AHI, the laboratory costs can be prohibitive in resource-limited settings. Therefore, targeting high-risk populations and utilizing a screening strategy that combines multiple samples together into fewer assays improves efficiency. AHI screening with pooled polymerase chain reaction (PCR) has been demonstrated as feasible in STI clinics and HIV care clinics in Malawi.[34]

The stage of HIV infection is an important biological tool for understanding infectivity and transmission of HIV. Because early HIV infection has implications for the infectivity of the virus, establishing the recency of HIV infections yields important information. Several iterations of biological assays and multi-assay algorithms incorporating viral loads, antibody avidity assays, and CD4 counts have been developed for different subtypes of HIV infection.[12,35] While their primary utility has been to estimate HIV incidence, the advent of the assays and subsequent algorithms enables a novel branch of research incorporating recency of infection into research questions. The current optimal algorithm for subtype C infections, which is the predominant HIV subtype in Malawi, uses a combination of cut-offs from the LAg avidity assay, a modified BioRad avidity assay, and HIV viral load to predict infections less than 248 days (95% confidence interval (CI): 218, 284) or about 8.3 months.[11,12,36] However, the modified BioRad avidity assay is not commercially available and so a majority of programs use a simpler algorithm incorporating the LAg avidity assay and HIV viral load (mean duration of subtype C infection: 142 days; 95% CI: 118, 167).[9-11] Using the LAg avidity assay alone yields a mean duration of infection of 130 days (95% CI: 118, 142).[9] Such assays allow for the identification of recent infection and persons with higher risk of forward transmission.

In summary, ending the HIV epidemic will require novel and aggressive strategies to identify and treat undetected cases of HIV. Contract partner notification, social contact referral, and AHI screening are good candidates for scalable strategies and focusing these efforts in STI clinics focuses resources among those at greatest risk. Furthermore, identifying persons with a recent HIV infection can improve our understanding of HIV stage of infection in populations that come into contact with medical care to help prioritize HIV testing strategies.

Significance

The realm of HIV research is at a critical point: ART regimens have evolved dramatically, both in their ability to improve health outcomes as well as prevent forward transmission.[37,38] But access to care and uptake of testing and treatment remains a challenge and engaging hard to

reach populations is critical. The goal of this dissertation is to assess the impact of a combination detection strategy on HIV diagnosis in the STI clinical setting. The intervention incorporates three well-documented strategies into one novel package, and the implications of the individual effects of the three strategies, as well as the potential synergies, have the potential to directly inform HIV testing strategies in resource-limited settings.[3,21,34]

The three strategies chosen for this combination intervention were selected based on their feasibility and their potential for synergistic effects: 1) contract partner notification 2) passive social contact referral and 3) AHI screening. Contract partner notification offers a high-reward methodology for finding undiagnosed HIV cases, and the strategy has the ability to be scalable on a larger level.[19,39,40] Furthermore, utilizing broader social networks and capitalizing on shared social norms among peer groups may improve the social acceptability of, and desire for, HIV testing. Increasing social referrals among groups with potentially higher levels of behaviors associated with STI transmission could lead to earlier and more frequent HIV testing. Finally, detecting HIV infection and linking persons to HIV care as early as possible can reduce the risk of forward transmission and improve overall health outcomes.[37,41] Though screening for AHI has historically been costly, inefficient, and largely impossible in resource-limited settings like Malawi, cheaper technology and more efficient screening strategies have brought this option back to the realm of possibility. Pooled PCR screening strategies in high-risk settings like an STI clinic, allow for a more concentrated effort with a relatively high yield.[34] Coupled with contract partner notification, identifying and testing the partners of persons with AHI has the potential to have even better effectiveness.

The combination intervention also makes significant contributions to HIV research that go beyond the impact of testing strategies. The social and sexual network defined by social contact referral, contract partner notification, and AHI screening provides a robust picture of interpersonal relationships between the participants and their referred contacts. This dissertation utilized the sociosexual network to assess duration of HIV infection among index

participants and their sexual partners and social contacts. While the scope of such an analysis is descriptive in nature, the information can contribute to future research questions and more targeted phylogenetic analyses.

The results of this dissertation have the potential to inform policy on HIV testing as well as to improve the scientific understanding of how HIV moves through sexual and social networks of persons seeking STI care.

Innovation

The proposed dissertation is innovative in several regards. Incorporating contract partner notification, AHI screening among HIV-seronegative and -serodiscordant patients, and passive social contact referral into one intervention joins three well-studied methodologies into one, potentially improving the effects of the whole beyond the simple sum of its parts. While contract partner notification has been more widely accepted as effective and feasible, integrating AHI screening among HIV-seronegative partners of HIV-positive persons holds promise for improved HIV detection. Additionally, improving referral rates among the partners of those newly diagnosed with AHI with contract partner notification, reaches a group that is either at high risk of HIV acquisition or may have just transmitted HIV. By combining contract partner notification and AHI screening, earlier infections and recent HIV transmissions can be targeted sooner during infection.

Generalizing the results of the internal study population to the Bwaila District Hospital STI Clinic population is also novel, as it utilizes data that is not often available in resource-limited settings. The EMR from the STI Clinic at Bwaila District Hospital is a resource that is not common in many clinical settings, as it is a biometrically linked longitudinal patient record. Generalizing the trial results to such a large and granular dataset is impactful and takes advantage of a new resource in an innovative way.

Finally, recency assays to detect ‘recent’ HIV infection were developed for HIV incidence estimation. Utilizing the assays to better understand stage of HIV infection at clinical encounters

comparing the duration of HIV infection (acute, recent, long-term) among sexual and social relationships has not yet been explored. This is one of the first analyses, to our knowledge, that uses the assays in this manner.

CHAPTER 3: METHODS

Overview

This dissertation is composed of three aims assessing the iKnow RCT in Lilongwe, Malawi. The iKnow study assessed a combination intervention intended to improve HIV detection among the sociosexual networks of PLWH seeking STI care, as compared to the Malawian standard of care. The intervention incorporated AHI screening, contract partner notification, and social contact referral; Malawian standard of care is rapid serological HIV tests and passive partner notification. The iKnow RCT was conducted in two STI clinics in Lilongwe between 2015 and 2019.

The first aim of this dissertation addresses the overall efficacy of the intervention through the primary outcome of newly diagnosed HIV infections per index participant, as compared to the standard of care in the control arm. We also included several other HIV care related outcomes including the number of persons referred, the number of sexual partners referred, and the number of PLWH referred (including those previously diagnosed with HIV). Additional sensitivity analyses assessed the impact of the intervention accounting for several study design characteristics.

The second aim of this dissertation focuses on the clinical implications of implementing the intervention as standard of care in the Bwaila District Hospital STI Clinic over six months. The main outcomes were the expected total persons referred to clinic and the expected number of new HIV diagnoses made. To achieve this, the expected number of acute HIV cases at the Bwaila clinic were calculated and the numbers of referrals per index participant were standardized to the Bwaila clinic population. Finally, the third aim utilizes recency testing assays

to assess the stage of HIV infection among iKnow index participants in the intervention arm as well as their referred sexual partners and social networks. Early HIV infection disproportionately contributes to the perpetuation of the HIV epidemic,[8] and using the SEDIA LAg avidity assay[9] we examined the patterns of duration of HIV infection and ART use among those newly and previously diagnosed with HIV. These patterns have the potential to inform our understanding of how PLWH are engaging with care in STI clinics and at what stage of infection they are being diagnosed with HIV.

Parent Study

The iKnow combination intervention was developed to improve detection of undiagnosed HIV cases beyond the Malawian standard of care. As described earlier, the intervention draws upon three established strategies for improved HIV diagnosis including contract partner notification, passive social contact referral, and AHI screening. By intervening among both HIV-seropositive patients (contract partner notification and social contact referral) and HIV-seronegative or -serodiscordant patients (AHI screening) in a high-risk clinical setting, outreach to persons unaware of their status is maximized. The resultant intervention was implemented in two STI clinics beginning in 2015.

Study Setting

The iKnow RCT was conducted in Lilongwe, Malawi. The city of Lilongwe was most recently estimated to include approximately 989,318 people in 2018, with a total of 1,637,583 people living in the greater district.[42] An estimated 9% of Malawian adults are living with HIV, though estimates for urban areas are considerably higher, at around 15%.[14] The iKnow study was housed at two STI clinics within the city center of Lilongwe.

The primary iKnow enrollment site was the STI clinic at Bwaila District Hospital, which is the primary public hospital for the Lilongwe District, and offers a variety of services including general outpatient and antenatal care alongside other specialty care clinics. The STI clinic at Bwaila provides medical services across approximately 13,000 patient visits each year, with an

estimated HIV prevalence of 12% among those visits. Approximately 600 people were newly diagnosed with HIV at the STI clinic in 2017 (internal records).

The second STI clinic was located at Kamuzu Central Hospital (KCH), which is less than four kilometers from Bwaila District Hospital. While Bwaila District Hospital is the primary public hospital, KCH is the main tertiary hospital in Lilongwe. The STI clinic at KCH offered outpatient STI services for several decades but closed in 2015 after the hospital transitioned to an exclusively referral basis of care. The STI patient population at KCH was subsequently referred to the STI clinic at Bwaila District Hospital for care, though enrollment into iKnow had already begun.

Malawian standard of care for persons with an STI-related concern allows patients to seek STI care on the day that they choose. Patients generally present to the clinic in the morning and are given a health education talk about STIs, HIV, and risk reduction strategies. Patients are then presented with a brief overview of the research studies taking place at the STI clinic before queueing for HIV testing services and a clinical visit with a nurse. The only requirement for clinical care at the STI clinic is a health passport, which is a small paper book where clinicians document the patient's medical history. Official identification is not required, and until April of 2019, no internal individual medical records were kept.

Since 2006, both STI clinics have employed opt-out strategies for HIV testing, meaning that all patients who present for care are given an HIV test by trained HTS counselors before being seen by a nurse, unless they specifically request not to. As HIV testing has become the standard of care in many health care settings across Malawi, patients rarely refuse HIV testing (only 3% of visits had a patient refuse a recommended HIV test in 2017; internal records).

Study Overview

iKnow was a two-arm RCT. STI patients were eligible for participation if they were at least 18 years old, lived in Lilongwe District, and reported sexual activity in the previous six

months. Per Malawian standard of care, STI patients were tested for HIV at the beginning of their visit using dual rapid serological tests (Figure 3.1).[43]

Persons newly diagnosed with HIV via rapid test were randomized 3:1 to the standard-of-care (control) arm and the intervention arm. Arm assignment was blocked in groups of four and eight by study personnel uninvolved with participant enrollment. An original sample size of 1,200 intervention arm participants and 3,330 control arm participants was calculated with sufficient power to detect a 0.04 difference in participants referring at least one newly diagnosed PLWH. After the KCH study site closed, persons previously diagnosed with HIV became eligible for participation in April 2017 and study randomization ratio switched to 1:1 in April 2018 to increase intervention arm enrollment. To allow for procedure-specific consent, informed consent was obtained after study arm assignment and neither study staff nor participants were blinded.[44]

Consenting HIV-seronegative and -serodiscordant participants were screened for AHI (see “Acute HIV Screening” below), and those found to have AHI were offered enrollment into the intervention arm.

HIV-seronegative Index Participants

After HIV serological testing, seronegative and serodiscordant participants were offered enrollment into the intervention arm, specifically for AHI screening procedures. After providing informed consent, participants were given a brief behavioral survey and asked to provide personal locator information so the clinic could contact them with their results. Approximately five mLs of blood were collected from each patient before he or she proceeded to a nurse for STI care.

Using pooled PCR (see section ‘Acute HIV Screening’), participants determined to have AHI were contacted by the study team, either by phone or in person, and asked to return to clinic. For privacy considerations, no clinical information was shared outside of the clinic. Upon returning to the clinic, the participant was provided with their HIV diagnosis, and asked if he or she would like to enroll further in the study with intervention study procedures. If he or she

provided informed consent, intervention arm study procedures were initiated, as described below (see section 'Intervention Arm Study Procedures – HIV-positive Index Participants').

Acute HIV Screening

Acute HIV infections and HIV-negative persons were classified based on a seronegative or serodiscordant rapid test(s) and followed by Abbott RealTime HIV-1 pooled PCR.[34] Those with no detectable HIV RNA were classified as HIV-negative, those with detectable HIV RNA ≥5000 copies/mL were classified as acute HIV, and those with HIV RNA <5,000 copies/mL were retested again with serologic tests to assess initial testing error. Community outreach personnel attempted to contact the person determined to have AHI within one day of the positive result.

Standard-of-Care Arm Study Procedures

HIV-seropositive Index Participants

After providing informed consent, HIV-seropositive participants randomized and enrolled in the standard-of-care arm were given a brief behavioral survey. They were also given five participant-specific partner notification cards to give to their sexual partners from the previous six months to refer them to the STI clinic. Participants then proceeded to a nurse for STI care, and upon completion of the visit, those who were newly diagnosed with HIV or who had fallen out of HIV care were escorted to a local HTS clinic for HIV care linkage.

Referred Sexual Partners

All referred sexual partners of participants in the standard-of-care arm were screened for study eligibility when they presented to clinic. Those who were eligible and enrolled in the study, completed a behavioral questionnaire. As part of the eligibility determination, referred partners were tested for HIV with rapid serological tests. If the partner tested HIV-seropositive, he or she was offered enrollment in the control arm of the study, following the same passive partner notification protocol as his or her referring partner. If the partner tested HIV-seronegative or -serodiscordant, he or she was offered enrollment and completed only a behavioral questionnaire.

Intervention Arm Study Procedures

HIV-positive Index Participants

After providing informed consent, all HIV-positive persons in the intervention arm, including both HIV-seropositive participants randomized to the intervention arm and persons with AHI assigned to the intervention arm, were given a comprehensive behavioral survey. Participants underwent study procedures for contract partner notification and social contact referral (see sections 'Contract Partner Notification' and 'Social Contact Referral'). Thirty mLs of blood were collected from participants before they proceeded to a nurse for STI care. Upon completion of the visit, participants who were newly diagnosed with HIV, or who had fallen out of HIV care, were escorted to a local HIV testing and counseling clinic for ART initiation.

Contract Partner Notification

Participants in the intervention arm were asked to name up to five sexual partners from the previous six months and provide information that would allow the study team to contact them in the community. This information included a physical description, phone number, and home or work location. If the partners failed to present to the STI clinic within seven days, members of the community tracing team used the provided contact information to reach the partner, either by phone or in person, to refer them to care. For privacy considerations, no medical diagnoses were given outside of the clinic and the identity of the person who named them as a sexual partner was never disclosed. Tracing attempts were made for each person for up to three weeks after the initial tracing attempt.

Social Contact Referral

Participants in the intervention arm were also asked to think of up to five friends and acquaintances that could benefit from STI and HIV testing. Names or pseudonyms were used to help participants identify potential social contacts, but no other information about the social contact was collected. An additional five participant-specific cards for social contacts were provided. The type of card (sexual partner vs. social contact) was distinguishable by color.

Referred Persons

All referred sexual partners and social contacts of participants receiving the intervention were screened for study eligibility when they presented to clinic. Referred persons of participants in the intervention arm were tested for HIV with rapid serological tests during eligibility determination. Eligible partners and social contacts that tested HIV-seropositive were offered enrollment in the intervention arm, and study procedures followed accordingly. Eligible partners and social contacts that were HIV-seronegative or -serodiscordant were offered enrollment and screened for AHI.

Regardless of study arm and further study participation, all persons with an HIV diagnosis were referred for care at a local HIV care clinic.

Measurement

The primary goal of the intervention assessed in the iKnow RCT was new HIV diagnosis among sexual and social networks, and thus the majority of measures used in this dissertation involve metrics describing persons referred to clinic as well as the natural and clinical course of HIV infection. As such, the documentation of referral chains of sexual partners and social contacts, as well as the documentation of the participants' HIV characteristics, was essential.

HIV Serostatus

Because the Malawian standard of care for HIV testing uses confirmatory rapid serological HIV tests, HIV serostatus was a critical distinction for HIV diagnosis. Per confirmatory testing procedures, patients were tested with an initial rapid test. Those who tested negative were considered HIV-seronegative. Those who tested positive on the initial test were given a second confirmatory test. HIV-seronegative and HIV-serodiscordant status did not preclude an HIV infection, as acute HIV infections are by definition among HIV-seronegative and HIV-serodiscordant persons.

HIV Diagnosis

Throughout the trial, participants could have been diagnosed with HIV in three different ways. At enrollment, participants could have reported having been previously diagnosed with HIV, they could have reported no previous HIV diagnosis and then tested positive via rapid test, or they could have reported no previous HIV diagnosis, tested HIV-negative or -serodiscordant via rapid test, and then tested positive for an acute infection during AHI screening. Based on these options, diagnoses were classified as previous, new HIV-seropositive, and new acute.

HIV Care

Participants who reported being previously diagnosed with HIV and reported taking ART were classified as such. This determination was based entirely on self-report and no further ART adherence information was collected.

Stage of HIV Infection

Stage of HIV infection was broken into four categories based on rapid serological tests, AHI screening, SEDIA HIV-1 LAg avidity enzyme immunoassay (EIA), and self-reported ART use: acute HIV, recent untreated infection, long-term untreated infection, and taking ART. Acute HIV was defined as HIV-seronegative or -serodiscordant rapid test results and a positive result from the Abbott RealTime HIV-1 assay as described above. PLWH taking ART were those who reported being previously diagnosed with HIV and taking ART. PLWH who did not have an acute infection and who did not report ART use were further broken down into recent and long-term untreated infections the SEDIA HIV-1 LAg Avidity performed on a provided blood sample.[9,10] Per the manufacturer's instructions, HIV-seropositive samples were tested to assess the normalized optical density (ODn) within the assay. Samples with an initial ODn >2.0 were considered long-term infections and those with an ODn ≤2.0 were tested again in triplicate and the median value was considered final. Final adjudication was made with an ODn cut-off of 1.5. Samples with an ODn ≤1.5 were classified as 'recent' with an expected mean duration of

infection of 130 days (95% CI: 118, 142).[9] Samples with an ODn >1.5 were considered to be 'long-term' infections.

Referred Persons

All persons who presented to the STI clinic with a sexual or social referral card were documented as referred by the participant whose code appeared on the card. Referred persons were classified based on the following attributes: presented to clinic, enrolled in the study, and linked to their referring index participant. All "successfully referred" persons were linked back to the original index participant seeking STI care, such that a person referred by a referred person was attributed to the original index participant. Persons who did not enroll in the study or who were unable to be linked to their referring participant during data analysis (e.g. due to computer error), were excluded from analysis.

Other Covariates

Demographic and other behavioral characteristics were collected during study procedures, including sex, age, and marital status. These pieces of information were collected during the eligibility determination portion (e.g. age and sex) or the behavioral questionnaire (e.g. marital status).

Data Analysis

Aim 1 Analysis

To estimate the effect of the iKnow intervention on HIV detection among the sociosexual networks of PLWH seeking STI care, we assessed the effect of the intervention on the number of new HIV cases detected per HIV-positive (seropositive or acutely infected) per index participant. However, to gain a more comprehensive understanding of the intervention, we also employed three other metrics (total persons referred, sexual partners referred, and PLWH referred). Each of these outcomes was assessed in the 'per index participant' format, as well as the second and third metrics described below.

Metric 1: 'Per HIV-positive Index Participant' Metrics (outcome assessed as a count per index participant)

Measures quantifying the absolute numbers of partners referred 'per HIV-positive index participant' were analyzed using use negative binomial regression to account for overdispersion of the data. In the case that a negative binomial model would not converge, we used Poisson regression with a scaled deviance.[45]

Metric 2: 'Proportion of HIV-positive Index Participants' Metrics (outcome assessed as a binary variable per index participant)

We quantified the proportion of HIV-positive index participants within each arm that referred at least one person in a given outcome category. Differences in proportions across arms were tested using Fisher's exact two-sided test (α=0.05).

Metric 3: Number Needed to Receive the Intervention (numeric outcome)

Our final analysis type assessed the number of people needed to receive the intervention (NNI), calculated as:

$$\frac{1}{\left({}^{\#\,referred}/_{\#\,index}\right)_{intervention} - \left({}^{\#\,referred}/_{\#\,index}\right)_{control}},$$

rounded up to the nearest integer.

Secondary Analyses

We conducted several sensitivity analyses to address specific aspects of the study design, each time reassessing our main outcome of new HIV diagnoses made per index participant. To account for any unintended trial arm imbalance, we repeated our analysis of the main outcome and adjusted for index participant sex, new versus previous HIV diagnosis, and marital status. To account for the inclusion of index participants with AHI in the intervention arm (but not control arm), we repeated our main analysis with restriction to HIV-seropositive participants. To assess potential differential effects of the intervention among participants with new versus previous HIV diagnoses, we repeated our main analysis with the addition of an interaction term for new (versus previous) HIV diagnosis. Finally, to determine if the travel

reimbursement differentially affected referrals across study arms by incentivizing those in the control arm to increase their referrals, we conducted the main analysis with an interaction term for study incentive (yes/no). Interactions were considered significant at $\alpha=0.05$.

All analyses were conducted using SAS v9.4 (Cary, North Carolina).

Aim 2 Analysis

In this analysis, we used quantitative generalizability methods to better understand the expected real-world impacts had the intervention and its components been implemented as the standard of care for all patients in the larger of the two study clinics. To achieve this, we standardized the iKnow RCT results to the Bwaila District Hospital STI Clinic visits between July 1, 2019 and December 31, 2019.

Trial Population: iKnow Study

iKnow participants in each study arm were the trial populations for assessing the impact of the intervention (intervention arm) and the standard of care (control arm). Persons previously diagnosed with HIV were excluded from study participation until April of 2017. We therefore restricted our analytic population to those enrolled after the inclusion criteria expanded. Study enrollment ended at the KCH STI Clinic when the hospital transitioned to exclusively referral-based care in January 2016. Thus, only iKnow participants enrolled at the Bwaila District Hospital study site were included in this analysis. Additionally, index participants with missing sex, age, and marital status data were excluded from the analysis as well as persons referred with an undocumented referral type (sexual partner versus social contact).

Target Population: Bwaila STI Clinic

Bwaila District Hospital is the largest public hospital in Lilongwe District, treating patients across a variety of outpatient and specialty clinics. The STI Clinic at Bwaila District Hospital provides outpatient STI care across approximately 13,000 patient visits per year. Patients are treated syndromically per Malawian standard of care on the day that they present to clinic.[4] No

previous scheduling of appointments is necessary, and there are no exclusions on who receives care.

In April 2019, an EMR was implemented in the Bwaila District Hospital STI Clinic to support clinical care and collect more detailed information about the patient population and care provided. At each visit, all patients provide basic demographic information, or confirm its accuracy if already in the system. Per Malawian standard of care, all patients are seen for HIV testing and counseling services before proceeding to a clinic nurse for examination.[46]

Our unit of analysis for the Bwaila STI Clinic was patient visits, as patients could seek care across multiple visits, and our visits of interest were those that occurred between July 1 to December 31, 2019. Bwaila STI patient encounters were further restricted to those in which patients were at least 18 years old and HIV testing and counseling occurred. We excluded patient visits that were designated as follow-up visits for earlier clinical care (e.g. a second weekly dose of prescribed treatment) as they were continuations of a recent encounter. And because we could not distinguish between STI clinic patients who were referred to care for STI diagnoses alone or STI and HIV diagnoses, we excluded encounters with patients who had been referred by a partner for care. The attributes for the participant at each visit were assigned to that visit. We restricted patient visits to those with a documented HIV status, sex, age, and marital status.

Statistical Analysis

To estimate the potential impact of the intervention as standard of care we first estimated the expected number of visits that were designated as HIV-negative or -seronegative in the Bwaila EMR but were potentially acute cases. To do this, we used the HIV-seronegative and -serodiscordant participants in the iKnow RCT. We calculated the percentages of HIV-seronegative and -serodiscordant iKnow participants that were found to have an acute infection in each strata of sex (male or female), age category (18-24 years old, 25-34 years old, 35-44 years old, and ≥45 years) and marital status (single, married, and divorced or widowed). We

applied these percentages to the frequencies of Bwaila visits with HIV-seronegative and -serodiscordant patients, also grouped into strata of sex, age category, and marital status. To account for the dynamic nature of a clinical population, we bootstrapped the Bwaila population (500 bootstraps) to estimate a 95% CI.

We combined these expected acute frequencies with the frequencies of newly HIV-seropositive and previously diagnosed PLWH in the Bwaila EMR, also categorized by sex, age category, marital status, as well as HIV status (acute infection, new HIV-seropositive infection, and previously diagnosed infection). As with the expected AHI cases, we bootstrapped 95% CIs to account for variability in patient population. The resultant dataset of frequencies and 95% CIs in each strata of HIV status, sex, age category, and marital status was the final set of Bwaila visits of PLWH where contract partner notification and social contact referral could be implemented.

Our outcomes of interest were expected persons referred (regardless of HIV status) and new HIV diagnoses made (including those with AHI). To estimate these totals for the intervention, the counts of total persons and undiagnosed PLWH referred in intervention arm were attributed to the original index participant who enrolled in the study while seeking STI care. These counts were summed for each iKnow participant in the intervention arm. Weights were calculated to standardize the iKnow intervention participants to the Bwaila STI patient population (observed frequencies and 95% CI) across strata of HIV status, sex, age category, and marital status, as described above. The weights for each stratum were then applied to the relevant referral totals and summed to estimate the total number of persons and newly diagnosed PLWH. The same standardization process was done with the iKnow control arm participants to estimate referrals through the standard of care.

In total, we examined a series of six scenarios: standard of care, each intervention component alone (contract partner notification, social contact referral, AHI screening), the

intervention among those newly diagnosed with HIV (as it was initially implemented), and the intervention among both those newly and previously diagnosed (as it was eventually modified).

To assess the effect of partial uptake if the intervention were adopted as standard of care, we estimated intervention impact in sensitivity analyses with 10% and 20% refusal rates for the intervention components in each scenario (acute HIV screening, contract partner notification, social contact referral). These refusal rates were applied in equal measure to HIV-seronegative and -serodiscordant persons hypothetically offered AHI screening across HIV status, sex, age, and marital status. All analyses were conducted using R, v. 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) or SAS v9.4 (Cary, North Carolina). See Appendix 1 for the list of packages used in the analysis.

Aim 3 Analysis

The goal of this analysis was to better understand the stage of infection among PLWH seeking STI clinical care as well as among their sexual and social contacts. As early HIV, including acute HIV, contributes disproportionately to the transmission of HIV, understanding when PLWH are diagnosed during the natural course of infection is important for understanding the strengths, limitations, and implications for HIV testing strategies.

Study Population

Only participants in the intervention arm provided blood samples in order to keep the control arm study procedures as close to the standard of care as possible. Therefore, the analytic population for this aim was restricted to intervention arm participants. Furthermore, due to the exclusion of previously diagnosed PLWH at the start of the trial, we restricted this study population to those enrolled after April 2017 when previously diagnosed PLWH were included. Because the KCH STI clinic ceased study enrollment prior to this date, all study participants included in this analysis were enrolled at the Bwaila District Hospital STI Clinic.

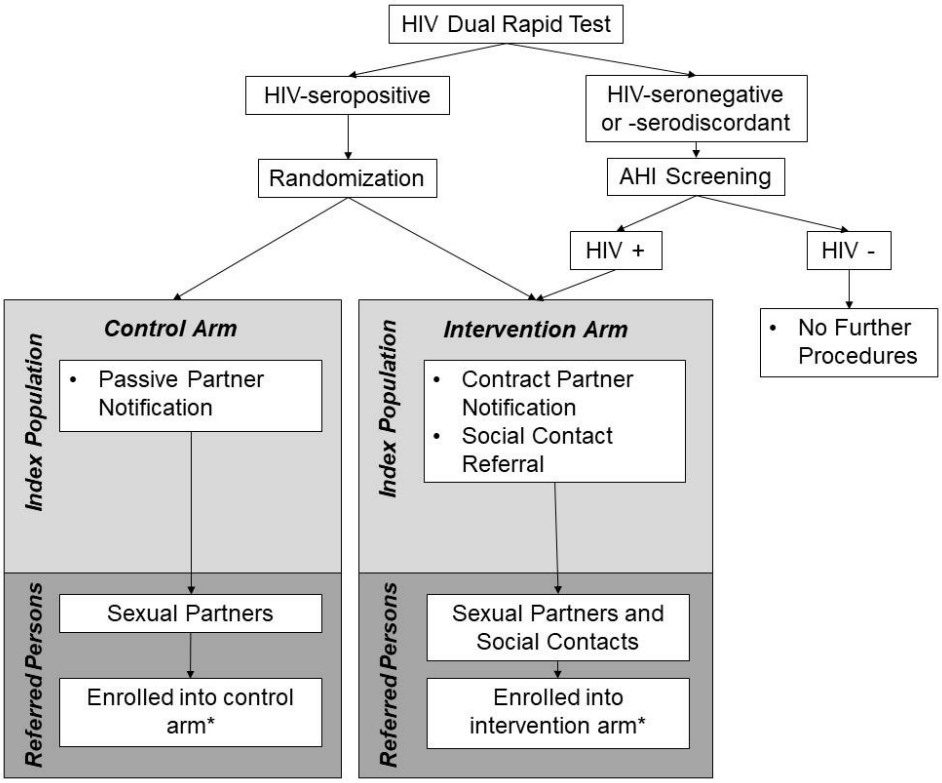
HIV Stage of Infection

As described above, participants' HIV statuses were classified as HIV-negative, acute untreated HIV infection, recent untreated HIV infection, long-term untreated HIV infection, and HIV-positive taking ART. Per protocol, all index participants and referred participants who enrolled as index participants were HIV-positive.

Statistical Analysis

We used descriptive statistics to describe patterns of HIV-infection stage among intervention arm participants and their referred sexual and social networks. All analyses were conducted using R, v. 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). See Appendix 1 for the list of packages used in the analysis.

Figure 3.1. iKnow RCT enrollment schema



*Per Malawian standard of care, rapid serological tests were used to determine HIV serostatus, and thus enrolled sexual partners in the control arm who tested HIV-seropositive were counselled on passive partner notification and enrolled sexual partners who tested HIV-seronegative were not eligible for further referral procedures. Enrolled HIV-seropositive sexual partners and social contacts in the intervention arm received contract partner notification and social contact referral procedures. Enrolled HIV-seronegative and -serodiscordant sexual partners and social contacts in the intervention arm received AHI screening.

CHAPTER 4: A RANDOMIZED CONTROLLED TRIAL EVALUATING COMBINATION DETECTION OF HIV IN MALAWIAN SEXUALLY TRANSMITTED INFECTIONS CLINICS

Introduction

HIV diagnosis is the essential first step for persons living with HIV (PLWH) to access HIV care, which can improve health outcomes and reduce the potential for onward transmission.[1,37,38] Although the scale-up of routine HIV testing in medical settings has improved HIV detection globally,[16] an estimated 19% of the nearly 38 million PLWH around the world are unaware of their HIV-positive status.[47] Improved HIV detection strategies are crucial for increasing HIV status awareness worldwide.

One key testing limitation is unrecognized acute HIV infection (AHI), which is the period of heightened transmission risk prior to the development of detectable antibodies.[29] Testing for AHI with RNA- or antigen-based approaches improves HIV detection by expanding the diagnostic window.[34] This approach has proven particularly efficient in populations with high HIV incidence, including sexually transmitted infections (STI) clinic patients.[16,34,48] Assisted partner notification (aPN) strategies, in which medical staff help PLWH refer sexual partners for HIV testing, are another efficient means of identifying PLWH unaware of their HIV infection.[19,20] Asking PLWH to refer their peers for HIV testing can also facilitate HIV diagnosis and offer a simple and inexpensive approach to widen health care engagement in a population that may be at risk of HIV.[3]

Combination detection strategies built from these approaches may be advantageous in high-burden settings like Malawi, where national HIV prevalence and incidence are estimated at 9% and 33,000 new HIV cases per year, respectively.[15] We developed an intervention incorporating AHI screening, the aPN strategy of contract partner notification, and social contact

referral, to increase HIV detection among STI clinic patients and their sociosexual networks. We assessed intervention efficacy through a randomized controlled trial (RCT) in two STI clinics in Lilongwe, Malawi.

Methods

Study Setting and Population

Patients seeking outpatient STI services at Bwaila District Hospital or Kamuzu Central Hospital (KCH) in Lilongwe, Malawi were recruited to enroll in the two-arm RCT between June 2015 and May 2019. Bwaila District Hospital is the largest public hospital in Lilongwe and KCH is the largest tertiary hospital. In January 2016, KCH transitioned to referral-based care, closing its STI clinic. All subsequent enrollment occurred only at Bwaila STI Clinic.

STI patients were eligible for study participation if they were ≥18 years old, lived in Lilongwe District, and reported sex in the previous six months. Per Malawian standard of care, STI patients were tested for HIV at the beginning of their visit using dual rapid serological tests.[43]

Study Design

Persons newly diagnosed with HIV via concordant positive rapid tests were randomly assigned as index participants in the standard-of-care (control) arm or the intervention arm. To allow for procedure-specific consent, informed consent was obtained after study arm assignment, and neither study staff nor participants were blinded.[44]

Consenting HIV-seronegative and -serodiscordant participants were screened for AHI (see below), and those found to have AHI were offered enrollment into the intervention arm as index participants.

In April 2018, a travel reimbursement was implemented for all study participants. The reimbursement and its amount were determined by the local ethics committee.

Randomization at the start of the trial was 3:1 (control: intervention), and arm assignment was blocked in groups of 4 and 8 by study personnel uninvolved with participant

enrollment to pursue an original target sample size of 1,200 intervention arm participants and 3,330 control arm participants. In response to recruitment reductions after the KCH study site closed, persons previously diagnosed with HIV became eligible for participation in April 2017, and the study randomization ratio switched to 1:1 in April 2018 to increase intervention arm enrollment specifically.

Control Arm

Control arm study procedures followed Malawian standard of care. Participants were asked to passively refer up to five sexual partners from the previous six months to the STI clinic for HIV testing with participant-specific referral cards (i.e., cards bearing the index participant's ID code). Participants also completed a short behavioral questionnaire.

Intervention Arm

Intervention arm procedures included contract partner notification, social contact referral, and AHI screening, as described below. Participants provided a blood sample and completed an in-depth behavioral survey.

For contract partner notification, intervention arm participants were asked to name up to five sexual partners from the previous six months. Participants were asked to refer these partners to the STI clinic for HIV testing with participant-specific referral cards. Locator information was collected for each partner, and if a partner did not present to the clinic within seven days, community outreach staff attempted to contact the partner for clinic referral. No index participant identifiers or clinical information were shared with partners.

For social contact referral, participants in the intervention arm were also asked to refer up to five friends and acquaintances who might benefit from HIV/STI services who they could refer to the STI clinic for HIV testing. Names or pseudonyms were used to help participants identify specific persons, but no contact information was collected. Participants were given participant-specific referral cards to give to these social contacts. Referral type (sexual partner versus social contact) was distinguishable by card color.

For Acute HIV screening, HIV-seronegative or -serodiscordant participants provided a blood sample for HIV RNA detection with Abbott RealTime HIV-1 PCR assays in pooled groups, as described elsewhere.[34] Persons who were seronegative or serodiscordant with HIV RNA ≥5000 copies/ml were considered to have AHI. Study staff initiated contact with all persons with AHI within one day of a positive AHI result. Contacted persons were referred to HIV care and offered enrollment into the intervention arm.

Referred Persons

All persons who presented to the STI clinic with a sexual or social referral card had their HIV serostatus determined per clinical standard of care. Study eligibility was assessed and enrollment was offered into the trial arm of the referring participant. Specifically, in the control arm, enrolled HIV-seropositive persons were encouraged to refer partners (i.e., passive partner notification), and enrolled HIV-seronegative and -serodiscordant persons received no further testing. In the intervention arm, enrolled HIV-seropositive persons received contract partner notification and social contact referral procedures, and enrolled HIV-seronegative and -serodiscordant persons underwent AHI screening. Finally, enrolled sexual partners and social contacts were recorded as "successfully referred" by the participant whose code appeared on the card. Due to changing enrollment criteria, persons previously diagnosed with HIV were not enrolled at the start of the trial and were therefore not counted in either arm. Systems were created to document any social harms reported during study procedures, though none were reported.

Study Outcomes

Because the study aimed to improve HIV testing engagement within the sociosexual networks of PLWH seeking STI care, all outcomes in this analysis were based on referred sexual partners and social contacts. Furthermore, all outcomes in this analysis were assessed among referred persons who enrolled in the study and were able to be linked to their referring participant, excluding sexual partners and social contacts who were ineligible for study

participation (due to age, HIV status, etc.) or unlinked to their referring index participant (e.g. computer error recording IDs).

Our primary outcome of interest was the number of new HIV diagnoses made per index participant among index participants' successfully referred sexual and social contacts, as determined by rapid tests in the control arm and both rapid tests and AHI screening in the intervention. We additionally assessed three other outcomes of interest: persons successfully referred irrespective of HIV status, sexual partners successfully referred, and PLWH (i.e., including those previously diagnosed with HIV) successfully referred. For each outcome, we examined three metrics: the number successfully referred per index, the proportion of index participants with ≥1 successful referral, and the number of index participants needed to receive the intervention (NNI) to result in one successful referral.

Statistical Analysis

To evaluate the intervention on the basis of "number referred per index participant" metrics, we used negative binomial regression or Poisson regression with a scaled deviance when a negative binomial model would not converge.[45] To test for differences between arms in proportions referring ≥1 person of a given outcome type, we used Fisher's exact test. Finally, we calculated the number of index participants needed to receive the intervention (NNI) to identify one person unaware of their HIV diagnosis, calculated as:

$$\frac{1}{\left(\frac{\#\ referred}{\#\ index}\right)_{intervention} - \left(\frac{\#\ referred}{\#\ index}\right)_{control}}$$, rounded up to the nearest integer.

We conducted several sensitivity analyses to address specific aspects of the study design, each time reassessing our main outcome of new HIV diagnoses made per index participant. To account for any unintended confounding during arm assignment, we repeated our main analysis adjusting for the sex, marital status, and new versus previous HIV diagnosis of the index participant. To account for the inclusion of index participants with AHI in the intervention arm only, we repeated our main analysis restricting to HIV-seropositive participants.

To understand how the inclusion of previously diagnosed persons midway through the trial affected the results, we repeated our main analysis with the addition of an interaction term for new (versus previous) HIV diagnosis. Finally, to determine if the travel reimbursement differentially impacted referrals across study arms, we repeated the main analysis with an interaction term for study incentive (yes/no) added. Interactions were considered significant at $\alpha=0.05$.

Analyses were conducted using SAS v9.4 (Cary, North Carolina). All study procedures received ethical approval from the University of North Carolina Institutional Review Board and the Malawian National Health Services Research Committee. The RCT is registered at clinicaltrials.gov (NCT number: NCT02467439).

Results

Between June 2015 and May 2019, 26,076 STI clinic patients were screened for study enrollment (Figure 4.1). Of those, 11,259 (43%) were ineligible or refused/terminated study participation (26% and 17%, respectively). The most common reasons for ineligibility were reporting no sex in the previous six months (42%), living outside Lilongwe (27%), and being <18 years old (23%). The most common reason for refusing participation was having no time (55%).

In total, 14,504 (56%) of the screened participants enrolled. Of those, 1,230 (8%) were HIV-seropositive participants randomized into the control arm, and 561 (4%) were HIV-seropositive participants randomized into the intervention arm. An additional 12,713 (88%) were HIV-seronegative or -serodiscordant participants who were screened for AHI. Within the latter group, 136 patients (1%) were diagnosed with AHI, 94 (69%) of whom enrolled into the intervention arm. This resulted in a total of 1,885 index participants, with 1,230 in the control arm and 655 (561 seropositive, 94 AHI) in the intervention arm.

Index Participants

The majority of randomized HIV-seropositive index participants were newly diagnosed with HIV (control: 71%; intervention: 65%), female (control: 60%; intervention: 62%) and married (control: 68%; intervention: 62%) (Table 4.1). Approximately half were 25-34 years old (control: 47%, intervention: 49%), with approximately one-quarter older and one-quarter younger. Seropositive participants in both arms reported a median of 1 (interquartile range (IQR): 1, 1) partner in the previous four weeks.

Within the intervention arm, index participants with AHI had a more even sex distribution (53% female versus 62%), were slightly younger (40% were 18-24 years old versus 25%) and were more likely to be single (23% versus 13%) compared with HIV-seropositive index participants. They reported a median of 1 (IQR: 1, 1) sexual partner in the previous four weeks (Table 4.1).

Referred Persons

Across all referral chains, 231 sexual partners were successfully referred to the STI clinic by control arm participants, and 320 sexual partners and social contacts were successfully referred by intervention arm participants (Figure 4.1). Of these successfully referred persons, 198 (86%) partners in the control arm, and 267 (83%) persons in the intervention arm were eligible and enrolled in the study. Among persons who did not enroll, more than half (65%) were ineligible due to having a known HIV diagnosis before study eligibility changed to include them. Of the 198 and 267 enrolled participants in the control arm and intervention arms, respectively, 185 (93%) and 240 (90%) were able to be linked to the referring index participant. Among linked referrals, the longest referral chain was four degrees of separation from the initial index patient, though the most common referral chain was one referred person.

Per protocol, all 185 linked persons in the control arm were sexual partners. In the intervention arm, 157 (65%) of the linked referrals were sexual partners, 81 (34%) were social contacts, and 2 were unrecorded (Table 4.2). Among sexual partners referred, 53% were men

in the control arm and 63% were men in the intervention arm. The majority were married (control: 81%; intervention: 85%). About half of sexual partners in both arms were 25-34 years old (control: 48%; intervention: 49%) and sexual partners in both arms reported a median of one partner and five sex acts in the past four weeks. About half of the sexual partners in both arms were HIV-negative (control: 56% HIV-seronegative; intervention: 51% HIV-negative), and approximately a quarter reported being previously HIV-diagnosed in both arms (control: 24%; intervention: 29%). While the majority of those previously HIV-diagnosed reported taking ART in both arms, more previously diagnosed persons in the control arm reported taking ART (control: 95%; intervention: 87%).

Among persons referred by participants in the intervention arm, a greater percentage of social versus sexual contacts were HIV-negative (70% versus 51%) and women (58% versus 37%). Fewer social contacts were newly diagnosed with HIV (7% versus 20%) and married (69% versus 85%) compared with sexual contacts. Both sexual partners and social contacts reported a median of one sexual partner and five sexual acts in the past four weeks.

Thirty-eight referred persons in each arm were newly diagnosed with HIV through their study participation (control: 21%, intervention: 16%), including five sexual partners in the intervention arm who were newly diagnosed with AHI (3% of referred persons screened for AHI).

Efficacy Analyses

The intervention was efficacious as measured by our primary outcome, with 1.9 times (95% confidence interval (CI): 1.2, 3.1) as many referred persons newly diagnosed with HIV per index participant in the intervention versus control arm (Table 4.3). The intervention was similarly efficacious in terms of total persons, sexual partners, and PLWH referred per index, with ratios comparing the intervention to control arm of 2.4 (95% CI: 1.9, 3.1), 1.6 (95% CI: 1.3, 1.9), and 2.3 (95% CI: 1.7, 3.2), respectively.

After adjusting for the index participant's sex, new versus previous HIV diagnosis status, and marital status, we estimated a ratio of 2.0 (95% CI: 1.2, 3.2) comparing the number of referred persons newly diagnosed with HIV per index participant in the intervention arm versus control (Table 4.4). When we restricted to HIV-seropositive participants (i.e., excluding participants with AHI), we estimated a smaller ratio of 1.6 (95% CI: 0.9, 2.6).

Though not statistically significant, we found that the intervention had differential effects among index participants with new versus previous HIV diagnoses (p=0.23). Specifically, our sensitivity analysis suggested that the intervention was more efficacious in finding persons unaware of their HIV status among index participants with a known HIV diagnosis (ratio: 3.3 (95% CI: 1.2, 9.5)) than those with a new HIV diagnosis (ratio: 1.6 (95% CI: 0.9, 2.8). We did not find differential effects of the travel reimbursement between trial arms (p=0.71, result not shown).

Index participants in the intervention arm were 1.6 times (95% CI: 1.0, 2.6) as likely to refer ≥1 person unaware of their HIV-positive status than index participants in the control arm, (Table 4.3). Participants receiving the intervention were also more likely than their control-arm counterparts to refer ≥1 person of any HIV status (ratio: 1.9 (95% CI: 1.6, 2.3)), ≥1 sexual partner (ratio: 1.7 (95% CI: 1.4, 2.1)) and ≥1 PLWH (ratio: 2.0 (95% CI: 1.5, 2.6)).

In expectation, 37 people would need to receive the intervention for one additional HIV case to be detected relative to the standard-of-care conditions. Five, would need to receive the intervention to refer an additional person, and twelve people would need to receive the intervention to refer an additional sexual partner or PLWH to the clinic (Table 4.3).

Discussion

We assessed the efficacy of an intervention incorporating contract partner notification, social contact referral, and AHI screening on HIV detection within the sociosexual networks of PLWH seeking STI care in Lilongwe, Malawi. This combination detection intervention increased

all referral outcomes of interest relative to the standard of care, including our primary outcome of new HIV diagnoses made.

Combining AHI screening with aPN in STI clinics is an efficient means of identifying new HIV diagnoses within sociosexual networks with substantial public health impact.[34] Testing sexual partners of persons with AHI reaches people who are either at especially high risk of HIV acquisition or who may have recently transmitted HIV, a critical population to test for HIV.[8] Testing social contacts and sexual partners of PLWH using AHI increases the diagnostic window, allowing for earlier identification of infection relative to rapid antibody testing.[32]

Assisted partner notification as a standalone service has been recommended by the WHO since 2016.[19,40] We built upon the strategy and assessed how additional services could improve HIV detection. Despite these additions, however, the NNI observed in our trial for a new diagnosis was larger than those seen in other aPN studies in Kenya[49], Mozambique[50], and Cameroon.[51,52] Due to constraints of the existing health care system in Malawi, index participants in our study were not contacted after their clinic visit, and HIV testing by partners and social contacts was only captured when performed at our study sites. In Kenya, Cameroon, and Mozambique, however, index participants had follow-up visits, and HIV testing could occur in non-study locations, including the home in Kenya and Cameroon. Such differences in study designs likely resulted in increased partner testing through greater study investment among index participants and easier testing logistics for their partners.[21]

Several participants receiving the intervention only referred social contacts, and 16% of new diagnoses made in the intervention arm were among social contacts. Though there may have been misclassification about referral type if a participant gave the social contact card to a sexual partner, social contact referral remains an inexpensive way to increase HIV testing, while simultaneously increasing discussion opportunities about HIV within social networks. For those who may not feel comfortable naming sexual partners, social contact referral offers a mechanism for PLWH to refer persons who may be at risk of HIV without needing to disclose

sexual relationships. This benefit is especially relevant given that 11% of our screened participants reported no sex in the past six months despite seeking care at an STI clinic. Enabling social contact referral may alleviate social pressures around discussing sexual health while maintaining the benefits of referral processes.

In our study, screening for AHI among STI clinic patients identified 136 new cases of HIV (1% of screened participants), and five AHI cases were identified among referred partners of PLWH (3% of screened referrals). Furthermore, when we excluded index patients with AHI from our analysis, the effect of the intervention decreased. We also did not include the number of index participants diagnosed with AHI in our outcomes to answer our research question, but this restriction limits our assessment of the total intervention impacts. Taken holistically, the AHI component of the intervention greatly increased HIV detection in our study population. PCR testing strategies to detect AHI may be cost-prohibitive in some settings, but 4th generation antigen-antibody tests may mitigate costs while still reducing the HIV window period relative to rapid tests.

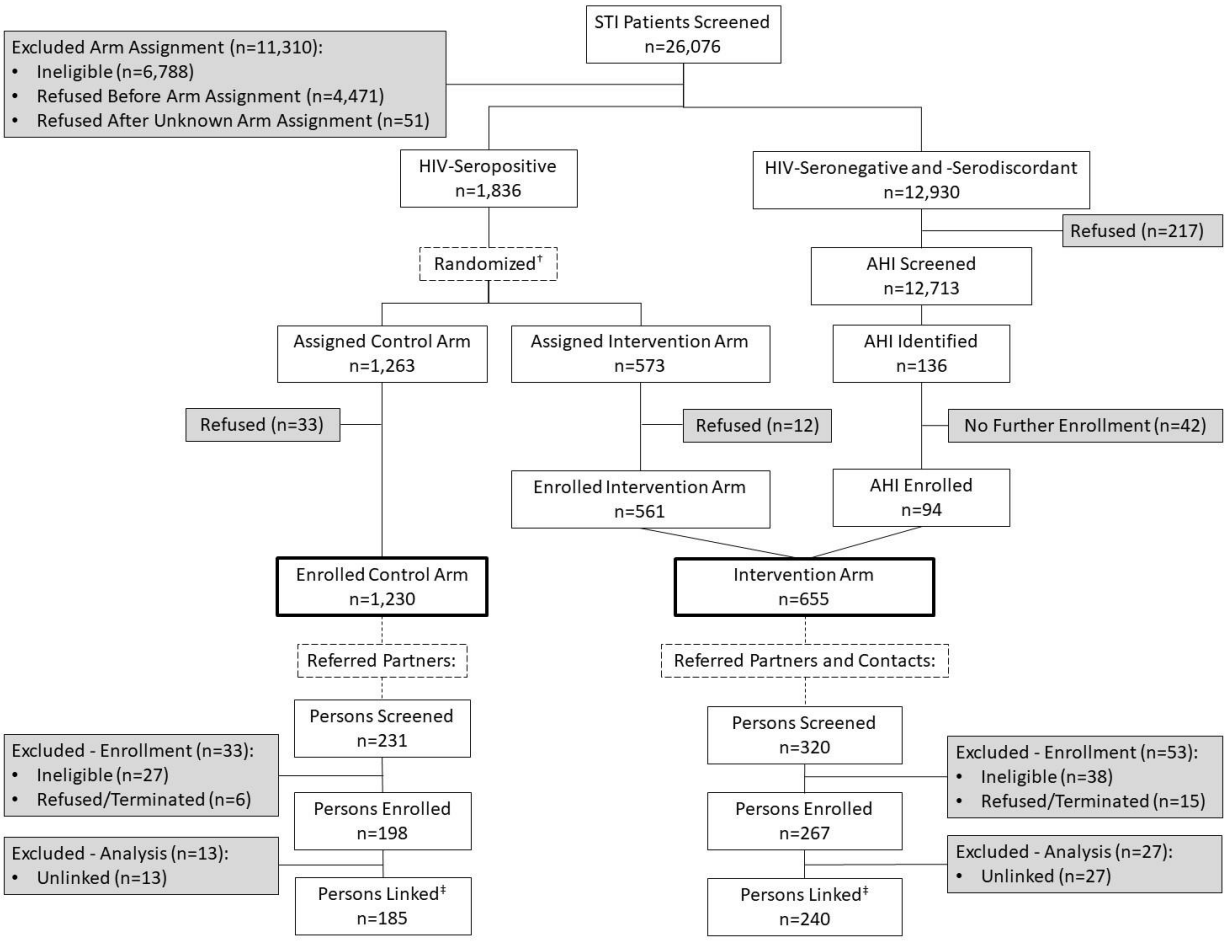
Just over half of the screened patients enrolled in the study, potentially limiting the generalizability of our findings. A high proportion of persons declined study participation citing a lack of time, likely due to the time required for informed consent and study procedures. Should the intervention be implemented as standard of care, these processes would be eliminated. While we found the intervention to be successful, barriers to widespread implementation remain. The necessary investment in personnel, patient tracking systems and lab facilities is significant and a cost-effectiveness analysis for combination detection would be informative for implementation.[6,53-56]

A unique component of our study was the inclusion of previously diagnosed PLWH, as other aPN studies tend to restrict to intervening among persons newly diagnosed with HIV.[49,52] Interestingly, we found that the intervention may have had a stronger effect among previously diagnosed index participants versus those newly diagnosed. Participants newly diagnosed with

HIV may feel a greater sense of urgency when passively referring partners, resulting in a higher level of referral through the standard of care. Meanwhile, those previously diagnosed may have already discussed their HIV status with their partners, and therefore may be less compelled to refer them through passive partner notification, causing a lower level of referral in the control arm and thus a greater potential for an intervention effect.

Our intervention combining AHI screening, contract partner notification, and social contact referral in STI clinics in Malawi was effective in bringing persons unaware of their HIV-positive status into the STI clinic for HIV testing. As the success of HIV treatment as prevention[38] and other HIV prevention strategies[57] shifts the HIV epidemic into vulnerable sub-populations[15,58], novel solutions for engaging difficult-to-reach persons unaware of their HIV status will become increasingly vital. Combination detection can improve HIV detection beyond the status quo.

Figure 4.1. Trial screening and enrollment, 2015 - 2019



*Randomization started as 3:1 (control:intervention) and changed to 1:1 in April 2018
†Linked to the original index participant and included in the analysis
Abbreviations: STI: sexually transmitted infections; AHI: acute HIV infection

Table 4.1. Demographic and behavioral characteristics for index participants by study arm

Characteristic	*Control - Index*		*Intervention - Index*					
	All		All		Seropositive		Acute	
Total	**1230**		**655**		**561**		**94**	
	n	(%)	n	(%)	n	(%)	n	(%)
HIV status								
Previously HIV-positive, ART user	306	(25)	165	(25)	165	(29)	0	(0)
Previously HIV-positive, no ART	50	(4)	31	(5)	31	(6)	0	(0)
New HIV-seropositive Diagnosis	874	(71)	365	(56)	365	(65)	0	(0)
Acute HIV Diagnosis	0	(0)	94	(14)	0	(0)	94	(100)
Sex								
Male	490	(40)	257	(39)	213	(38)	44	(47)
Female	725	(60)	395	(61)	345	(62)	50	(53)
Missing	15		3		3		0	
Age								
18-24	305	(25)	175	(27)	137	(25)	38	(40)
25-34	571	(47)	303	(47)	265	(49)	38	(40)
35-44	281	(23)	138	(22)	123	(23)	15	(16)
45+	58	(5)	24	(4)	21	(4)	3	(3)
Missing	15		15		15		0	
Marital Status								
Single	152	(12)	93	(14)	72	(13)	21	(23)
Married	828	(68)	388	(60)	344	(62)	44	(48)
Divorced/Widowed	237	(19)	166	(26)	140	(25)	26	(29)
Missing	13		8		5		3	
	Median	(IQR)	Median	(IQR)	Median	(IQR)	Median	(IQR)
Behavioral Characteristics								
Number partners in past 4 weeks	1	(1, 1)	1	(1, 1)	1	(1, 1)	1	(1, 1)
Number sex acts in past 4 weeks	4	(2, 12)	5	(2, 10)	5	(2, 12)	4	(1, 8)

Abbreviations: IQR: interquartile range

Table 4.2. Demographic and behavioral characteristics of referred, enrolled, and linked persons, by study arm

	Control		*Intervention*					
Characteristic	Sexual Partners		Sexual Partners and Social Contacts		Sexual Partners*		Social Contacts*	
Total	**185**		**240**		**157**		**81**	
	n	(%)	n	(%)	n	(%)	n	(%)
Referral Type								
Sexual Partner	185	(100)	157	(66)	157	(100)	0	(0)
Social Contact	0	(0)	81	(34)	0	(0)	81	(0)
Missing	0		2				0	
HIV status**								
HIV Seronegative/Serodiscordant	103	(56)	-	--	-	--	-	--
HIV Negative	-	--	138	(58)	80	(51)	57	(70)
Acute HIV	-	--	5	(2)	5	(3)	0	(0)
New HIV-seropositive Diagnosis	38	(21)	33	(14)	26	(17)	6	(7)
Previously HIV-positive, ART User	42	(23)	55	(23)	40	(25)	15	(19)
Previously HIV-positive, No ART	2	(1)	9	(4)	6	(4)	3	(4)
Sex								
Male	98	(53)	133	(56)	97	(63)	34	(43)
Female	86	(47)	104	(44)	58	(37)	46	(58)
Missing	1		3		2		1	
Age								
18-24	52	(28)	52	(23)	30	(20)	20	(26)
25-34	88	(48)	106	(46)	72	(49)	34	(44)
35-44	36	(20)	56	(25)	39	(26)	17	(22)
45+	8	(4)	14	(6)	7	(5)	7	(9)
Missing	1		12		9		3	
Marital Status								
Single	27	(15)	25	(10)	13	(8)	10	(12)
Married	148	(81)	189	(79)	133	(85)	56	(69)
Divorced/Widowed	7	(4)	25	(10)	10	(6)	15	(19)
Missing	3		1		1			
	Median	(IQR)	Median	(IQR)	Median	(IQR)	Median	(IQR)
Behavioral Characteristics								
Number partners in past 4 weeks	1	(1, 1)	1	(1, 1)	1	(1, 1)	1	(1, 2)
Number sex acts in past 4 weeks	5	(2, 12)	5	(1, 9)	5	(2, 10)	5	(1, 8)

*Two referred persons were unable to be classified as a sexual partner or a social contact

**HIV testing protocols in each arm resulted in different results in each arm. In the control arm, persons were only tested with dual rapid tests, per standard of care, resulting in serologic diagnoses only. In the intervention arm, HIV-seronegative and HIV-serodiscordant participants were further screened for AHI, resulting in virologic diagnoses.

Table 4.3. Count, proportion, and NNI outcomes

Outcome	*N*	*Number Referred Per Index*		*Proportion Referred ≥1*		*NNI*
		Count (95% CI)	Ratio (95% CI)	%	Ratio (95% CI)	
Persons with New HIV Diagnosis Referred						
Control Arm	38	0.03 (0.02, 0.04)		3.0%	--	--
Intervention Arm	38	0.06 (0.04, 0.08)	1.9 (1.2, 3.1)	4.9%	1.6 (1.0, 2.6)	37
Total Persons Referred						
Control Arm	185	0.15 (0.13, 0.18)	--	12.9%	--	--
Intervention Arm	240	0.37 (0.31, 0.43)	2.4 (1.9, 3.1)	24.9%	1.9 (1.6, 2.3)*	5
Sexual Partners Referred						
Control Arm	185	0.15 (0.13, 0.17)	--	12.9%	--	--
Intervention Arm	157	0.24 (0.21, 0.27)	1.6 (1.3, 1.9)	22.2%	1.7 (1.4, 2.1)*	12
Persons Living with HIV Referred						
Control Arm	82	0.07 (0.05, 0.08)	--	6.3%	--	--
Intervention Arm	102	0.16 (0.12, 0.19)	2.3 (1.7, 3.2)	12.3%	2.0 (1.5, 2.6)*	12

*Statistically significant (α=0.05) with Fisher's Exact Test

Acronyms: NNI: Number needed to receive the intervention

Table 4.4 Sensitivity analyses of the main outcome (new HIV diagnoses per index participant)

Sensitivity Analysis	*Index (N)*	*New HIV Diagnoses Per Index (95 CI)*	*Ratio (95% CI)*	*Interaction Term P Value*
Adjusted Model*				--
Control Arm	1,202	0.03 (0.02, 0.06)	--	
Intervention Arm	644	0.07 (0.03, 0.13)	2.0 (1.2, 3.2)	
Restricted to HIV-seropositive Participants **(acute participants and their referrals excluded)**				--
Control Arm	1,230	0.03 (0.03, 0.03)	--	
Intervention Arm	561	0.05 (0.03, 0.07)	1.6 (0.9, 2.6)	
Persons with New HIV Diagnosis Referred **(with interaction term for new HIV diagnosis for index participant)**				0.23
Control Arm, Previous Positive	356	0.02 (0.01, 0.04)	--	
Control Arm, New Positive	874	0.04 (0.03, 0.05)	2.2 (0.9, 5.4)	
Intervention Arm, Previous Positive	196	0.06 (0.03, 0.11)	3.3 (1.2, 9.5)	
Intervention Arm, New Positive	459	0.06 (0.04, 0.09)	3.5 (1.4, 8.8)	
Intervention Arm, New Positive (ref: control, New Positive)	--	--	1.6 (0.9, 2.8)	
Intervention Arm, New Positive (ref: intervention, Previous Positive)	--	--	1.0 (0.5, 2.3)	

*Adjusted for sex, new or previous HIV diagnosis, marital status of the index participant

CHAPTER 5: ESTIMATING EXPECTED IMPACTS OF A COMBINATION HIV DETECTION INTERVENTION IN A MALAWIAN SEXUALLY TRANSMITTED INFECTIONS CLINIC

Introduction

Randomized controlled trials (RCT) are generally considered the gold standard for testing the efficacy of health-related interventions because the random allocation of study participants to study arms theoretically balances all potential confounders, reducing or eliminating a critical threat to internal validity.[59] As a result, RCTs are often employed to assess the efficacy of biomedical interventions across preventative, therapeutic, and behavioral facets of health.[38,57,60] While RCT estimates typically have strong internal validity, inclusion criteria and patient sampling in RCT study protocols often shift study populations away from the broader patient population, resulting in findings that may not be directly generalizable to their respective settings.[50,61]

Between 2015 and 2019, we conducted the iKnow RCT to test a combination intervention to improve HIV detection within the sociosexual networks of people living with HIV (PLWH) seeking sexually transmitted infections (STI) care in Lilongwe, Malawi.[62] The intervention integrated contract partner notification,[21] social contact referral,[3] and acute HIV infection (AHI) screening and was compared to the Malawian standard of care.[34] The intervention was shown to be efficacious in increasing HIV detection (ratio: 1.9 (95% confidence interval (CI): 1.2, 3.1), but due to study ineligibility and refusal, only 56% of those screened for study participation were enrolled, raising questions about the study's external validity. Furthermore, the distribution of HIV diagnosis among index participants (acutely diagnosed, newly serologically diagnosed, and previously diagnosed) were not representative of the clinic

population, over representing those acutely and newly serologically diagnosed. Finally, outcomes were only assessed among referred persons, without consideration of the additional acute HIV diagnoses made among index participants, limiting understanding of the intervention's full impact on HIV detection in the entire study population.

In this analysis, we use quantitative generalizability methods to better understand the expected clinical impacts had the intervention or components thereof been implemented as standard of care for all patients in the larger of the two trial clinics in Lilongwe, Malawi.

Methods

Trial Population: iKnow Study

Between June 2015 and May 2019, a combination intervention to increase HIV detection was assessed in the outpatient STI clinics at Bwaila District Hospital and Kamuzu Central Hospital (KCH) in Lilongwe, Malawi. As detailed elsewhere,[62] the intervention consisted of contract partner notification, social contact referral, and AHI screening. Briefly, STI clinic patients who tested HIV-seropositive via dual rapid serological HIV tests were randomized to receive the intervention or the standard of care. Participants in both trial arms were asked to refer recent sexual partners to the clinic for HIV testing, and they were given paper referral cards containing clinic information for up to five recent sexual partners. Consistent with the assisted partner notification strategy of contract partner notification,[21] participants in the intervention arm additionally provided their partners' contact information so that trained clinic staff could reach out to the partners if they did not present to the clinic for HIV testing within one week. Participants in the intervention arm further received referral cards to give to up to five social contacts. Participants who tested HIV-seronegative or -serodiscordant with the dual rapid tests were offered AHI screening, and those found to have an acute HIV infection were contacted and offered enrollment into the study in the intervention arm.

Referred sexual partners in both study arms and social contacts in the intervention arm were documented over the course of the study along with their participant referral chains. All

referred persons who tested HIV-seropositive were additionally offered enrollment into the study in the same arm as the person who referred them. Referred HIV-seronegative and -serodiscordant persons in the intervention arm were offered AHI screening while those in the standard-of-care arm received no additional HIV testing. Those found to have AHI were offered enrollment as intervention-arm index participants.

Persons previously diagnosed with HIV were excluded from study participation until April of 2017. We therefore restricted our analytic population to those enrolled after the inclusion criteria expanded. Study enrollment ended at the KCH STI clinic when the hospital transitioned to exclusively referral-based care in January 2016. Thus, only iKnow participants enrolled at the Bwaila District Hospital study site were included in this analysis. Additionally, index participants with missing sex, age, and marital status data were excluded from the analysis as well as persons referred with an undocumented referral type (sexual partner versus social contact).

Target Population: Bwaila STI Clinic

Bwaila District Hospital is the largest public hospital in Lilongwe District, treating patients across a variety of outpatient and specialty clinics. The STI Clinic at Bwaila District Hospital provides outpatient STI care to approximately 13,000 patient visits per year. Patients are treated syndromically per Malawian standard of care on the day they present to clinic.[4]

In April 2019, an electronic medical record (EMR) was implemented in the Bwaila STI Clinic to support clinical care and collect more detailed information about the patient population and care provided. At each visit, patients provide basic demographic information, or confirm its accuracy if already in the system. Per Malawian standard of care, all patients are seen for HIV testing and counseling services before proceeding to a clinic nurse for examination and care.[46]

Our unit of analysis for the Bwaila STI Clinic population was patient visits rather than individual patients, who can have multiple visits each. Our target visits of interest were those that occurred July 1 through December 31, 2019. Bwaila patient encounters were further restricted to those in which patients were at least 18 years old and HIV testing and counseling

occurred. We excluded patient visits that were designated as follow-up visits for earlier clinical care (e.g. a second weekly dose of prescribed treatment), and because we could not distinguish between STI clinic patients who were referred to care for STI diagnoses alone or for both STI and HIV diagnoses, we excluded encounters with patients who had been referred by a partner for care. Finally, we restricted patient visits to those where the patient had HIV status, sex, age, and marital status documented.

Statistical Analysis

To estimate the potential impact of the intervention if it were to be adopted as standard of care, we first estimated the number of visits that were designated as HIV-seronegative or -serodiscordant in the Bwaila EMR but were potentially acute cases. To do this, we calculated the percentages of HIV-seronegative and -serodiscordant iKnow participants who were found to have an acute HIV infection in each stratum of sex (male or female), age category (18-24 years old, 25-34 years old, 35-44 years old, and ≥45 years) and marital status (single, married, and divorced or widowed). We then applied these percentages to the corresponding frequencies of Bwaila visits with HIV-seronegative and -serodiscordant patients to estimate the expected number of persons with undetected AHI. To account for the dynamic nature of a clinical population, we bootstrapped the observed Bwaila population (500 bootstraps) and applied the same observed iKnow percentages to estimate a 95% CI.

We combined these expected acute frequencies with the frequencies of newly HIV-seropositive and previously diagnosed PLWH in the Bwaila EMR, also categorized by sex, age category, marital status, and HIV status (acute infection, new HIV-seropositive infection, and previously diagnosed infection). As with the expected AHI cases, we bootstrapped 95% CIs to account for variability in the patient population. The resultant dataset of frequencies and 95% CIs in each strata of HIV status, sex, age category, and marital status was considered the set of Bwaila visits of PLWH where contract partner notification and social contact referral could be implemented.

Standardization weights were then calculated to standardize the iKnow intervention participants to the Bwaila STI patient population (observed frequencies and 95% CI) across strata of HIV status, sex, age category and marital status, as described above.

Our outcomes of interest were expected persons referred (regardless of HIV status) and new HIV diagnoses made (including those with AHI among index participants). To estimate these totals for the intervention, the counts of total persons and undiagnosed PLWH referred in the iKnow intervention arm were attributed to the original index participant who enrolled in the study while seeking STI care and summed. The previously calculated weights for each stratum were then applied to the relevant referral totals and summed to estimate the total number of persons and newly diagnosed PLWH. The same standardization process was done with the iKnow control-arm participants to estimate referrals through the standard of care, as the HIV specific referrals are unable to disentangled from other STI-related referrals in the Bwaila EMR and to provide consistency across our estimates for comparison purposes.

We examined a series of six scenarios: standard of care, each intervention component alone (contract partner notification, social contact referral, AHI screening), the intervention if offered only to those newly diagnosed with HIV (as it was initially implemented), and the intervention offered to both those newly and previously diagnosed (as it was eventually modified) (Table 5.1) AHI case finding among all HIV-seronegative and -serodiscordant persons was the same in both of the latter two scenarios.

To assess the effect of incomplete uptake if the intervention were adopted as standard of care, we estimated intervention impact in sensitivity analyses with 10% and 20% opt-out rates for the intervention components in each scenario (acute HIV screening, contract partner notification, social contact referral). These refusal rates were uniformly applied to HIV-seropositive persons hypothetically offered contract partner notification and social contact referral as well as HIV-seronegative and serodiscordant persons hypothetically offered AHI screening across HIV status, sex, age, and marital status.

Analyses were conducted using R, v. 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) or SAS v9.4 (Cary, North Carolina). All study procedures received ethical approval from the University of North Carolina Institutional Review Board and the Malawian National Health Services Research Committee.

Results

Patient Populations

Between July 1 and December 31, 2019, there were 5584 visits meeting our analytic requirements among 5563 patients at the Bwaila STI Clinic. Of those, 4739 (85%) visits had a documented session with an HIV testing and counseling counselor, with 4730 (>99%) having complete demographic and HIV status information. Across these 4730 visits that were eligible for inclusion in analyses, 4719 individual patients were represented, only 11 (<1%) of whom had more than one visit in the study period.

Eligible visits (n=4730) at the Bwaila STI Clinic during the analysis period were predominantly with patients who were HIV-seronegative (n=4150, 88%), with 12% (n=580) of visits being with newly or previously diagnosed PLWH (Table 5.2). Similarly, the majority of iKnow participants (n=7425, 87%) were enrolled as HIV-seronegative, though 76 of these participants (1%) were found to have AHI. iKnow included 1075 enrolled index participants living with HIV.

Characteristics of with HIV-seronegative and -serodiscordant Bwaila patients varied slightly from those of iKnow participants (Table 5.2). The majority of Bwaila visits with those who were HIV-negative were with women (58%) whereas enrollments in iKnow were more evenly distributed across genders (Female: 47%). Similar percentages of encounters in Bwaila and iKnow were with married patients (69% and 67% respectively), though distributions of unmarried patients varied slightly with Bwaila having fewer encounters with divorced or widowed patients (5% vs 10%) compared to iKnow. The age distribution across both sets of encounters was similar with the most common age group being 25-34 years old (42% for Bwaila and iKnow).

Among encounters with HIV-seropositive persons, one notable difference between Bwaila clinical care and iKnow participants was the timing of HIV diagnosis with respect to the visit date. Among Bwaila patient encounters, 78% were with persons who had been previously diagnosed with HIV, with the remaining 22% having a new HIV diagnosis via rapid test (Table 5.2). In contrast only 50% of iKnow enrollments were with persons previously diagnosed with HIV, 44% were with a person newly diagnosed with HIV via rapid test. An additional 5% were with a participant with an acute HIV diagnosis. iKnow enrollments with PLWH also tended to have a younger age distribution (70% were less than 35 years old, as compared to 57% among Bwaila encounters) and have more divorced and widowed participants (18% versus 12%).

Expected Results: Standard of Care

When the standard-of-care arm referral outcomes from iKnow were standardized to the Bwaila patient encounters over the six-month period under consideration, we estimated that 119 (95% CI: 74, 170) sexual partners would have been passively referred by HIV-seropositive patients over the period (Table 5.3). Of those, we estimated that 12 (95% CI: 7, 18) would have been new HIV diagnoses.

Expected Results: Individual Intervention Components

If contract partner notification alone been implemented during the six-month period under consideration, we estimated that 199 (95% CI: 130, 278) sexual partners would have been referred and that 23 (95% CI: 16, 33) would have been newly diagnosed with HIV (Table 5.3). Compared to our standard-of-care estimates, contract partner notification would have brought 1.7 times the referred persons to the STI clinic and would have yielded 2.0 times the number of new HIV diagnoses as the standard of care. When considering passive social contact referral alone, the clinic could expect an additional 79 (95% CI: 47, 117) social contacts in addition to the 119 (95% CI: 74, 170) sexual partners referred through the standard of care, yielding 1.7 times the number of referrals and 2.0 times the number of new HIV diagnoses made. Had an AHI screening program been implemented in the clinic, we estimated that 40

(95% CI: 35, 46) additional new diagnoses would have been identified beyond the standard of care, 4.4 times the number of new diagnoses made under the standard of care.

Expected Results: Combination Intervention

When we estimated the impacts of the intervention on patient referrals if AHI screening had been implemented in the clinic, contract partner notification and social contact referral had been provided only to those encounters with patients newly diagnosed with HIV, and AHI screening, contract partner notification, and social contact referral had been provided for their referrals, we estimated that 122 (95% CI: 79, 174) sexual partners would be referred, 21 (95% CI: 13, 31) social contacts would be referred, and 56 (95% CI: 44, 70) new HIV diagnoses would be made (Table 5.3). Compared to our standard-of-care estimates, this implementation strategy would result in 1.2 times the number of referrals and 4.7 times the number of new HIV diagnoses, including the 40 diagnosed with AHI. Finally, when we estimated the impact of the intervention on clinic referrals and diagnoses if AHI screening had been implemented in the clinic, contract partner notification and social contact referral had been provided to both newly and previously diagnosed PLWH, and AHI screening, contract partner notification, and social contact referral had been provided for all of their referred persons, we estimated that 210 (95% CI: 139, 293) sexual partners and 97 (95% CI: 60, 140) social contacts would be referred, leading to 79 (95% CI: 61, 102) new HIV diagnoses. Thus, the full intervention would yield 2.6 times the number of referrals and 6.7 times the number of new HIV diagnoses compared to the standard of care. When we excluded the index participants diagnosed with AHI in our new HIV diagnoses, an estimate comparable to the iKnow trial result, we estimated that 36 (95% CI: 26, 56) new diagnoses would be made, which is 3.3 times the expected number in the standard of care.

Participant Refusal

All estimates of sexual partners, social contacts and new HIV cases decreased when potential refusals were incorporated in sensitivity analyses (Figure 5.1). If the intervention were

only offered to persons with new HIV diagnoses with 20% refusal in AHI screening, and 20% refusal in contract partner notification and social contact referral, we would expect 114 (95% CI: 73, 165) sexual contacts referred, 16 (95% CI: 9, 25) social contacts referred and 54 (95% CI: 43, 68) new HIV diagnoses. Similarly with 20% refusal for the intervention had it been offered to all PLWH, we could expect 167 (95% CI: 105, 243) sexual partners referred, 76 (95% CI: 45, 115) social contacts and 71 (95% CI: 54, 92) new HIV diagnoses, yielding 4.6 and 6.0 times the number of new HIV diagnoses as seen in the standard of care, respectively.

Discussion

We assessed the potential impacts of separately implementing AHI screening, contract partner notification, and social contact referral, as well as two versions of an integrated combined intervention, into the Bwaila District Hospital STI Clinic between July and December 2019. We compared these results to expected standard-of-care outcomes and found that all intervention scenarios would be expected to increase the number of new HIV diagnoses beyond the standard of care. We found that among the three individual components, the AHI screening component provided the greatest overall increase in the absolute number of HIV cases detected among the stand alone strategies, and that the fully integrated intervention offered to all PLWH was the most effective overall.

In the original RCT the number of newly HIV diagnosed persons per index in the intervention arm was 1.9 (95% CI: 1.2, 3.1) times that in the standard-of-care arm.[62] Because the index participants' sociosexual networks members were the population of interest and AHI screening had already been found to be effective in identifying new HIV cases in the same clinical setting,[34,48] the AHI cases identified among index participants were not considered as part of the outcome in the primary trial analysis. While excluding those outcomes was a necessary analytical choice to answer the trial's research question, including those new HIV diagnoses in the outcomes of this analysis was important to understand the larger diagnostic impacts of the full intervention in the STI clinic. As such, the AHI cases among STI index

patients were the primary driver of our finding that 6.7 times the number of HIV cases would be identified as compared to the standard of care, which was notably higher than the RCT estimate. This was further exemplified by our finding that AHI screening alone was estimated to increase HIV detection more than four-fold without any additional referrals.

We excluded patient encounters in which the patient had already been referred to clinic by someone else, as well as those that were follow-up visits for previous care. In this context, these exclusions meant that we excluded patients who had been referred for STI management or HIV testing and likely may have had undiagnosed HIV. Additionally, we excluded 15% of patient visits that did not have documented HIV testing services (HTS) information as they likely did not meet with an HTS counselor and would therefore not have received AHI screening or referral procedures had they been implemented in the clinic. While these persons were potentially missed opportunities for outreach, implementing the intervention services at the HTS stage of the patient visit is the most feasible approach within the existing clinic flow. Anecdotally, the majority of these participants were previously diagnosed with HIV and already taking ART, so all three of these exclusions removed only low-risk persons from our target population.

Furthermore, while our analysis used all eligible encounters, programmatic decisions about how to implement an intervention with patient population that may have multiple clinical care visits over time are important. Offering contract partner notification to a patient who already participated in contract partner notification procedures during a previous STI related visit, may not be an efficient use of resources. Conversely, asking returning patients to refer social contacts or screening returning HIV-seronegative participants for AHI may be a worthwhile effort. We did not have enough electronic medical record data on repeated patient visits to fully explore these possible scenarios.

Our simulated refusal rates unsurprisingly influenced the potential impacts of the intervention in sensitivity analyses. While there was a markedly high refusal rate (56% participation) to enroll in the iKnow RCT, we hypothesize that this was driven by the opt-in

nature of study recruitment as well as the additional time required for written informed consent and research-based behavioral questionnaires. While we would not expect 100% participation if the intervention or its components were implemented as standard of care, we presume that opt-out protocols and reduced time demands relative to a research study would improve the refusal rate beyond that observed in the iKnow trial.

The results of this analysis show that the intervention has great potential to improve detection of HIV among STI clinic patients and members of their sociosexual networks. By standardizing our results across strata of gender, HIV status, age category and marital category, we were able to weight our trial results to account for study refusals and differences in study population to show that we what could expect a discernable and meaningful absolute difference in the number of HIV cases detected within our time period of consideration when comparing to the standard of care. Furthermore, by including the new HIV diagnoses made through AHI screening among index participants in our outcomes, we described a comprehensive view of the intervention's impacts. Though our estimates had wide confidence intervals, this likely reflects the temporal variability in patient population that can be seen in such a dynamic health care setting. STI clinics are a critical place for HIV testing as the HIV epidemic increasingly affects key populations.[15]

Because the primary aim of this analysis was to estimate the HIV detection effect of the intervention relative to the current standard of care, we did not take cost into account. Furthermore, the complexity surrounding decisions about cost, absolute case counts, and patient engagement remains a nuanced issue that requires input from local stakeholders and policy makers. Local clinical, laboratory, and personnel resources are not easily generalizable and vary greatly across settings, while having a critical impact on the feasibility of implementing the intervention as standard of care. Additionally, travel reimbursement was provided to iKnow participants, potentially increasing sexual partner and social contact referral during the trial, and therefore overestimating intervention impacts through standardization. The inclusion of the

travel reimbursement, as well as the amount, were determined by the local ethics committee, and thus while it may have biased our estimates, it was a necessary part of the study protocol.

Our analysis offers insight into the impacts and expected outcomes of incorporating AHI screening and partner services interventions into routine care in a resource-limited setting. While research has shown across a variety of studies that AHI screening, assisted partner notification, and partner services are effective in doing improving HIV detection, understanding how those results translate to the populations who would receive the services is more nuanced. Thinking beyond specific trial results to a more comprehensive view of intervention impacts can lead to more informed programmatic decisions.

Table 5.1. Implementation scenarios considered at Bwaila District Hospital STI Clinic, July 2019 - December 2019

Scenario	***Screening***	***Referral strategy for HIV-positive participants who were…***					
	HIV-seronegative*	Previously Diagnosed		Newly Diagnosed via Rapid Test		Acutely Diagnosed	
	AHI Screening	Sexual Partners	Social Contacts	Sexual Partners	Social Contacts	Sexual Partners	Social Contacts
1. Standard of Care	--	Passive	--	Passive	--	--	--
2. Contract Partner Notification	--	***Contract***	--	***Contract***	--	--	--
3. Social Contact Referral	--	Passive	✓	Passive	✓	--	--
4. Acute HIV Screening	✓	Passive	--	Passive	--	--	--
5. Intervention for New HIV Dx	✓	Passive	--	***Contract***	✓	***Contract***	✓
6. Intervention for All HIV Dx	✓	***Contract***	✓	***Contact***	✓	***Contract***	✓

*and HIV-serodiscordant

Acronyms: Dx - Diagnosis

Table 5.2. Demographic characteristics of iKnow index participants and the Bwaila District Hospital STI Clinic patient population from July 2019 - December 2019, stratified by HIV status

Demographic Characteristic	*HIV-seronegative (Screen for AHI)*				*HIV-positive (Referrals)*			
	Bwaila		iKnow		Bwaila		iKnow	
Total Encounters	4150		7425		580		1075	
	n	(%)	N	(%)	n	(%)	n	(%)
HIV Status								
HIV-negative/discordant	4150	(100)	7349	(99)	--	--	--	--
Previously HIV-positive	--	--	--	--	452	(78)	542	(50)
New HIV-seropositive	--	--	--	--	128	(22)	478	(44)
*New acute HIV diagnosis**	--	--	76	(1)	--	--	55	(5)
Sex								
Male	1747	(42)	3902	(53)	198	(34)	358	(33)
Female	2403	(58)	3523	(47)	382	(66)	717	(67)
Age								
18-24	1414	(34)	2740	(37)	110	(19)	270	(25)
25-34	1742	(42)	3145	(42)	222	(38)	485	(45)
35-44	733	(18)	1201	(16)	196	(34)	261	(24)
45+	261	(6)	339	(5)	52	(9)	59	(5)
Marital Status								
Single	1062	(26)	1708	(23)	106	(18)	129	(12)
Married	2870	(69)	4969	(67)	407	(70)	712	(66)
Divorced/Widowed	218	(5)	748	(10)	67	(12)	234	(22)

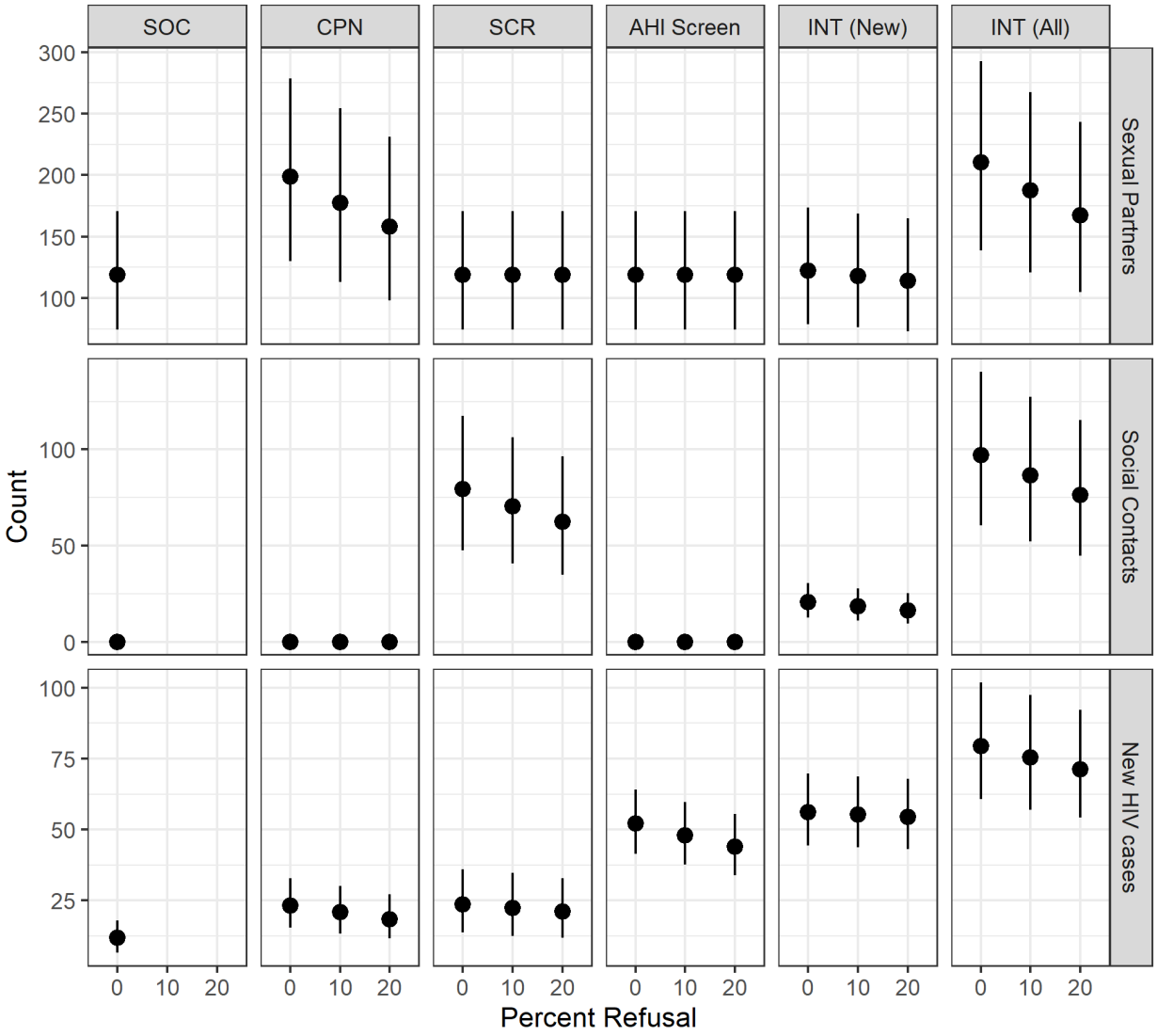
*iKnow participants with acute HIV are included in both denominators as they were HIV-seronegative or serodiscordant during their initial visit to receive AHI screening but were found to be HIV-positive and therefore offered further enrollment into the study to referred sexual partners and social contacts.
Acronyms: AHI: acute HIV infection

Table 5.3. Expected referred persons and new HIV diagnoses under six implementation scenarios with bootstrapped confidence intervals

Scenario	*AHI identified*		*Sexual Partners Referred*		*Social Contacts Referred*		*New HIV Dx's Made*		*Relative Extra Persons*	*Relative Extra New Dx*
	N	(95% CI)	N	(95% CI)	N	(95% CI)	N	(95% CI)	N	N
SOC	0	(0, 0)	119	(74, 170)	0	(0, 0)	12	(7, 18)	--	--
CPN	0	(0, 0)	199	(130, 278)	0	(0, 0)	23	(16, 33)	1.7	2.0
SCR	0	(0, 0)	119	(74, 170)	79	(47, 117)	24	(14, 36)	1.7	2.0
AHI Screen	40	(35, 46)	119	(74, 170)	0	(0, 0)	52	(41, 64)	1.0	4.4
INT (New Dx)	40	(35, 46)	122	(79, 174)	21	(13, 31)	56	(44, 70)	1.2	4.7
INT (All Dx)	40	(35, 46)	210	(139, 293)	97	(60, 140)	79	(61, 102)	2.6	6.7

*Acronyms: SOC: standard of care, CPN: contract partner notification; SCR: social contact referral; AHI screen: acute HIV screening; INT (New Dx): full intervention among patients newly diagnosed with HIV; INT (All Dx): full intervention among all persons living with HIV

Figure 5.1. Expected referred sexual partners, social contacts, and new HIV diagnoses with 10% and 20% refusal rates at Bwaila District Hospital STI Clinic July 2019 - December 2019



Acronyms: SOC: standard of care, CPN: contract partner notification; SCR: social contact referral; AHI screen: acute HIV screening; INT (New): full intervention among patients newly diagnosed with HIV; INT (All): full intervention among all persons living with HIV

CHAPTER 6: HIV DIAGNOSIS, INFECTION STAGE, AND ART USE AMONG SEXUALLY TRANSMITTED INFECTION PATIENTS AND THEIR SEXUAL AND SOCIAL CONTACTS IN LILONGWE, MALAWI

Introduction

HIV diagnosis and linkage to care benefit persons living with HIV (PLWH) at all stages of HIV infection.[1] HIV care and antiretroviral therapy (ART) improve health outcomes for PLWH and drastically reduce the potential for onward transmission.[37,38] While every diagnosis is important, the timing of diagnosis and the timing of linkage to HIV care during the natural course of infection have varying implications for health outcomes, transmission, and the HIV epidemic.[29]

The infectiousness of HIV evolves over time, with peak transmission probability occurring at the beginning of infection during early HIV.[8] Acute HIV infection (AHI), which is the first stage of early HIV, prior to seroconversion, is characterized by peak viral loads and high infectiousness.[30] Characterized by fleeting non-specific symptoms and undetectable by serological rapid tests, acute infections often go undiagnosed. Meanwhile, the deleterious impacts of an untreated HIV infection on the immune system continue to increase over time.[30] As such, early HIV contributes disproportionately to HIV transmission but often goes undiagnosed, and delays in diagnosis lead to potentially poorer long-term health outcomes for PLWH.[8] Additionally, not all newly diagnosed PLWH access available care and treatment right away, and thus persons who are aware of their HIV infection but not receiving care are also an important group to link to sustainable ART.[38]

Despite its importance for HIV transmission, determining HIV infection stage has been a diagnostic challenge until recently.[63,64] Recency testing, which includes a wide range of

biological assays, was developed to estimate HIV incidence in populations by categorizing infections within a given 'recent' window of time and determining the proportion of recent infections in the population.[35,65] Though generally summarized in aggregate within a population, recency tests yield results at individual level that can be linked with other patient characteristics. When recency tests are paired with HIV care information, a more granular understanding of HIV infection in specific clinical contexts can be achieved in pursuit of UNAIDS 90-90-90 goals.[66]

Patient populations seeking care at sexually transmitted infections (STI) clinics are known to be at increased risk of HIV transmission and acquisition through the biological synergy and similar risk factors.[2,18,48] Furthermore, research has shown that STI patient populations are effective recruiters of persons undiagnosed with HIV.[3,21,34] However, such clinics rely on standard serological HIV testing to identify and refer PLWH to care, and a more nuanced understanding of HIV in the STI clinical care setting is needed.[46]

We examined HIV diagnosis status, infection stage, and ART use among PLWH seeking STI care in Lilongwe, Malawi to better understand when and how PLWH are engaging with STI clinical care.

Methods

The following analysis is nested within the iKnow randomized controlled trial (RCT) conducted between October 2015 and May 2019 in Lilongwe, Malawi, as described elsewhere.[62] Briefly, eligible patients seeking care at the outpatient STI clinics at Bwaila District Hospital and Kamuzu Central Hospital were offered enrollment into a two-arm RCT assessing the efficacy of a combination intervention to improve HIV detection among participants' social contacts and sexual partners. The intervention included AHI screening,[34] contract partner notification,[21] and social contact referral,[3] and was compared to rapid serological HIV tests and passive partner notification, which is the Malawian standard of care.[46]

As part of routine clinical care, STI clinic patients were tested for HIV at the beginning of their visit with dual rapid HIV antibody testing (e.g. a positive initial test was confirmed with a

second rapid test).[46] Patients were eligible for study participation if they were at least 18 years old, resided in Lilongwe District, and reported sex within the previous six months. Consenting HIV-seropositive persons were randomly assigned (3:1 at the trial start, switching to 1:1 in April 2018) to the control or intervention arm.[62] Consenting HIV-seronegative and -serodiscordant participants were screened for AHI with a pooled polymerase chain reaction (PCR) approach (see below). Those who were determined to have AHI were contacted and offered enrollment into the intervention arm.

To ensure that control arm procedures remained close to the standard of care, no blood specimens were collected from control arm participants, precluding the potential for HIV recency testing. Thus, only index participants and referred persons in the intervention arm were included in the analysis. During their enrollment study visit, intervention arm participants were asked to refer up to five of their sexual partners from the previous six months to the STI clinic for HIV testing. Per contract partner notification procedures, participants also provided contact information for each sexual partner such that if he or she did not present to the clinic within seven days, clinic staff specialized in community outreach could attempt to contact them and refer them to the clinic directly. Intervention arm participants were also asked to passively refer up to five of their social contacts for HIV testing. For easy referral, participants were given paper cards to give to their sexual partners and social contacts that could be presented when they attended clinic. The type of referral card (sexual partner versus social contact) was distinguishable by color. Intervention arm participants also completed an in-depth behavioral questionnaire and provided a blood sample for HIV laboratories, including recency testing.

All referred sexual partners and social contacts in the intervention arm who presented to the clinic were tested for HIV with rapid serological tests. Those who tested HIV-seropositive were offered enrollment into the intervention arm, participating in contract partner notification and social contact referral. Consenting individuals completed the same in-depth behavioral questionnaire and provided a blood sample. Those who tested HIV-seronegative or -

serodiscordant were offered AHI screening and those found to have AHI were offered enrollment into the intervention arm, receiving the same contract partner notification and social contact referral as described above. Referral chains of sexual partners and social contacts were documented throughout the study.

Analytic Population

In addition to restricting our analytic population to participants in the intervention arm, we further restricted our analytic population to those enrolling after April 2017, when study inclusion criteria expanded from only newly diagnosed PLWH to all PLWH, including those previously diagnosed. Because the STI Clinic at Kamuzu Central Hospital had ceased study enrollment prior to this date, all participants in the current analysis were enrolled at Bwaila District Hospital's STI Clinic.

HIV Stage of Infection

Participants were categorized by HIV infection stage as HIV-negative, acute HIV infection, recent HIV infection, long-term HIV infection, or HIV-infected and taking ART.

All participants received rapid serological HIV tests during enrollment. Among those who tested HIV-seronegative or -serodiscordant, distinctions between those who were HIV-negative and acutely infected with HIV were made via Abbott RealTime HIV-1 pooled polymerase chain reaction.[34] Those with no detectable HIV RNA were classified as HIV-negative, those with detectable HIV RNA ≥5000 copies/mL were classified as acute and those with HIV RNA <5,000 copies/mL were retested again with serologic tests to assess initial testing error.

Among participants who tested HIV-seropositive at enrollment, distinctions between recent and long-term untreated HIV infections were made by the SEDIA HIV-1 LAg Avidity enzyme immunoassay (EIA) performed on the provided blood sample.[9,10] Per the manufacturer's instructions, HIV-seropositive samples were tested to assess the normalized optical density (ODn) of the processed sample. Samples with an initial ODn >2.0 were considered long-term infections and those with an ODn ≤2.0 were tested again in triplicate and

the median value was considered final. Final adjudication was made with an ODn cut-off of 1.5. Samples with an ODn ≤1.5 were classified as 'recent' with an expected mean duration of infection of 130 days (95% confidence interval (CI): 118, 142).[9]

HIV Diagnosis

HIV infections were further categorized based on how and when the diagnosis was made. The three diagnosis types were: acute infection detected through AHI screening processes described above, new serological diagnosis with rapid tests, and a previous HIV diagnosis. New serological diagnosis and previous diagnosis were determined based on participants' self-reported HIV testing history and the results of their rapid tests at the study visit.

Statistical Analysis

We used descriptive statistics to summarize HIV diagnosis status, HIV stage, and ART usage among intervention arm participants and their referred sexual partners and social contacts. We further examined patterns of referral among persons stratified by stage of HIV infection of the referring participant. All analyses were conducted using R, v. 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). All study procedures received ethical approval from the University of North Carolina Institutional Review Board and the Malawian National Health Services Research Committee.

Results

Between April 2017 and May 2019, a total of 388 HIV-positive persons enrolled into the intervention arm of the iKnow study and had available recency testing and ART information (Table 6.1). Among these index participants, 56 (14%) had an acute HIV infection, 10 (3%) had a recent infection, 165 (43%) had a long-term infection, and 157 (40%) reported taking ART. The majority of index participants were women (n=251, 65%), married (n=230, 59%), and about half were between the ages of 25-34 (n=179, 46%).

When stratified by diagnosis type, necessarily, 100% of the 56 PLWH diagnosed through AHI screening had an acute infection and were not taking ART (Figure 6.1). Among those who

were newly diagnosed with HIV via rapid serological testing, 6% had recent untreated infections and 94% had long-term untreated infections. Among those previously diagnosed with HIV, 85% reported taking ART, 15% reported not taking ART and had a long-term infection, and 1% reported not taking ART and had a recent infection.

Referred Sexual Partners and Social Contacts

During enrollment, 109 sexual partners and 76 social contacts were referred (Figure 6.2). Approximately half of referred sexual partners (52/109, 48%) were HIV-negative, with another 39% (n=42) having a previous HIV diagnosis, and 14% (n=15) having a new HIV diagnosis. Among those sexual partners previously diagnosed, 93% (n=39) reported taking ART. The remaining 7% (n=3) had a long-term infection and reported ne ART use. Among those newly diagnosed with HIV, 80% (n=12) had a long-term infection, 7% (n=1) had a recent infection, and 13% (n=2) had an acute HIV infection.

Among referred social contacts, the majority (54/76, 71%) were HIV negative, 24% (n=18) were previously diagnosed with HIV, and 5% (n=4) were newly diagnosed with HIV. Among social contacts newly diagnosed, 100% (n=4) had long-term infections and reported no ART use. Among social contacts previously diagnosed, 83% (n=15) reported taking ART, and the remaining 17% (n=3) had a long-term infection and did not report ART use.

Referral Dyads

Among sexual partners, 105 (96%) were referred by a person of a different sex and 4 (4%) were referred by someone of the same sex (Table 6.2). The vast majority of referred sexual partners were married persons referred by other married persons (n=86, 79%), 7 (6%) sexual partners were unmarried persons referred by other unmarried persons, and 13% of sexual partners were referred by someone of a different marital status. Among social contacts, 17 (22%) were referred by someone of a different sex and 59 (78%) were referred by someone of the same sex. About half of social contact referrals (46%, n=35) were married persons referred other married persons, 9 (12%) were unmarried persons referred by another unmarried

person, and the remaining 32 (42%) were referred by referring someone of a different marital status.

Among the 109 referred sexual partners, 9 (8%) were referred by persons with an acute untreated HIV infection, 1 (1%) was referred by a person with a recent untreated HIV infection, 37 (34%) were referred by a person with a long-term untreated HIV infection, and 62 (57%) were referred by a PLWH already taking ART (Figure 6.3). When stratified by the HIV status of the referring participant, about half of each group of referred persons were HIV-negative: 44%, 49%, and 48% among sexual partners referred by persons with acute HIV, long-term HIV infection and those taking ART, respectively. Among those referred by persons with AHI, the other half was mostly comprised of people with a long-term HIV infection (33%) followed by those with AHI (11%) and those taking ART (11%). Among sexual partners referred by persons with a long-term HIV infection or those taking ART, the next most common referral group were those taking ART (27% and 44%, respectively) and those with long-term infections (19% and 8%, respectively). Persons with a recent HIV infection only referred one sexual partner who was HIV-positive and reported taking ART.

Among the 76 social contact referrals made, 10 (13%) were made by persons with AHI, 28 (37%) were made by persons with a long-term untreated HIV infection, and 38 (50%) were made by a PLWH already taking ART (Figure 6.3). When stratified by the HIV status of the referring participant, the majority of referred social contacts were HIV-negative: 90%, 71%, and 66% among social contacts referred by persons with acute HIV, long-term infection, or those taking ART. Persons with acute HIV only referred one other person who had a recent infection. Among those referred by persons with a long-term infection or those taking ART, the next most common groups were social contacts taking ART (18% and 26%, respectively) and those with long-term untreated HIV infections (11% and 8%, respectively). Those with a recent infection did not refer any social contacts.

Discussion

We examined HIV infection stage and ART use among PLWH seeking STI care in Lilongwe, Malawi, along with their sexual partners and social contacts. We leveraged a novel biological assay to assess patterns of HIV diagnosis and infection. Among STI patients seeking care, we found that most patients newly diagnosed with HIV had a long-term infection. We also found that while the majority of persons previously diagnosed with HIV reported being on ART, prevalence of ART use in this group fell short of the second 90 in the UNAIDS 90-90-90 goals.[1]

Among index participants newly diagnosed with HIV, only 6% had recent infections. Therefore, the remaining 94% unknowingly had HIV for at least 4 months, if not considerably longer. Because the Malawian standard of care does not include AHI screening, this distribution of HIV infection stage suggests that in the absence of AHI screening, most STI patients newly diagnosed with HIV went undiagnosed during early HIV infection, which is a highly infectious stage that contributes disproportionately to HIV transmission.[29,46] Our results show that HIV serological testing at STI clinics largely finds long-term infections, after the critical early months of HIV infection are over. Importantly, study participants were enrolled while seeking care at an STI clinic, usually to seek treatment for an STI symptom(s).[4] Active STI symptoms can suggest recent condomless sex, which is a potential opportunity for undiagnosed HIV infection to transmit.

Encouragingly, our analyses also show that 85% of persons previously diagnosed with HIV and seeking STI care, reported taking ART. Though not quite reaching the UNAIDS 90-90-90 goal of 90% of persons diagnosed with HIV accessing ART, the majority of persons previously diagnosed were linked to care.[1] The remaining 15% are a critical group for care engagement. Engaging these untreated PLWH in a discussion about the importance of ART use for both personal health reasons and transmission risks is an opportunity for outreach in the context of STI care.[16]

When we examined the HIV status and stage of infection of sexual partners, approximately half of the referrals were HIV-negative. While HIV detection is a primary goal of contact partner notification, engaging HIV-negative persons in risk-reduction education and potentially linking them to PrEP services strengthens and expands HIV-prevention services.[57,67] Among social contacts referred, nearly 3 out of 4 were HIV-negative. While we do not know the HIV status of their sexual partners, engaging these contacts in HIV testing and counseling may promote open conversations within social networks about HIV risk and promote more open dialogues around HIV prevention.[25,27,68]

We had a small sample size and therefore cannot draw definitive conclusions about referral patterns within each HIV stage, however, our data show that nearly half of sexual partners referred by persons taking ART were also taking ART, which was a higher percentage than among the other groups. Conversely, those sexual partners referred by persons with AHI had the highest percentage of new HIV diagnoses and the lowest percentage of persons taking ART. Our results show that the sexual partners of those with AHI are a critical group to bring into care as the vast majority were either unaware of their HIV infection or HIV-negative and potentially at risk of acquiring HIV from their partner sexual with AHI.

Perhaps the most generalizable finding of this study was the relatively low frequency of recent infections among STI clinic patients and their referred sexual partners and social contacts compared to the other stages of infection. Early HIV diagnosis is beneficial and STI clinics are generally considered to be a medical setting that has high engagement with PLWH.[3,16] Through this study, we have improved our understanding of the HIV stages at which PLWH are engaging in STI care. As has been demonstrated before,[34,48] STI clinics are efficient medical settings in which to find acute HIV cases, and long-term infections. Though the LAg avidity assay has imperfect sensitivity and specificity,[11] our results show that PLWH engage in STI care during the AHI stage when they may have another symptomatic coinfection, and later during long-term infection when they may need STI care.

This analysis leveraged a biological assay designed to estimate HIV incidence to better understand the stage of HIV infection among STI clinic patients and their sexual partners and social contacts. A more granular understanding of HIV stage at HIV testing in the STI clinical setting has the potential to improve our understanding of HIV infection patterns and transmission dynamics and to optimize HIV testing strategies.

Table 6.1. Demographic characteristics of index participants enrolled into the intervention arm of the iKnow RCT at the Bwaila District Hospital STI Clinic, 2017-2019

Characteristic	*n (%)*	
Total	388	
	n	(%)
HIV Duration of Infection		
Acute Untreated HIV Infection	56	(14)
Recent Untreated HIV Infection	10	(3)
Long-term Untreated HIV Infection	165	(43)
In Care	157	(40)
HIV Diagnosis		
Acute HIV Screening	56	(14)
New Rapid Test Diagnosis	147	(38)
Previous HIV Diagnosis	185	(48)
Sex		
Male	137	(35)
Female	251	(65)
Marital Status		
Single	60	(15)
Married	230	(59)
Divorced/Widowed	92	(24)
Missing	6	(2)
Age		
18-24	103	(27)
25-34	179	(46)
35-44	88	(23)
45+	18	(5)

Figure 6.1. HIV infection stage and ART usage among HIV-positive index participants enrolled in the iKnow intervention arm, Bwaila District Hospital STI Clinic 2017 - 2019

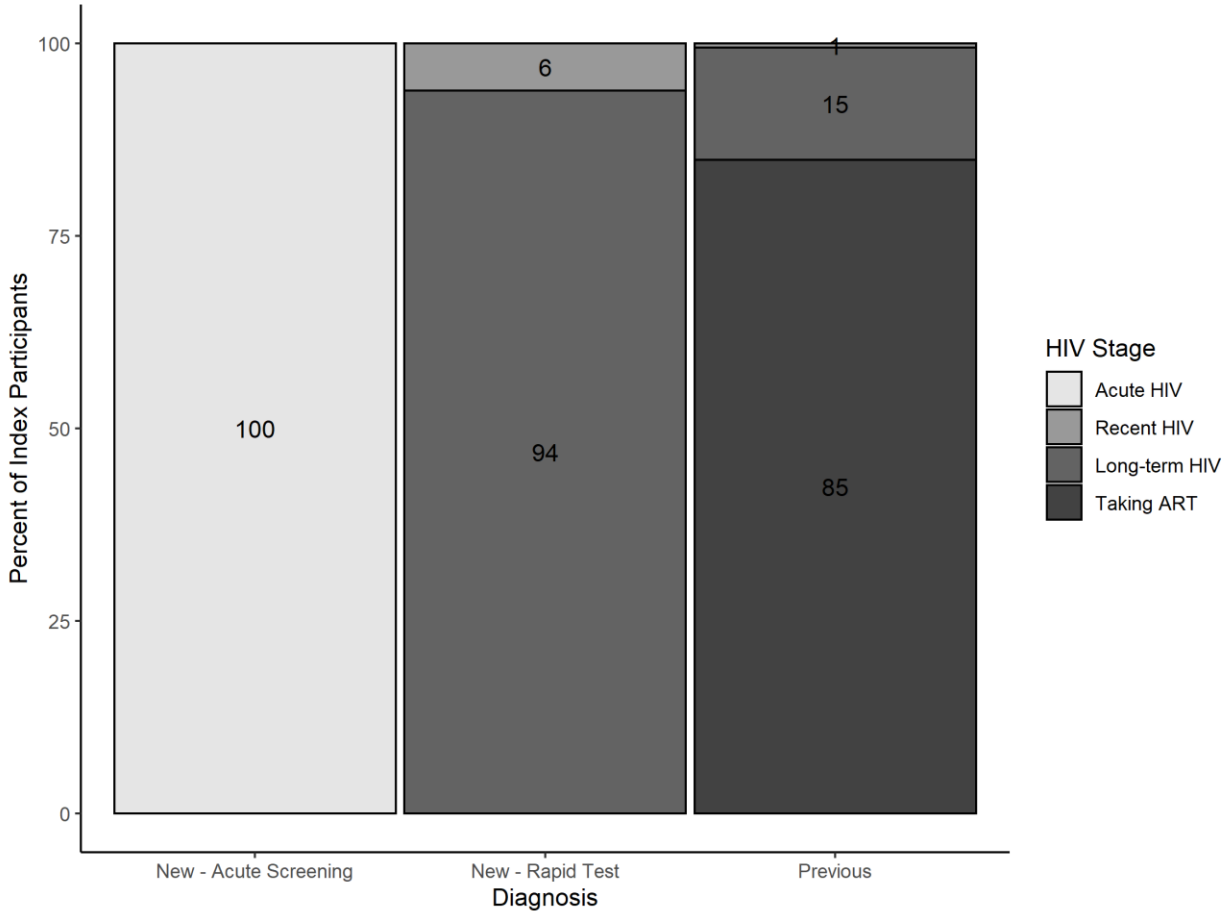


Figure 6.2. HIV infection stage and ART usage among sexual partners and social contacts referred in the iKnow intervention arm, Bwaila District Hospital STI Clinic, 2017 - 2019

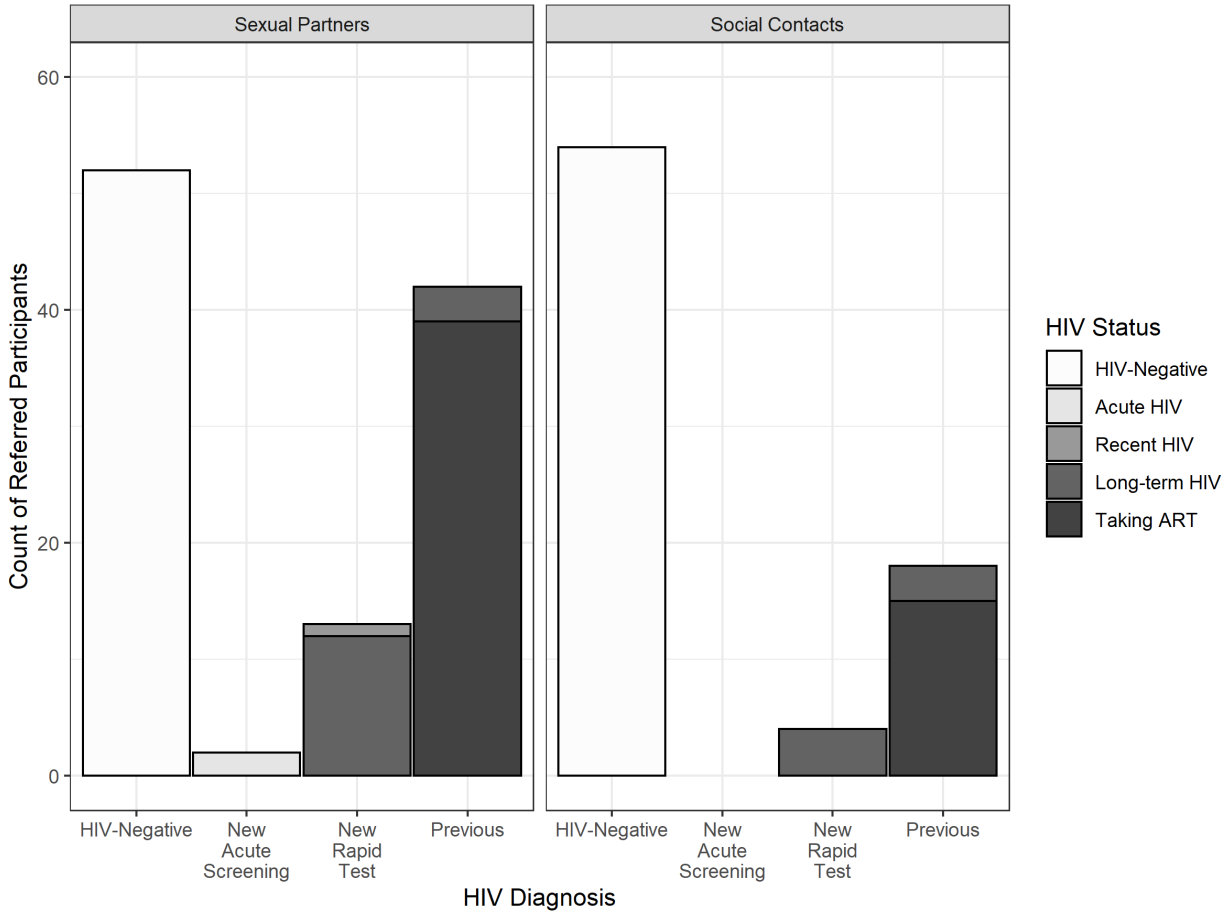
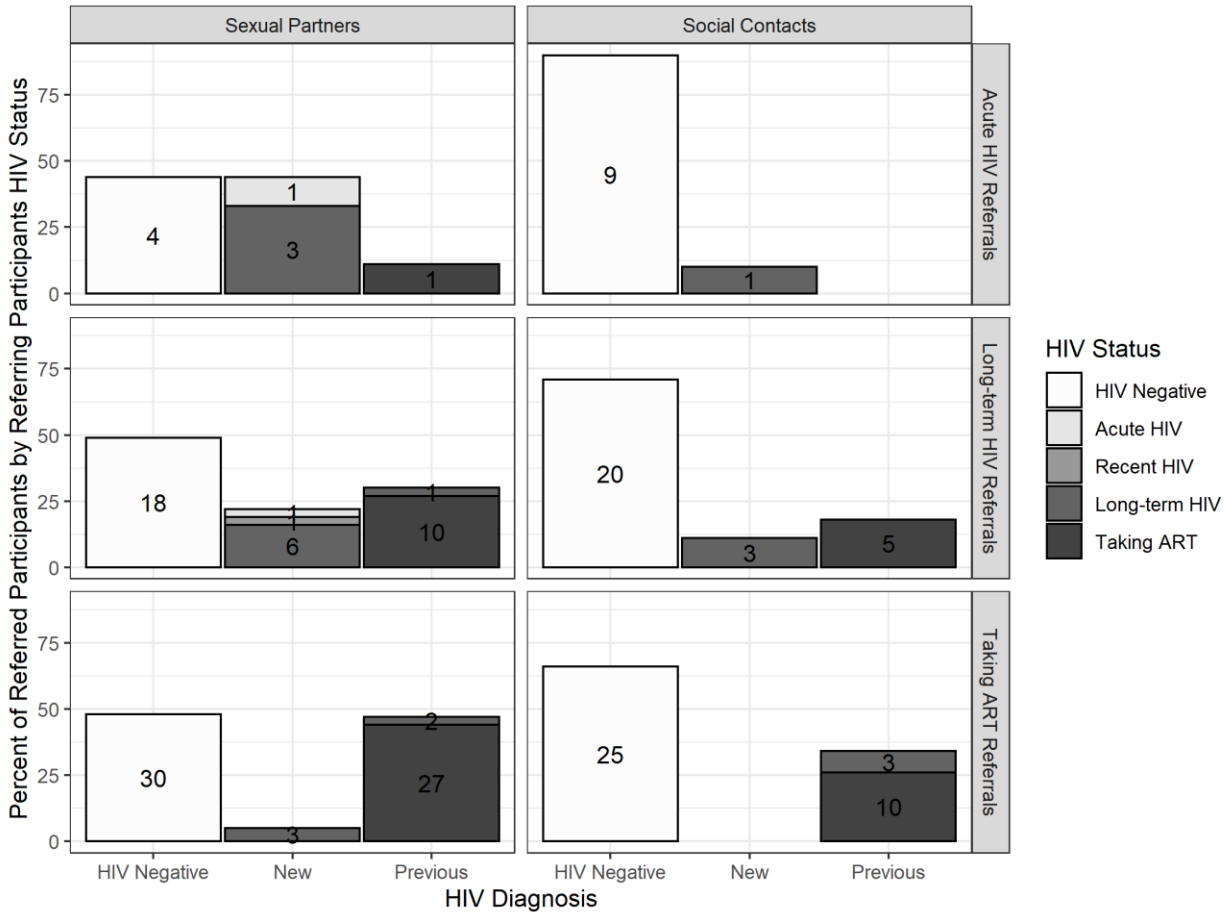


Table 6.2. Referral dyad characteristics, stratified by sexual and social relationships at the Bwaila District Hospital STI Clinic, 2017-2019

Characteristic	*Sexual Partnerships*		*Social Contacts*	
Total	109		76	
	n	(%)	n	(%)
Sex				
Referred Person of a Different Sex	105	(96)	17	(22)
Referred Person of the Same Sex	4	(4)	59	(78)
Missing	0	(0)	0	(0)
Marital Status*				
Married Person Referred Married Person	86	(79)	35	(46)
Unmarried Person Referred Unmarried Person	7	(6)	9	(12)
Person Referred Someone of a Different Marital Category	14	(13)	32	(42)

*Missing 2 with unknown marital status

Figure 6.3. HIV infection stage and ART usage among referred sexual partners and social contacts, stratified by the HIV status of the referring participant, Bwaila District Hospital STI Clinic, 2017 - 2019



NOTE: Text labels show counts.

NOTE: Referrals by persons with a recent infection are not presented due to small numbers (n=1 participant).

CHAPTER 7: CONCLUSIONS

The central theme of this dissertation is premised on ending the HIV epidemic through the UNAIDS 90-90-90 goals, with a specific focus on the first 90: 90% of PLWH knowing their status.[1] Though reaching this goal will require a multifaceted approach across prevention and treatment strategies, we chose to specifically explore how the known synergy between HIV and other STIs can be capitalized upon to increase HIV detection in resource-limited contexts.[2,18]

Due to the non-specific and fleeting nature of symptoms during early HIV infection, PLWH may not have any physiological indicators to seek HIV testing.[32] However, the field of HIV research has shown that early diagnosis is critical to improving long-term health outcomes and preventing transmission.[37,38] Because of the synergy between HIV and other STIs, testing for HIV among patients diagnosed with other symptomatic STIs has been successful in identifying and diagnosing HIV.[48] As such, this dissertation is set in the STI clinical setting and is centered around persons seeking STI care.

Previous research done by this study team and others has shown that AHI is readily detected among patients seeking STI care, highlighting the importance of intervening in this population as well as the feasibility of diagnosing HIV as early as possible.[34,48] Contract partner notification has also been shown to be effective in detecting new HIV cases in the STI clinical context, and STI clinic patients have been found to be effective recruiters of PLWH.[3,21] Thus, an intervention integrating all three strategies was designed to improve HIV detection among the social and sexual networks of PLWH seeking STI care.[62] Combining contract partner notification and AHI screening has great potential since focusing on the sexual partners of those with AHI

tests those at the highest risk of transmitting and acquiring HIV.[29] Conversely, testing the sexual partners of PLWH for AHI improves the ability to detect HIV with greater sensitivity.[32]

The first goal of this dissertation was to determine if the intervention improved HIV detection among the sociosexual networks of PLWH seeking STI care (Aim 1). We found that the intervention was successful in increasing HIV detection relative to the current Malawian standard of care. This result has positive implications for the intervention's utility and supports integrated strategies to find persons unaware of their HIV infection. We additionally found that each facet of the intervention contributed to the efficacious result making the integration of the strategies important to the overall success.

The second goal of this dissertation was to determine if the trial results would translate to the clinical setting had the intervention been implemented as the standard of care (Aim 2). We used six months of documented patient visits to the Bwaila District Hospital STI Clinic to assess potential impacts. Importantly, we also included those PLWH diagnosed with AHI through the intervention as part of our outcome in this analysis, unlike the initial trial analysis. Results of Aim 2 showed that the intervention could be even more successful than had been suggested by the clinical trial results and that AHI screening among patients at the STI clinic was a notable contributor to the new HIV diagnoses made through the intervention. We found that AHI screening, as has been shown in the past, is an effective way to detect patients as early as possible in the STI clinical setting.[34,48]

Because both rapid serological HIV tests and AHI screening have been effective in diagnosing PLWH in the STI clinical context, and AHI can be resource intensive, our third aim was to better understand how and when PLWH are being diagnosed in the STI clinical setting (Aim 3). In short, we sought to understand how AHI screening was contributing to HIV case detection when added to the standard of care. Using the SEDIA LAg avidity assay,[9] a novel biological assay that estimates recency of HIV infection based on avidity properties of anti-HIV antibodies, we assessed the recency of HIV infection among iKnow RCT participants, both

those enrolled as index patients while seeking STI care, as well as those who were referred through contract partner notification and social contact referral. Results of this analysis showed that PLWH who are diagnosed in the STI clinical setting are largely in the long-term stage of infection, meaning that they have been unaware of their HIV infection through at least the first four months of infection, including the most infectious stage of infection. We also found that while the majority of people who previously received their HIV diagnosis are in care, more than one in eight are not taking ART.

When considered holistically, the three aims of this dissertation form a narrative about HIV diagnosis in the STI clinic. Our synthesis begins with a more nuanced understanding of the standard of care. Firstly, using only standard rapid serological tests, a notable number of PLWH are seeking care at the STI clinic with acute HIV and are not being diagnosed because their infection is not detectable with standard diagnostics (Aims 1 and 2). Thus, they are engaging with STI care but the necessary tests to detect their HIV infection are not available through the standard of care. These individuals represent a critical missed opportunity because they test HIV-seronegative or HIV-serodiscordant instead of HIV-positive. Secondly, among those who are diagnosed with HIV via rapid test, the vast majority have a long-term infection (Aim 3). While we cannot assume that these are the same people who were missed in the STI clinic during acute HIV infection, we can infer that HIV diagnosis during recent infection is uncommon in STI clinics under the standard of care.

We further confirmed that contract partner notification, social contact referral, and AHI screening have the potential to increase HIV diagnosis and that social contact referral and contract partner notification generally referred more people to the clinic for HIV testing as compared to the standard of care (Aims 1 and 2). Our results also showed that implementing the integrated intervention across the clinic yielded the greatest number of new HIV diagnoses, beyond each of the individual strategies (Aim 2). Furthermore, while reaching PLWH improves diagnosis, there are positive impacts of reaching those who are HIV-negative, as it offers an

opportunity for linkage to preventative services.[67] Based on our findings in Aim 3, we also know that while those who are taking ART are most often referred by others taking ART, those with AHI are the most likely to refer PLWH not currently taking ART. Thus, the integrated strategy among those with AHI has the added benefit of increasing linkage to care.

In short, while this dissertation initially assessed the effect of one combination intervention for improved HIV detection, the implications of the full analysis have broader applications beyond the scope of the intervention.

Strengths and Limitations

Strengths and limitations of this dissertation are discussed below across four sources of epidemiological bias.

Confounding

Because of the randomized nature of the trial (Aim 1), we did not have any confounding in expectation for the trial results. However, due to practical reasons surrounding ethics and informed consent, participants gave informed consent after receiving their study arm assignment per the Zelen method,[44] giving opportunity for differential consent into each arm, and thereby breaking randomization. However, when we adjusted for potential confounders in a sensitivity analysis, we found similar results to the original estimate.

Additionally, our standardization set of variables (HIV status, sex, age category, and marital status) for our analysis in Aim 2 may have been incomplete. Omitting characteristics in the standardization set, such as number of sexual partners in the last four weeks or condom use at last sexual encounter, may have resulted in biased estimates. However, we were able to standardize across four characteristics that we hypothesize lead to the most accurate estimates without making assumptions about sexual behavior and referral patterns.

Finally, due to the descriptive nature of Aim 3, we did not assess any associations or test any hypotheses that could have been confounded.

Measurement

The key study variables in this dissertation included a variety of HIV tests (rapid serological tests for HIV serology, PCR for HIV RNA detection, avidity EIAs for duration of HIV infection), self-reported variables regarding HIV testing and treatment history, and persons referred.

The test characteristics of rapid serological HIV tests are such that diagnostic error is not a primary concern for HIV serology,[69,70] and similarly with AHI screening, the test characteristics of PCR in series with the other AHI screening procedures mitigate concerns about measurement error of HIV RNA detection.[34] The EIAs assessing antibody avidity for HIV recency however, are more prone to diagnostic error than the previously discussed laboratories.[9-11] By classifying those taking ART as a separate group, we avoided the diagnostic error from ART and antibody avidity, but potential misclassification remains. Incorporating antibody avidity assays into multi-assay algorithms would reduce this measurement error. Additionally, the reliance on self-report for HIV testing and ART history is vulnerable to social desirability bias.

Another source of measurement error is the detection of referred persons. The STI clinics at Kamuzu Central Hospital and Bwaila District Hospital are busy and their primary concern is providing the best clinical care. As such, referred persons who came to clinic through the study may have been gone undocumented. This measurement error would undercount our primary outcomes of interest, though we hypothesize that this misclassification would not be differential with respect to the exposure (i.e. those who received the intervention or the standard of care).

Missingness

Missingness for our exposure and outcome was not a primary concern for the Aim 1 analysis of the iKnow RCT: the exposure was randomized, and the outcome included missing referred partners as part of its definition. As noted above, the potential misclassification of

referred persons was a greater limitation. However, we did have missingness when it came to linking the referred sexual partners and social contacts to their referring participant. Because we needed to be able to attribute each referred sexual partner or social contact to an individual, unlinked referred persons were subsequently removed from the analysis and thus, our outcomes were undercounted. However, as with the misclassification, we do not believe this missingness was differential across arms.

In Aim 2, there was missingness within the Bwaila STI Clinic EMR, specifically surrounding HIV testing and counseling. While this missingness may have been a function of the clinic flow and those with a missing HIV status did not have a visit with the HTS counselors, this missingness potentially excluded a notable portion of the population. Anecdotally however, we surmise that those who had a missing HIV status did not have an HTS visit and therefore would not have been able to receive the intervention anyway. Thus, our estimates still reflect the potential impacts of the intervention as standard of care. We also had missingness among iKnow participants among our standardization set characteristics. Less than 1% of participants had missing data however, and so we believe that our results remain robust.

Missingness in Aim 3 is a potential concern because missing duration of HIV infection limited our sample size. Importantly however, we assume that the samples were missing completely at random as the laboratory personnel who selected the samples to test were completely blinded to any patient information when selecting the samples to test. Thus, our results are still likely representative of the population.

Generalizability

Perhaps the greatest concern regarding generalizability is the clinical setting in which this study and the subsequent analyses are housed. Our study settings were STI specialty care clinics in an urban center of a country with relatively high HIV prevalence (see section ‘Study Setting’ above). In communities where STI care is included in general outpatient or primary care, these results may not be generalizable. Furthermore, AHI screening, which is a central

pillar to this RCT and dissertation, requires laboratory resources and personnel that may not be available in other contexts. Thus, our study findings may not easily be implemented elsewhere.

Finally, due to inclusion and exclusion criteria and enrollment refusals, the analysis results of the RCT in Aim 1 are likely not generalizable as is. However, we used our analysis in Aim 2 to show that standardization methods could be used to reach more locally specific, and therefore more applicable, results.

Public Health Implications and Future Directions

This dissertation adds to the current general understanding of how a combination intervention, such as the one evaluated in this dissertation, can affect HIV diagnosis in an STI clinical setting. Decades of research have shown that the synergy between HIV and other STIs is a critical underpinning of the perpetuation of the HIV epidemic through both biological and behavioral processes.[2] Patients seeking STI care are therefore an imperative population for HIV testing, counseling, education, and prevention.

We further observed through this dissertation that AHI screening is an impactful component of HIV diagnosis among patients seeking STI care (Aim 2). Furthermore, identifying persons with AHI at STI clinical settings may be one of the earliest points of contact with the medical system after acquiring HIV, which is a critical moment for diagnosis. Due to the episodic nature of medical care, these PLWH may not need to interact with a medical professional for a long period of time after this, leaving them undiagnosed and untreated through the most infectious period of HIV infection (Aim 3). We also observed that persons with AHI are effective recruiters of other PLWH through social contact referral and contract partner notification (Aim 1) and thus engaging them in care during the acute phase has the potential to bring more PLWH into contact with the medical system.

As such, the future directions for the area of research should involve a cost-effectiveness analysis to better improve our understanding of the financial implications of the intervention. Furthermore, phylogenetic research within sexual and social networks is an

important next step in understanding viral transmission patterns. Paired with geospatial analyses of these partnerships, phylogenetic analyses can push the field forward and improve our understanding of HIV transmission and how to prevent it.

Finally, while this dissertation is centered around HIV and STI synergy, we did not include any variables in our analyses about STI diagnosis or treatment. We assumed that participant referral for HIV testing was done non-differentially toward any other STI diagnosis participants may have received. Future research should incorporate these STI comorbidities and further examine how HIV and STI synergy affects referral patterns and HIV diagnosis in STI clinics. If the STI clinical setting is an effective place to identify new HIV diagnoses, more information about the interplay between the HIV and STI care provided there is needed.

APPENDIX

R Packages Used in Aims 2 and 3:

- *R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.*

Package	**Version**
lubridate	1.7.4
tidyverse	1.2.1
sas7bdat	0.5
stringr	1.4.0

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