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HIV PREVENTION, TREATMENT, AND CARE IN SUB-SAHARAN AFRICA

John A. Crump, MB, ChB (Otago), DTM&H (London), FRACP, FRCPA, FRCP

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1. ABSTRACT

Access to HIV care and treatment services, including antiretroviral therapy, was expanded rapidly in sub-Saharan Africa from 2004 with multi-lateral support from a range of donors. This expansion raised a wide range of highly pragmatic research questions relating to HIV counseling and testing services, diagnosis and management of HIV co-infections, HIV care and treatment service delivery, and the optimal use of antiretroviral therapy. I was fortunate to lead a collaborative health research, training, and service program in northern Tanzania from 2002-11. The body of work in this thesis represents our joint efforts to anticipate and respond to key health policy and clinical management research questions arising during this extraordinary period of transformation in management of HIV infection and disease in sub-Saharan Africa.
2. ACKNOWLEDGEMENTS

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3.1. LIST OF ABBREVIATIONS

3TC  lamivudine
ABC  abacavir
ACTG  AIDS Clinical Trials Group
AIDS  acquired immune deficiency syndrome
aOR  adjusted odds ratio
ART  antiretroviral therapy
CCFCC  Child Centred Family Care Clinic
CHBC  community home-based care
CDC  United States Centers for Disease Control and Prevention
CMV  cytomegalovirus
CRF  circulating recombinant form
DUMC  Duke University Medical Center
EBV  Epstein-Barr virus
ELISA  enzyme-linked immunosorbent assay
ESR  erythrocyte sedimentation rate
HCT  HIV counseling and testing
HHV-8  human herpes virus type 8
HIV  human immunodeficiency virus
HPV  human papilloma virus
HSV  herpes simplex virus
IRIS     immune reconstitution inflammatory syndrome

IMPAACT International Maternal Pediatric and Adolescent AIDS Clinical Trials group

INH      isoniazid

ITT      intention to treat

KCMC     Kilimanjaro Christian Medical Centre

KCRI     Kilimanjaro Clinical Research Institute

KIWAKKUKI Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI

KS       Kaposi’s sarcoma

LMIC     low- or middle-income country

MAC      Mycobacterium avium complex

MSM      men who have sex with men

MTB      Mycobacterium tuberculosis complex

NAAT     nucleic acid amplification test

NHL      non-Hodgkin’s lymphoma

NIH      United States National Institutes of Health

NNRTI    non-nucleoside reverse transcriptase inhibitors

NRTI     nucleoside and nucleotide reverse transcriptase inhibitors

NTS      non-typhoidal Salmonella

OHL      oral hairy leukoplakia

OR       odds ratio
PGL  persistent generalized lymphadenopathy
PI   protease inhibitor
PML  progressive multifocal leukoencephalopathy
SAE  serious adverse event
SIV  simian immunodeficiency virus
SXT  trimethoprim-sulfamethoxazole
TAWREF Tanzania Women’s Research Foundation
TLC  total lymphocyte count
UNAIDS Joint United Nations Programme on HIV/AIDS
VZV  varicella zoster virus
WHO  World Health Organization
ZDV  zidovudine
4. INTRODUCTION

4.1. Historical background

In 1981, a series of previously healthy patients with *Pneumocytis carinii* (now *P. jiroveci*) pneumonia and Kaposi’s sarcoma were reported from California and New York in the United States.¹⁻⁵ These patients had new acquired immunodeficiency that was later named the Acquired Immunodeficiency Syndrome (AIDS), caused by the human immunodeficiency virus (HIV).⁶⁻⁷ Early epidemiologic information suggested that the disease predominantly affected men who have sex with men (MSM) in North America and Europe. However, subsequent work confirmed a much larger epidemic among heterosexual persons in sub-Saharan Africa.⁸⁻¹⁰

Since the initial description of AIDS and HIV, a large and growing body of basic science, clinical, and epidemiologic research has been directed at improving patient outcomes and curbing the expansion of the pandemic. Key achievements of this research have included the development and demonstration of clinical efficacy of antiretroviral therapy (ART) for the treatment of established HIV infection¹¹ and the widespread availability and success of ART to HIV-infected persons, first in the west¹² and then in heavily affected low- and middle-income countries (LMICs) including in sub-Saharan Africa.¹³ Major achievements in controlling the spread of HIV include the development of rapid diagnostic tests that have allowed HIV counseling and testing (HCT) services to identify infected persons while providing education directed at promoting behavior change;¹⁴ effective national campaigns that promote behavior change and condom use;¹⁵ expansion of programs to prevent mother-to-child transmission of HIV;¹⁶¹⁷ demonstration of the dramatic effect of male circumcision on reduction of male HIV acquisition;¹⁶¹⁹ evidence that early diagnosis of HIV with immediate use of suppressive ART, also known as ‘test and treat,’ reduces HIV transmission from infected persons;²⁰ and the first glimmers of hope for an effective HIV-1 transmission blocking vaccine.²¹
4.2. Origin and taxonomy of HIV

Retroviral infections of humans are understood to be zoonoses that were transmitted to humans from non-human primates. The closest relatives of HIV-1 are simian immunodeficiency viruses (SIVs) that infect wild chimpanzees (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla gorilla*) in west central Africa. Phylogenetic analyses indicate that the original hosts of the HIV-1 precursor viruses were chimpanzees. HIV-2, which is largely restricted to west Africa, is most closely related to SIVs of sooty mangabey (*Cercocebus atys atys*) communities in western Ivory Coast. Four HIV-1 lineages have arisen by independent cross-species transmissions to humans from chimpanzees; one or two of these cross-species transmissions may have occurred via gorillas. HIV-1 strains can be divided into three distinct groups. HIV-1 groups N and O are rare and recorded in Cameroon and in other west African countries. HIV-1 group M accounts for >95% of HIV-1 infections worldwide. HIV-1 group M is further subdivided into subtypes A, B, C, D, E, F, G, H, J, and K. HIV-1 group M subtypes may recombine; some of these may become established in an area and are termed circulating recombinant forms (CRFs). HIV-1 group M subtype diversity is greatest in west central Africa where the infection is thought to have originated. Sub-type diversity is lower in other parts of the world, consistent with the founder effect, or the loss of genetic variation that occurs when a new population is established by a small number of individuals from a larger population. Using molecular clocks, the common ancestor of HIV-1 group M strains has been dated to around the 1920s.

4.3. Epidemiology

In 2009, an estimated 2.6 million people became newly infected with HIV. This is 19% fewer than the estimated 3.1 million people newly infected in 1999, and 21% fewer than the estimated 3.2 million infected in 1997, the year that annual new infections peaked. As access to services for preventing the mother-to-child transmission of HIV has increased, the total number of children being born with
HIV has decreased. In 2009, 370,000 children were estimated to be newly infected with HIV, 24% fewer than in 2004.\textsuperscript{27}

Worldwide AIDS-related deaths peaked in 2004 at 2.1 million. Increasing availability of ART, combined with declining HIV incidence, has led to a steady decline in AIDS-related deaths since then, with an estimated 1.8 million deaths reported in 2009.\textsuperscript{27}

UNAIDS estimated that 33.3 million people were living with HIV at the end of 2009; 52% were women and 2.5 million were children. This compares with 26.2 million people living with HIV in 1999 and represents a 27% increase over the previous decade. HIV prevalence has continued to increase because the reduction in AIDS-related deaths associated with the scale-up of ART since 2004 have offset the decline in HIV incidence during the same period.\textsuperscript{27}

\textbf{4.3.1. Geography}

Of the 33.3 million persons living with HIV in 2009, the majority or 22.5 million (67.6\%) lived in sub-Saharan Africa. South and southeast Asia carry the next highest regional burden of infections with 4.1 million. North America has an estimated 1.5 million HIV-infected persons, Eastern Europe and central Asia and Central and South America each have 1.4 million, western and central Europe 820,000, east Asia 770,000, the Middle East and North Africa 460,000, Caribbean 240,000, and Oceania 57,000.\textsuperscript{27}

\textbf{4.3.2. Mode of transmission and risk factors}

On a worldwide basis, HIV is predominantly sexually transmitted. Unprotected heterosexual intercourse accounts for the majority of HIV infections in sub-Saharan Africa and in some countries in the Caribbean and Asia. Penile-vaginal intercourse is a relatively inefficient method for transmission of HIV, with an overall unadjusted probability of HIV-1 transmission per coital act of 0.0011 (95\% CI 0.0008–0.0015) in Rakai, Uganda.\textsuperscript{28} However, sexual transmission of HIV is amplified by high levels of viremia,\textsuperscript{29} as occur in acute
HIV infection or advanced, untreated HIV disease, and by receptive anal intercourse, sex during menses, lack of male circumcision, and the presence of other sexually transmitted infections.

Globally, mother-to-child transmission of HIV during pregnancy, delivery, or breastfeeding is a major mode of transmission. Meta-analysis indicates that the frequency of breast milk transmission during acute HIV infection of the mother is 29% (95% confidence interval [CI] 16%, 42%). In established HIV infection, breastfeeding contributes an additional 14% (95% CI 7%, 22%) to the risk of transmission in utero and during delivery. Exclusive formula feeding is known to prevent transmission of HIV via breast milk, but mixed feeding of breast milk with supplemental solids or liquids is associated with a greater risk for transmission of HIV via breast milk than is exclusive breastfeeding. Furthermore, mixed feeding and formula feeding are associated with increased risk of illness and death from non-HIV conditions such as diarrhea. Consequently, formula feeding is recommended in low-resource areas only if safe water and sanitation are assured; the mother or the caregiver can reliably provide sufficient infant formula milk to support the normal growth and development of the infant; can prepare formula cleanly and frequently enough so that it is safe and carries a low risk of diarrhea and malnutrition; can in the first six months exclusively give infant formula milk; can access health care that offers comprehensive child health services; and the family is supportive of formula feeding.

Injecting drug use is an important mode of HIV transmission outside sub-Saharan Africa, although injecting drug use is now increasingly reported from Africa as well. It is estimated that 15.9 million (range 11.0-21.2 million) people inject drugs worldwide, with the largest numbers of injectors living in China, the United States, and Russia, where mid-estimates of HIV prevalence among injectors were 12%, 16%, and 37%, respectively. Interventions to reduce HIV transmission among injecting drug users include programs that promote the
use of sterile needles for injection, drug use treatment including methadone maintenance, and community outreach.

Transfusion transmitted HIV infection continues to occur in some parts of the developing world. Underlying causes include failure to develop and implement national policies for transfusion, the recruitment of family members and paid donors, inadequate screening of collected blood, lack of strategies for the rational use of collected blood, a high prevalence of blood-borne agents, poverty, and sometimes organizational deficits.\textsuperscript{36}

The risk of HIV transmission through needle stick exposure is estimated to be 0.23% (95% CI 0.00%, 0.46%).\textsuperscript{37} Transmission of HIV through injection with non-sterile syringes and needles continues to occur both within the healthcare system and outside it. The contribution of nosocomial HIV transmission to global transmission rates is poorly quantified. However, notable outbreaks have been reported, including among paid plasma donors in several Chinese provinces associated with contaminated equipment used in the collection of plasma and reinjection of blood cells.\textsuperscript{38}

4.3.3. Access to care

As of December 2009, an estimated 5.2 million people in low- and middle-income countries were receiving ART.\textsuperscript{39} This represented an increase of 1.2 million people, or 30%, over the number receiving ART 12 months earlier. The number of children aged <15 years receiving ART increased by approximately 80,000 or 29% in 2009, from 275,000 to 354,000. However, only 28% of eligible children were accessing ART compared with 37% of eligible adults. It is estimated that 90% of the world's children living with HIV live in sub-Saharan Africa.\textsuperscript{27}

In sub-Saharan Africa, approximately 37% of people eligible for treatment were able to access ART in 2009. The proportion of eligible persons accessing ART was 42% in Central and South America, 51% in Oceania, 48% in the Caribbean, and 19% in Eastern Europe and Central Asia.\textsuperscript{27}
In 2010 WHO changed the recommended CD4 count threshold for the initiation of ART from $<200$ cells/mm$^3$ to $<350$ cells/mm$^3$ to reflect evidence of improved patient survival with earlier ART initiation. The new criteria increased the total number of people eligible for ART antiretroviral by approximately 50%, from 10 million to 15 million in 2009.

In 2009, there were an estimated 380,000 deaths from tuberculosis among people living with HIV. In sub-Saharan Africa, which accounts for 78% of people with HIV-related TB, the HIV prevalence among people with tuberculosis is as high as 80% in some countries. However, only 79,000 (0.2%) people living with HIV received isoniazid preventive therapy, a treatment that can greatly reduce a person's risk of developing TB disease. Current WHO guidelines, recommend that all patients with tuberculosis and HIV co-infection should receive ART regardless of CD4 count. In 2009, 1.6 million people with tuberculosis or 26% of the total were tested for HIV. This is compared with 22% tested in 2008 and 4% tested in 2003. Of the people tested, 450,000 were HIV-infected. Seventy five percent of those who were HIV-infected received trimethoprim-sulfamethoxazole prophylaxis against opportunistic infections and 37% received ART.

4.4. Viral transmission and life cycle

4.4.1. Biology of transmission

The early events during HIV exposure and successful transmission are incompletely understood. The majority of heterosexual HIV transmissions involve a single founder virus; a smaller proportion of heterosexual transmissions involve 2-5 founder viruses. Due to the high rate of mutation that occurs in HIV, founder viruses rapidly diversify into clusters of related viral species. Larger numbers of founder viruses are identified in patients infected via penile-anal HIV transmission compared with those infected via penile-vaginal transmission, and more still during transfusion transmission events. Transmitted viruses typically use the interaction of the viral glycoprotein gp120
with the cellular CD4 receptor and the chemokine receptor CCR5 to gain entry to the cell.

Following initial infection, there is a rapid rise in plasma HIV-1 RNA concentration\textsuperscript{44} within days associated with widespread dissemination of the virus targeting the lymphoreticular and central nervous systems. During this phase the patient may experience a seroconversion illness sometimes associated with a maculopapular rash and influenza-like symptoms. Plasma virus levels then fall to a steady state, probably related to host-mediated antivirus cellular immune responses. The innate immune response predominates in the early stage of infection; at least three HIV inhibitory chemokines are produced within hours of initial infection. High levels of potent HIV-1 specific cytotoxic T lymphocytes may correlate with the decline of virus prior to the production of neutralizing antibody through the adaptive immune response.\textsuperscript{45} The HIV-1 RNA steadying state level, or viral set point, is closely related to the subsequent rate of CD4-positive T-lymphocyte depletion and progression to symptomatic illness and death. Patients with high HIV-1 RNA set points tend to progress more rapidly to AIDS, whereas those with low set points progress slowly.

4.4.2. Life cycle

The life cycle of HIV-1 is often divided into two phases. In the first phase, virions attach to the host cell, enter into the cytoplasm, viral RNA is reverse transcribed to DNA that passes to the nucleus, and is then integrated into the host cell double-stranded DNA as the pro-virus. The second phase takes place over the lifetime of the cell. Viral and cellular proteins regulate the production of viral proteins and new infectious virions. Infection is initiated by the binding of the virion gp120 Env surface protein to CD4 molecules present on some T cells, macrophages, and microglial cells.
4.5. The pathogen

The mature infectious virus buds from the host cell membrane forming a sphere with an outer lipid bilayer and a nucleocapsid with a cone-shaped core. The outer membrane contains up to 72 spiked knobs that are trimers of the outer envelope protein gp120 bound to the transmembrane portion gp41. Within the nucleocapsid are two molecules of single-stranded RNA surrounded by three gag gene cleavage products; the p17 matrix protein; the p24 major capsid protein; and the p7 nucleoprotein. Other proteins required for early phases of infection are included in the virion, such as reverse transcriptase and integrase; tRNA; and Vpr. HIV-1 proviral DNA is 9.7 kb in length follows the structure gag-pol-env flanked by long terminal repeats containing transcriptional regulatory sequences, RNA processing signals, packaging sites, and integration sites.

4.5.1. Pathogenesis

The pathogenesis of HIV infection is related to the rate of production of HIV virions and loss of CD4-positive T-lymphocytes. In untreated patients, billions of HIV-1 virions are produced per day and billions of T-lymphocytes are turned over per day. These large numbers account for the rapid emergence of viral variants and the fluctuating and progressive nature of T cell depletion in HIV infection. One product of the interaction is a highly activated immune system that is attempting to both control HIV-1 and to renew itself. While ART drastically reduces the plasma HIV-1 RNA level, proviral DNA persists in lymph nodes and peripheral blood mononuclear cells. The pool of latently infected cells is established very early in HIV-1 infection and is refractory to elimination, even with prolonged courses of suppressive ART.

4.6. Clinical features

4.6.1. Primary HIV infection

Primary HIV infection begins when the founder virus or viruses breach the mucosal surface. Fiebig divides primary HIV infection into six stages based on
the evolution of viral markers including HIV-1 RNA, p24 antigen, HIV-1 antibody, and the appearance of bands on HIV Western blot. Clinically, approximately one half to two thirds of infected persons will develop an acute retroviral syndrome, sometimes referred to as a seroconversion illness, 2-4 weeks after primary infection. The acute retroviral syndrome corresponds with the development of HIV-1 antibodies during Fiebig Stage V. First described in 1985, the acute retroviral syndrome is characterized by an acute mononucleosis-like syndrome that is usually associated with fever, lymphadenopathy, pharyngitis, rash, and myalgia or arthralgia. Some persons may also develop thrombocytopenia, leukopenia, diarrhea, headache, nausea, vomiting, and elevated hepatic transaminase levels. Rarely hepatomegaly, thrush, or encephalopathy may occur.

4.6.2. Established HIV infection

Following acute HIV infection, the remainder of the course of HIV disease is referred to as established HIV infection. Established HIV infection is staged using two major staging systems; that of the US Centers for Disease Control and Prevention (CDC) and that of the World Health Organization. The current CDC staging system relies on the availability of a method for determining CD4 count and percent, supplemented by clinical features. The WHO staging system is suited to low-resource settings without access to a method for determining CD4 count and relies primarily on clinical features supplemented by the results of CD4 enumeration if available. In both systems stage 1 disease is defined as a CD4 count \( \geq 500 \text{ cells/mm}^3 \); the CDC system includes CD4 percent \( \geq 29 \). Stage 2 is defined as CD4 count 350-499 cells/mm\(^3\) by WHO, and CD4 count 200-499 cells/mm\(^3\) or CD4 percent 14-28 by the CDC. Stage 3 is defined by CD4 count 200-349 cells/mm\(^3\) by WHO, and CD4 count <200 cells/mm\(^3\) or CD4 percent <14 by the CDC. Only the WHO staging system includes stage 4 which is defined as CD4 count <200 cells/mm\(^3\) or CD4 percent <15. The features of WHO clinical stage 1 are asymptomatic infection and persistent generalized lymphadenopathy. WHO clinical stage 2 includes
moderate unexplained weight loss <10% presumed or measured; recurrent respiratory tract infections; herpes zoster; angular cheilitis; recurrent oral ulceration; papular pruritic eruptions; seborrheic dermatitis; or fungal nail infections. Features of WHO clinical stage 3 include unexplained severe weight loss >10% presumed or measured; unexplained chronic diarrhea >1 month; unexplained persistent fever >37.6°C intermittent or constant for >1 month; persistent oral candidiasis; oral hairy leukoplakia; current pulmonary tuberculosis; severe bacterial infection; acute necrotizing ulcerative stomatitis; gingivitis, or periodontitis; unexplained anemia <8 g/dL; neutropenia <0.5 x 10^6/L; or chronic thrombocytopenia <50 x 10^6/L. WHO clinical stage 4 includes the HIV wasting syndrome; *Pneumocystis jiroveci* infection; recurrent severe bacterial pneumonia; chronic *Herpes simplex* infection; esophageal candidiasis; extrapulmonary tuberculosis; Kaposi's sarcoma; cytomegalovirus infection; central nervous system toxoplasmosis; HIV encephalopathy; extrapulmonary cryptococcosis; disseminated non-tuberculous mycobacterial infection; progressive multifocal leukoencephalopathy; chronic cryptosporidiosis with diarrhea; chronic isosporiasis; disseminated mycosis; recurrent non-typhoidal *Salmonella* bacteremia; lymphoma or other solid HIV-associated tumors; invasive cervical carcinoma; atypical disseminated leishmaniasis; or symptomatic HIV-associated neuropathy or cardiomyopathy.49

4.6.3. Major HIV-associated infections and malignancies

HIV-associated conditions can be divided into three broad groups; those related to primary infection; immune-mediated conditions associated with the host immune response to HIV infection (e.g., lymphadenopathy, thrombocytopenia); and opportunistic infections resulting from damage to cell mediated immunity.

Persistent generalized lymphadenopathy (PGL) was described soon after the recognition of HIV disease.50,51 PGL is defined as the presence of two or more extrainguinal sites of lymphadenopathy for ≥3 months with no alternative explanation. Lymph node enlargement is generally symmetrical. Nodes remain
mobile, up to 2cm in size, and non-painful. Lymphadenopathy in HIV has a broad differential diagnosis that includes a number of HIV-associated infections and neoplasms. Lymph node biopsy in PGL shows follicular hyperplasia without evidence of specific pathogens.

Following primary HIV infection and before the onset of currently described opportunistic infections, patients may experience nonspecific constitutional symptoms. These may include fatigue, fevers <38°C, intermittent night sweats and diarrhea, and weight loss <10% of body weight. Development of weight loss ≥10% and fever >2 weeks duration may herald the onset on an AIDS-associated condition.

Risk for the major HIV-associated infections and malignancies increases as the CD4 count declines. Some of these infections and malignancies are listed below.

4.6.3.1. Extra-pulmonary and disseminated tuberculosis

Risk for development of active tuberculosis is markedly increased among persons with HIV infection. This may occur via primary infection or reactivation of latent infection. Persons with CD4-counts >400 cells/mm³ tend to develop similar clinical manifestations of tuberculosis to those without HIV infection. In immunologically advanced HIV infection, patients are more likely to develop extrapulmonary and disseminated disease with prominent constitutional symptoms. When present, pulmonary manifestations may diverge from those expected among immunocompetent persons. Mediastinal and hilar lymphadenopathy, lower lobe involvement, diffuse and widespread infiltrates, and pleural effusions may be more common. Sputum samples are more often negative for acid-fast bacilli although may ultimately be positive by mycobacterial culture. Alternative diagnostic approaches such as mycobacterial blood culture, bone marrow culture, and tissue culture for mycobacteria may be needed to confirm the diagnosis.
4.6.3.2. Disseminated non-tuberculous mycobacterial infections

Non-tuberculous mycobacteria, particularly those belonging to *Mycobacterium avium* complex (MAC) may cause disseminated infections in persons with immunologically advanced HIV disease. Disseminated MAC is uncommon in those with CD4 counts >100 cells/mm³. Infection is by ingestion or inhalation with penetration to infect the reticuloendothelial system. Gut involvement leads to thickening of the bowel wall with associated mechanical complications. Bloodstream infection is common and any organ may be seeded. Diagnosis is by mycobacterial blood culture, bone marrow culture, or culture of involved tissues.

4.6.3.3. Kaposi's sarcoma, lymphoma, and other tumors

Kaposi's sarcoma (KS) is a vascular malignancy of the skin and visceral organs caused by *human herpes virus type 8* (HHV-8). HHV-8 is transmitted sexually. KS lesions are generally painless and non-pruritic, and appear as firm, slightly raised, or nodular tumors. HIV-associated KS occurs in a younger population than the classic, non-HIV-associated form of KS that usually occurred in elderly men. It is also markedly more aggressive. HIV-infected persons have a 200-fold increased risk of developing non-Hodgkin’s lymphoma (NHL) compared with those without HIV. HIV-associated NHL tends to be more often B-cell type, advanced stage, and extra-nodal disease. The incidence of primary central nervous system lymphoma is 1,000-fold higher among HIV-infected persons than those without HIV and is associated with Epstein Barr virus (EBV) infection. Human papilloma virus (HPV) and HIV co-infection increase the risk for cervical neoplasia and anal neoplasia.

4.6.3.4. Cytomegalovirus infection

HIV-infected persons previously infected with CMV may develop CMV-associated disease, particularly as the CD4-count falls below 50 cells/mm³. Retinitis is the most commonly recognized form of CMV infection in HIV
infection and causes painless, progressive visual loss. CMV may also cause esophagitis, enteritis, colitis, pneumonitis, and infection of the central nervous system in HIV infected person.

4.6.3.5. **Chronic Herpes simplex infection**

*Herpes simplex* virus (HSV) infection is common in HIV-infected persons. HSV-1 and HSV-2, may cause more prolonged or more severe orolabial or genital lesions in HIV-infected persons compared with those without HIV. Genital HSV-2 increases risk for HIV-1 acquisition and transmission. Subclinical HSV shedding is more common among HIV-infected persons.

4.6.3.6. **Herpes zoster infection**

*Varicella zoster* virus (VZV) may recur as shingles or herpes zoster, often involving multiple dermatomes, in the presence of HIV-related immune suppression. Complicated infection including post-herpetic neuralgia and scarring occurs more often than among persons without HIV infected persons.

4.6.3.7. **Central nervous system toxoplasmosis**

*Toxoplasma gondii* infection is common but is usually not associated with disease. However, in HIV-infected persons *T. gondii* may reactivate to cause encephalitis, and less often pneumonitis and chorioretinitis. Both trimethoprim-sulfamethoxazole prophylaxis and ART-associated immune reconstitution significantly reduce the risk for *T. gondii* reactivation in HIV.

4.6.3.8. **Non-typhoidal Salmonella bacteremia and other invasive bacterial disease**

Non-typhoidal serovars of *Salmonella enterica* (NTS) cause bloodstream infections much more frequently among persons with HIV infection than among those without HIV. Furthermore, persons with HIV infection are at risk for developing recurrent bacteremia. Extended courses of initial antimicrobial therapy, lasting 4-6 weeks, may be used to reduce the risk for recurrence. In
addition to increasing risk for NTS bacteremia, HIV infection is associated with increased risk for other bloodstream infections most notably *Streptococcus pneumoniae*.67

4.6.3.9. **Cryptosporidiosis and isosporiasis**

*Cryptosporidium* and *Isospora belli* are coccidian parasites that cause diarrhea in humans. The organisms may be spread from person-to-person, via water or food, and sometimes from animals and their environments to humans. Diarrhea may be acute or persistent, but in immunologically advanced HIV disease chronic and severe diarrhea may occur.68 Occasionally extra-intestinal sites such as the biliary and respiratory tract may be involved.69 Effective ART greatly reduces the risk for disease.68

4.6.3.10. **Cryptococcosis and disseminated mycoses**

*Cryptococcus neoformans* is a fungus that usually enters the host through the lungs and has a predilection for invading the central nervous system. It is predominantly a cause of disease in immunocompromised hosts, and patients with immunologically advanced HIV disease are at particular risk for cryptococcosis and cryptococcal meningitis.70 HIV-infected patients may be at increased risk for other disseminated fungal infections, including histoplasmosis in endemic areas.71

4.6.3.11. **Esophageal candidiasis**

*Candida* spp. are yeasts that are normal commensals of the skin, gastrointestinal tract, and female genital tract. *Candida* spp. may establish infections of these and other sites, especially in immunocompromised individuals. *Candida* esophagitis occurs commonly in advanced HIV disease.72

4.6.3.12. **Pneumocystis jiroveci infection**

*Pneumocystis jiroveci* are unicellular fungi of low virulence. Infants become colonized in the respiratory tract73 and adults experience transient subclinical
colonization. Immunocompromised patients including those with HIV infection are at risk for developing *Pneumocystis* pneumonia, classically developing bilateral infiltrates extending from the perihilar regions. Both trimethoprim-sulfamethoxazole prophylaxis and ART-associated immune reconstitution significantly reduce risk for *Pneumocystis* pneumonia in HIV infected persons.

### 4.6.3.13. Oral hairy leukoplakia

Oral hairy leukoplakia (OHL) is a raised white lesion usually seen on the lateral side of the tongue. OHL is likely due to Epstein-Barr virus replication in the epithelium and becomes more common as CD4 count declines. The diagnosis of OHL is established by visual inspection, failure to scrape off the lesion with a tongue blade, lack of response to antifungal therapy, and biopsy.

### 4.6.3.14. Progressive multifocal leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is caused by the polyoma virus JCV. In HIV-infected persons PML usually develops with CD4 counts <200 cells/mm$^3$ and is most often associated with limb weakness, altered mental status, ataxia, or visual symptoms. PML lesions cause patchy or confluent areas of low attenuation on computerized tomogram or hyperintensity of T$_2$-weighted magnetic resonance imaging. There is no specific treatment for PML, and optimization of ART remains the best strategy for improving outcomes.

### 4.7. Therapy

#### 4.7.1. Prevention of opportunistic infections

Trimethoprim-sulfamethoxazole prophylaxis with one single strength tablet per day substantially reduces the risk for development of *P. jiroveci* pneumonia. Subsequently it was found the trimethoprim-sulfamethoxazole also reduces the risk of hospitalization and death among persons living with HIV in sub-Saharan Africa. In sub-Saharan Africa, the benefits of trimethoprim-sulfamethoxazole prophylaxis are thought to extend beyond prevention of *P. jiroveci* pneumonia.
to include prevention of invasive bacterial disease, bacterial pneumonia, malaria, and diarrhea.\textsuperscript{80-83} Treatment of latent tuberculosis infection reduces risk for the development of active tuberculosis among HIV-infected persons whether or not it is targeted to those with positive tuberculin skin tests.\textsuperscript{84,85}

4.7.2. Antiretroviral therapy

Antiretroviral therapy (ART) has evolved from early research demonstrating the effectiveness but lack of durability of response to individual antiretroviral drugs\textsuperscript{86-88} or combinations of two drugs\textsuperscript{89,90} for improving patient survival and reducing plasma HIV-1 RNA levels. The discovery that ART combinations using three or more drugs from multiple classes produces sustained suppression of HIV-1 RNA and greatly improved patient outcomes has revolutionized the management of HIV infection.\textsuperscript{91}

Antiretroviral drugs in common use belong to the three classes: 1. nucleoside and nucleotide reverse transcriptase inhibitors (NRTI); 2. non-nucleoside reverse transcriptase inhibitors (NNRTI); and 3. protease inhibitors (PI). Of NRTIs, zidovudine, stavudine, didanosine, lamivudine, emtricitabine, abacavir, and tenofovir are mostly widely used. Commonly used NNRTIs include nevirapine, efavirenz, delavirdine, and etravirine. The PIs ritonavir, saquinavir, indinavir, nelfinavir, amprenavir, fosamprenavir, lopinavir, atazanavir, tipranavir, and darunavir are used. Other classes of antiretroviral drugs in less widespread use to date include entry inhibitors such as enfuvirtide and maraviroc, and integrase inhibitors such as raltegravir.

At present, national antiretroviral therapy programs in low- and middle-income countries usually offer a standard first line regimen consisting of two NRTIs, such as stavudine and lamivudine, in combination with an NNRTI such as nevirapine.\textsuperscript{40} Alternative drugs may be available for substitution in special circumstances, such as zidovudine if stavudine-associated peripheral neuropathy develops or efavirenz to minimize nevirapine-related drug interactions for patients requiring simultaneous treatment for tuberculosis.
Second line regimens are usually based on a boosted PI regimen, such as lopinavir and ritonavir, in combination with NRTIs not included in the first line regimen and selected to minimize the risk for co-selected antiviral resistance, such as zidovudine, didanosine, and abacavir. Few HIV care and treatment programs in LMICs have access to routine antiretroviral drug resistance testing services. Most programs do not have additional classes of drugs available for patients who fail first- and second-line therapy.

4.8. Laboratory diagnosis

4.8.1. HIV counseling and testing

HIV Counseling and Testing (HCT) is promoted to increase serostatus awareness and entry into HIV care and treatment programs, particularly in LMICs. Although uncertainty remains about its efficacy in producing behavior change, the role of HCT in linking HIV-infected persons to care and treatment services is undisputed. Since patients seeking healthcare services are at increased risk for HIV infection relative to the general population in countries with generalized HIV epidemics, many countries have a program of provider initiated HCT in place and patients are routinely offered HCT services.

4.8.2. Antibody testing

Enzyme-linked immunosorbent assays (ELISAs) that detect circulating antibodies against HIV and Western blots that detect circulating antibodies to specific HIV proteins derived from viral lysates have formed the basis of HIV diagnosis after infancy for many years. Both achieve sensitivities and specificities exceeding 99% for the diagnosis of established HIV infection.

More recently, the development, refinement, and deployment of reliable rapid HIV antibody tests using agglutination and immunochromatographic strip formats have allowed the expansion of access to HIV testing and counseling services in remote, rural, and poor areas where more complex and expensive
means of diagnosing HIV infection are difficult to implement. The use of rapid HIV antibody tests has been endorsed by the World Health Organization and they have been adopted into national guidelines for HIV counseling and testing (HCT) in many countries in sub-Saharan Africa.

4.8.3. Nucleic acid amplification and genotyping

Nucleic acid amplification tests (NAATs) that detect or quantify HIV-1 proviral DNA or HIV-1 RNA are widely available in resource-rich settings and are increasingly available in LMICs. HIV-1 DNA NAATs may be used for the early diagnosis of HIV infection in infants. During the period from birth until 18 months, diagnosis of HIV infection is critical to avert mortality, yet the diagnosis of HIV in exposed infants using antibody testing is complicated by the presence of maternal HIV antibody as well as by the possibility of primary HIV infection prior to the development of antibody. HIV-1 RNA NAAT may also be used for early diagnosis of HIV infection in infants, but is more often used to measure HIV-1 RNA levels when monitoring patient response to ART. During successful ART, HIV-1 RNA levels will be suppressed to <400 copies/mL and often to <40 copies/mL, the lower limit of detection of HIV-1 RNA NAATs in common use.

In addition to determining HIV-1 subtype, sequencing of portions of the HIV-1 genome can be used to identify the presence of mutations associated with resistance to antiretroviral drugs. Clinically relevant drug resistance mutations in the reverse transcriptase gene, the protease gene, as well as the envelope and integrase genes are regularly published and updated. The ability to obtain genotypic resistance profiles from HIV-infected patients is available in most resource-rich countries, but is rarely available in LMIC. Information on genotypic resistance mutations can be invaluable for the management of patients who have failed ART and for whom careful selection of agents with the greatest probability of virologic success is needed.
4.8.4. Virus isolation

Isolation of HIV by *in vitro* cultivation in peripheral blood mononuclear cells may be used to confirm established HIV infection, but the technique is largely restricted to research and reference laboratories.

4.9. Background to this thesis

This MD thesis includes selected scientific contributions from almost a decade of research on HIV prevention, treatment, and care in Africa conducted from 2002 through 2011. Much of the work was done in northern Tanzania with specific emphasis on research questions related to HCT, HIV co-infections, and the early roll-out of ART in a resource-limited area. The period of this research began in 2002, prior to the availability of free HCT and HIV care and treatment services providing ART in most of sub-Saharan Africa. In 2002 HIV clinical care focused on the inpatient management of HIV co-infections, usually in patients presenting late with immunologically advanced HIV disease. Through international efforts free ART began to be available at tertiary hospitals in Tanzania from 2004 and HCT services became increasingly available in the community. During subsequent years ART provision was expanded to the regional and then district hospital level. Today in Tanzania HIV care and treatment services providing ART are available even at local health centers. This rapid and remarkable transformation of HIV prevention, care, and treatment in Tanzania and elsewhere in sub-Saharan Africa generated numerous practical research questions. The body of work reflected in this thesis attempts to address some of these questions.

4.9.1. Developing research infrastructure

The infrastructure required to do the research reported in this thesis did not exist in 2002 when I moved to Moshi, Tanzania as inaugural director of the Kilimanjaro Christian Medical Centre (KCMC)-Duke University Collaboration. Although not directly reflected in the thesis papers, the experience of developing a comprehensive clinical research site in a low-income country is an
important back-story to the thesis research. In order to provide background to the research findings in this respect, a brief history of the KCMC-Duke University Collaboration is presented here.

Duke University, based in Durham, North Carolina, in the United States, has collaborated with partners in Tanzania since the mid-1980s, initially in the Coast Region, where the Division of Infectious Diseases and International Health began a collaborative relationship with Muhimbili National Hospital in Dar es Salaam. When one of the major Tanzanian collaboration partners at Muhimbili National Hospital moved to northern Tanzania in the mid-1990s to be Executive Director of KCMC in Moshi, the collaboration moved with him. During the rest of the 1990s, the collaboration primarily provided opportunities for Duke internal medicine residents to do rotations in Tanzania in order to experience the practice of medicine there. In 2002 it was jointly agreed to scale up the collaboration. I was recruited as a fulltime Duke faculty member to be based at KCMC and to develop the program along research lines. Since that time, additional research partners have been added, including Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI; Women Against AIDS in Kilimanjaro), and more recently the Tanzania Women’s Research Foundation (TAWREF); the Kibong’oto National Tuberculosis Hospital; and inpatient and outpatient research with Mawenzi Regional Hospital. While the initial focus of the collaboration was on HIV prevention, treatment, and care research, work has expanded into other infectious diseases as well as into other disciplines including women’s health, chronic diseases, injury, health policy, and mental health.

From humble beginnings in 2002 with just one staff member and no research funding, by 2011 the collaboration employed more than 50 full-time staff and generated more than USD 2 million per year in research revenue. Staff expertise covers the spectrum needed to support health research including clinical and epidemiologic research, data management, laboratory sciences, pharmacy, and research administration. The US National Institutes of Health
(NIH) is the major sponsor of research providing major awards in the areas of research training for Tanzanian persons (AIDS International Training and Research Program); HIV co-infections (International Studies on AIDS-Associated Co-infections); HIV vaccine basic science research (Center for HIV/AIDS Vaccine Immunology); and clinical trials on optimization of management of HIV infected persons (AIDS Clinical Trials Group and International Maternal Pediatric and Adolescent AIDS Clinical Trials Group). In addition to NIH support, the KCMC-Duke Collaboration receives funding for research on global health ethics from the Wellcome Trust; typhoid fever epidemiology from the Bill & Melinda Gates Foundation; and a wide range of investigator initiated research projects.

Besides developing a large team of local research staff and successfully competing for research grants, the collaboration has been instrumental in a number of key infrastructure developments at KCMC over the past decade. The construction of the Child Centred Family Care Clinic (CCFCC), co-funded by the collaboration, houses the entire non-laboratory staff of the KCMC-Duke University Collaboration and integrates with family-oriented HIV care and treatment services as well as teaching space. The Kilimanjaro Clinical Research Institute (KCRI) Biotechnology Laboratory on the KCMC campus was built with support from the Bill & Melinda Gates Foundation. However, the early development of laboratory services within the building was led by the KCMC-Duke University Collaboration. Today, the KCMC-Duke University Collaboration operates a full-service clinical laboratory to the same standards as a US clinical laboratory including Hematology, Chemistry, Immunology, Microbiology, and Molecular Sections that provide patient care diagnostic services and also at-cost, fee for service laboratory support to numerous research groups and studies in northern Tanzania. In addition, information technology infrastructure has been improved with the establishment of a data management unit that has evolved from key-punch data entry in 2002 to scanned case report form data entry, personal digital assistant data entry, and remote data entry today.
Internet access has evolved from a dial-up service in 2002 through satellite internet, to a fiber optic cable that was connected in 2011.

The KCMC-Duke University Collaboration follows a model of 'research with service,' which means that the research program should provide training to personnel, should clearly improve patient care or public health, and that partners should strive for true collaboration with interdependence of partners and mutual benefit. As such, many of the studies reported in publications in this thesis involve Tanzanian and foreign students who wished to develop careers working on research in global health.
5. PUBLICATIONS
5.1. Sociodemographic and clinical characteristics of clients presenting for HIV voluntary counseling and testing in Moshi, Tanzania


CONTRIBUTION

My position as second author reflects my role providing on-site, day-to-day guidance and mentorship to Ms. Chu, a medical student who worked on this project. With Dr. Thielman, I co-developed the database that formed the basis of the analysis, assisted with seeking and obtaining funding, co-designed the project, and co-supervised the research including the study team members responsible for participant enrollment, data collection, and data management. Ms. Chu conducted the analysis and wrote the first draft of the manuscript with guidance from Dr. Thielman and me. Ostermann assisted with analyses. Oenga was Chu’s local student partner on the project. Itemba, Mgonja, and Mtweve coordinated research activities with HIV counseling and testing service delivery. Bartlett assisted with institutional relationships and helped to obtain funding for Chu’s year in Tanzania and for the research. All authors contributed to revisions of the manuscript.

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Sociodemographic and clinical characteristics of clients presenting for HIV voluntary counselling and testing in Moshi, Tanzania

H Y Chu MD, J A Crump MB ChB, J Ostermann PhD, R B Oenga, D K Itemba BA, A Mgonja, S Mtweve MD MPH, J A Bartlett MD, J F Shao MD PhD and N M Thielman MD MPH

1 Department of Medicine, Division of Infectious Diseases and International Health, Box 3152, Duke University Medical Center, Durham, NC 27710, USA; 2 Kilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania; 3 Health Inequalities Program, Sanford Institute of Public Policy, Duke University, Durham, NC, USA; 4 Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI, Women Against AIDS in Kilimanjaro), Moshi, Tanzania

Summary: HIV voluntary counselling and testing (VCT) reduces high-risk sexual behaviour. Factors associated with HIV infection in VCT clients have not been well characterized in northern Tanzania. We prospectively surveyed 813 VCT clients in Moshi, Tanzania. Clients were administered a questionnaire on sociodemographic characteristics, sexual behaviour, and health status. Blood was taken for rapid HIV antibody testing. Factors associated with HIV seropositivity were identified using multivariate logistic regression analysis. Of 813 clients, the seroprevalence was 16.7%. The strongest associations with seropositivity were reporting diarrhoea (odds ratio [OR] 10.4, 95% confidence interval [CI] 3.6-29.9), an ill sexual partner (OR 6.3, 95% CI 3.0-12.9), or being a woman (OR 3.5, 95% CI 2.0-6.3). In a separate regression, the number of symptoms also predicted HIV infection (OR 2.1, 95% CI 1.6-2.6). VCT clients who tested positive had more HIV-related symptoms suggesting presentation at a later stage of HIV infection.

Keywords: Tanzania, HIV seroprevalence, voluntary counselling and testing, sexual behaviour, risk factors, sociodemographic characteristics

Introduction

As of December 2003, there were an estimated 34-46 million HIV-infected individuals worldwide, of whom 90% were unaware of their infections. The epidemic has disproportionately affected sub-Saharan Africa, which bears 70% of the worldwide HIV/AIDS burden. At the end of 2002, HIV prevalence among blood donors in Tanzania was estimated at 9.7%, with 82% of the cases transmitted by heterosexual sex. Most AIDS cases fall within the age group 20-49 years, with highest rates of infection in young women aged 25-34 years. Estimates of numbers of children orphaned by AIDS range from 1.5 to over two million. Epidemic modelling has suggested that when about 5% or more of a country’s adult population becomes HIV infected, as is the case in Tanzania, the adult HIV prevalence rate tends to grow exponentially over time. In the Kilimanjaro Region, even in a hospital setting, 44% of patients found to be HIV infected in a point prevalence serosurvey on adult medicine and paediatric wards were unaware of their infections. With the advent of cheaper, rapid, and simple HIV testing kits, universal voluntary testing in Africa has been advocated.

Voluntary counselling and testing (VCT) is an effective method of reducing high-risk sexual behaviour in sub-Saharan Africa, ideally identifying infected persons early in HIV disease. The development and expansion of VCT centres in Uganda and elsewhere has been associated with significant reductions in HIV seroprevalence. Research in Kenya, Trinidad, and Tanzania has shown that unprotected sex with a non-primary partner decreases from 30% to 18% in men receiving VCT and from 22% to 12% among women receiving VCT. Those found to be HIV infected may be more likely to protect themselves and others from HIV and to seek medical attention for early symptoms of AIDS-related illnesses. Those who test negative are more likely to change their behaviour to maintain their negative status by...
using condoms and/or by encouraging their partners to test for HIV.\(^8\)

As well as promoting behaviour change, VCT can also serve as a point of referral for preventive services, including the prevention of mother to child transmission (PMTCT), and as an entry point for treatment of sexually transmitted infections (STIs), prophylaxis of opportunistic infections, diagnosis and treatment of tuberculosis (TB),\(^11\) and initiation of highly active antiretroviral therapy (HAART).\(^7\) It is estimated that less than 1% of sexually active urban populations have been tested for HIV,\(^12\) highlighting the urgent need to increase access to VCT.

The characteristics of VCT clients and risk factors for infection have not been previously reported in northern Tanzania. Consequently, we conducted a study among attendees of a newly established VCT centre in downtown Moshi, a city in the Kilimanjaro Region of Tanzania.

**Methods**

**Location and context**

An existing health-care centre in Moshi, Tanzania known as KIWAKKUKI (Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI; Women Against AIDS in Kilimanjaro) was chosen as the site for the establishment of an HIV-VCT programme and for collection of the sociodemographic characteristics of VCT clients. This decision was based on KIWAKKUKI’s strong history of HIV care in the community and the established presence of a counselling network. KIWAKKUKI supports persons living with HIV/AIDS by providing home-based care, counselling and information about HIV infection, and orphan care and assistance. Health-care counsellors at KIWAKKUKI include both volunteers and paid staff members. Volunteers and staff members are trained with classroom teaching and practical experience in accordance with the Tanzania Ministry of Health Guidelines for VCT testing. KIWAKKUKI VCT charges 1000 Tanzania shillings (US$ 0.95 at 2003 exchange rates) for VCT, and offers free testing for clients whose age is 24 years and younger, and for KIWAKKUKI members (estimated to be <4.4% of all tests). The VCT programme was initiated in March 2003, and data collection for this report began on 19 May 2003.

**Voluntary HIV counselling, interviewing, laboratory procedures, and follow-up**

Clients presenting for VCT received a confidential pre-test counselling session with a trained counsellor that lasted from 25 to 45 minutes. After obtaining informed consent, the counsellor interviewed each client using a structured questionnaire. VCT was not contingent on patient consent to participate in the survey. The questionnaire was designed to obtain sociodemographic characteristics, reasons for testing, sexual behaviour, including number of sexual partners in the past year, whether a sexual partner has other partners, condom use, alcohol use, exchange of gifts or money for sex, personal risk perception, and health status. Client response data were recorded on a paper questionnaire by the counsellor.

After pre-test counselling, a 2 mL blood sample was drawn by syringe into a glass test tube, labelled with a code, and tested using both Capillus (Trinity Biotech PLC, Bray, County Wicklow, Ireland) and Determine (Abbott Laboratories, Abbott Park, IL, USA) rapid HIV1/2 antibody tests. If the two test results were contradictory, the blood sample was sent to the zonal referral hospital for confirmatory testing via Vironostika HIV-1 microELISA assay (Organon Teknika, Charlotte, NC, USA) in accordance with World Health Organization recommendations.\(^13\) In addition, for quality control purposes repeat testing was done on every 20th blood sample at the zonal referral hospital using the Vironostika HIV-1 microELISA assay. The client received the result of the HIV test in approximately 30 min. Appropriate post-test counselling was provided according to Tanzania Ministry of Health guidelines, and clients testing positive were referred to the zonal hospital HIV clinic for care and offered home-based care through the KIWAKKUKI home-based care network.

When HIV test results returned negative, the post-test counselling focused mainly on prevention of transmission of HIV, and each client was encouraged to return for repeat HIV testing in three and six months. Regular testing of the sexual partner was also emphasized.

Ethical approval for the study was granted by the Kilimanjaro Christian Medical Centre (KCMC) Research Ethics Committee, the Institutional Review Board of Duke University Medical Center (DUMC), and the Tanzania National Institute of Medical Research (NIMR) National Medical Research Coordinating Committee.

**Analysis**

Data from questionnaires were entered into an electronic database constructed with EpiInfo 2002 software (Centers for Disease Control, Atlanta, GA, USA). Data were validated by randomly sampling 10% of the questionnaires, with acceptable error rate being less than one error per five forms. Data analysis was done using EpiInfo 2002 and Stata 8.0 (Stata Corporation, College Station, TX, USA). Multivariate logistic regression analysis was used to evaluate the association of demographic characteristics, HIV risk factors, and HIV symptoms with seropositivity. The Bonferroni correction, in which a level of significance of \(P<0.05\) is reduced to \(P<0.0014\) for these data, was used to account for the 36 comparisons.
Results

From 19 May 2003 to 23 November 2003, 813 (>99%) of individuals who presented to KIWAKKUKI for testing consented to participate in the study. The median client age was 29 years (range 13-80 years). The sociodemographic characteristics of the attendees are summarized in Table 1. Nearly equal numbers of men and women (379 and 418, respectively) presented for testing. A large proportion were employed in business (24%) or farming (21%), educated to the primary level (69%), and either single (48%) or married (29%). The most frequently cited reasons for seeking VCT were for marriage planning, illness, unfaithful sexual partner, and previous high-risk sexual behaviour. A majority of clients reported fewer than two sexual partners in the past year (86%), while 48% had sexual partners with other sexual partners. On direct questioning of symptoms of fever, weight loss, cough, rash, and diarrhoea over the previous two months, 27% of clients reported experiencing at least one symptom.

The overall seroprevalence of HIV in this population presenting for self-initiated HIV testing was 16.7%. Table 2 highlights characteristics of clients who tested seropositive versus those who tested seronegative. A majority of HIV-infected persons reported symptoms, and 48% presented for testing with symptoms they believed were specifically related to HIV infection. Being women, older, divorced, or not being single; or having an ill child or an ill sexual partner or knowing a person living with or died from HIV; or reporting symptoms or a higher perceived risk of HIV infection were all significantly associated with HIV-seropositivity. In multivariate logistic regression analysis, women were significantly more likely to test positive for HIV, as were clients with children (Table 3). Persons who were previously tested were half as likely to be positive. Having an ill sex partner or child was associated with significantly increased odds of seropositivity. Clients' perceived HIV symptoms and any of the specific symptoms asked about in the survey were associated with higher odds of seropositivity, but the effect was significant only for diarrhoea. Persons who had previously received TB treatment were three times as likely to test positive as those who had not received such treatment. The level of self-perceived risk of HIV infection, ranging from none to high, was independently positively associated with a higher likelihood of testing positive, as was the objective risk behaviour index. The objective risk behaviour index is defined as the sum of the indicator variables for more than one sex partner in the past year, concurrent partners with other partners, and exchange of gifts or money for sex.

In a separate regression in which cardinal symptoms were collapsed into a variable accounting for the number of these symptoms reported, the strongest predictors of seropositivity (P<0.001) were women (odds ratio [OR] 3.5, 95% confidence

### Table 1 Characteristics of clients presenting for VCT (n=813), KIWAKKUKI, May-November 2003

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV seropositive</strong></td>
<td>135</td>
<td>16.7</td>
</tr>
<tr>
<td><strong>Sociodemographic</strong></td>
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<td></td>
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<tr>
<td>Sex</td>
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<tr>
<td>Male</td>
<td>379</td>
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</tr>
<tr>
<td>Female</td>
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</tr>
<tr>
<td>Age</td>
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<td></td>
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<tr>
<td>Less than 25 years</td>
<td>266</td>
<td>32.7</td>
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<tr>
<td>25-34 years</td>
<td>274</td>
<td>33.7</td>
</tr>
<tr>
<td>35-44 years</td>
<td>135</td>
<td>16.6</td>
</tr>
<tr>
<td>Greater than 44 years</td>
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<td>10.7</td>
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<td>Occupation</td>
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<tr>
<td>Peasant</td>
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</tr>
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<td>11.5</td>
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<tr>
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<tr>
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</tr>
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<td>Divorced/separated/widowed</td>
<td>96</td>
<td>12.4</td>
</tr>
<tr>
<td>Cohabiting</td>
<td>89</td>
<td>11.5</td>
</tr>
<tr>
<td>Have children</td>
<td>443</td>
<td>92.5</td>
</tr>
<tr>
<td>Reason for testing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premarriage/preconception/reunion</td>
<td>250</td>
<td>31.4</td>
</tr>
<tr>
<td>Knowledge of serostatus for future</td>
<td>127</td>
<td>15.9</td>
</tr>
<tr>
<td>Unfaithful sexual partner</td>
<td>118</td>
<td>14.8</td>
</tr>
<tr>
<td>High-risk sexual behaviour</td>
<td>97</td>
<td>12.2</td>
</tr>
<tr>
<td>Ill</td>
<td>79</td>
<td>9.9</td>
</tr>
<tr>
<td>Ill sexual partner</td>
<td>56</td>
<td>7.2</td>
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<tr>
<td><strong>Sexual behaviour</strong></td>
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<td></td>
</tr>
<tr>
<td>No sex partners in past 12 months</td>
<td>136</td>
<td>20.2</td>
</tr>
<tr>
<td>One sex partner in past 12 months</td>
<td>441</td>
<td>65.4</td>
</tr>
<tr>
<td>More than one sex partner in past 12 months</td>
<td>97</td>
<td>14.4</td>
</tr>
<tr>
<td>Co/omds used in the last five years</td>
<td>288</td>
<td>36.5</td>
</tr>
<tr>
<td>Exchange gifts or money for sex</td>
<td>282</td>
<td>35.2</td>
</tr>
<tr>
<td>Sexual partner with other partners</td>
<td>358</td>
<td>48</td>
</tr>
<tr>
<td>Any alcohol use</td>
<td>218</td>
<td>27.1</td>
</tr>
<tr>
<td><strong>Health status</strong></td>
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<td></td>
</tr>
<tr>
<td>Previous HIV test</td>
<td>260</td>
<td>32.5</td>
</tr>
<tr>
<td>Prior treatment for TB</td>
<td>31</td>
<td>3.9</td>
</tr>
<tr>
<td>Perceived symptoms from HIV/AIDS</td>
<td>115</td>
<td>14.6</td>
</tr>
<tr>
<td>Specific symptoms (past two months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>123</td>
<td>15.3</td>
</tr>
<tr>
<td>Cough</td>
<td>112</td>
<td>13.8</td>
</tr>
<tr>
<td>Weight loss</td>
<td>97</td>
<td>12.2</td>
</tr>
<tr>
<td>Rash</td>
<td>68</td>
<td>8.5</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>41</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Number of symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One or more</td>
<td>216</td>
<td>26.9</td>
</tr>
<tr>
<td>Two or more</td>
<td>124</td>
<td>15.3</td>
</tr>
<tr>
<td>Three or more</td>
<td>61</td>
<td>7.5</td>
</tr>
<tr>
<td>Four or more</td>
<td>29</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Table 2 Characteristics of VCT clients testing negative and those testing positive (%), May-November 2003

<table>
<thead>
<tr>
<th>Table 2 Characteristics of VCT clients testing negative and those testing positive (%)</th>
<th>Negative (n=672)</th>
<th>Positive (n=135)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>47.6</td>
<td>77.1*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>30.2</td>
<td>35.1*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Married</td>
<td>28.5</td>
<td>29.2</td>
<td>0.8635</td>
</tr>
<tr>
<td>Cohabiting</td>
<td>10.2</td>
<td>17.7</td>
<td>0.0141</td>
</tr>
<tr>
<td>Divorced</td>
<td>9.2</td>
<td>28.5*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Single</td>
<td>52.1</td>
<td>24.6*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Any children</td>
<td>91.6</td>
<td>96.6</td>
<td>0.0710</td>
</tr>
<tr>
<td>Primary education</td>
<td>67.0</td>
<td>77.2</td>
<td>0.0245</td>
</tr>
<tr>
<td>Secondary or higher education</td>
<td>33.0</td>
<td>22.8</td>
<td>0.0245</td>
</tr>
<tr>
<td>Exposure and knowledge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously been tested for HIV</td>
<td>32.3</td>
<td>30.4</td>
<td>0.6627</td>
</tr>
<tr>
<td>Know anyone living with or died from HIV</td>
<td>76.0</td>
<td>83.7</td>
<td>0.0525</td>
</tr>
<tr>
<td>Ill sexual partner</td>
<td>5.8</td>
<td>31.9*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ill child</td>
<td>1.6</td>
<td>9.6*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any symptoms client thinks might be from HIV/AIDS</td>
<td>8.1</td>
<td>48.4*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight loss</td>
<td>7.2</td>
<td>38.0*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1.4</td>
<td>24.1*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rash</td>
<td>3.9</td>
<td>31.8*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cough</td>
<td>9.1</td>
<td>37.8*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fever</td>
<td>10.0</td>
<td>42.1*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Any symptoms</td>
<td>20.6</td>
<td>60.0*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of symptoms (n)</td>
<td>0.3</td>
<td>1.7*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of symptoms if any symptoms (n)</td>
<td>1.5</td>
<td>2.8*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ever been treated for TB</td>
<td>2.3</td>
<td>12.2*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Perceived risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>33.9</td>
<td>11.3*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Some</td>
<td>15.1</td>
<td>6.6</td>
<td>0.0205</td>
</tr>
<tr>
<td>Small</td>
<td>26.0</td>
<td>19.8</td>
<td>0.1828</td>
</tr>
<tr>
<td>Moderate</td>
<td>10.9</td>
<td>12.3</td>
<td>0.6818</td>
</tr>
<tr>
<td>High</td>
<td>13.0</td>
<td>42.5*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Perceived risk index if known (O=none; 4=high)</td>
<td>1.5</td>
<td>2.7*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always used condoms during the last five years</td>
<td>8.0</td>
<td>3.9</td>
<td>0.0999</td>
</tr>
<tr>
<td>Occasionally used condoms during the last five years</td>
<td>29.4</td>
<td>29.2</td>
<td>0.9769</td>
</tr>
<tr>
<td>Never used condoms during the last five years</td>
<td>62.7</td>
<td>66.9</td>
<td>0.3609</td>
</tr>
<tr>
<td>Number of sex partners</td>
<td>1.8</td>
<td>1.7</td>
<td>0.7070</td>
</tr>
<tr>
<td>in past five years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than one sex partner in past five years</td>
<td>36.3</td>
<td>41.5</td>
<td>0.2571</td>
</tr>
<tr>
<td>Concurrent (overlapping) partners in past one year</td>
<td>33.2</td>
<td>45.5</td>
<td>0.0066</td>
</tr>
<tr>
<td>Has sexual partner who had other partners</td>
<td>27.5</td>
<td>32.6</td>
<td>0.2343</td>
</tr>
<tr>
<td>Ever exchanged money or gift for sex</td>
<td>48.1</td>
<td>62.2</td>
<td>0.0027</td>
</tr>
<tr>
<td>Risk index (sum of previous four items; O=none; 4=high)</td>
<td>1.5</td>
<td>1.8</td>
<td>0.0078</td>
</tr>
</tbody>
</table>

Note: Numbers are percentages, unless otherwise indicated
*P<0.05 after Bonferroni correction
1n1 and n2 may differ from 672 and 135, respectively, due to missing values for some variables

interval [CI, 2.0-6.3], reporting an ill sexual partner (OR 6.3, 95% CI, 3.0-12.9), and the number of cardinal symptoms reported (2.1, 95% CI 1.6-2.6). Several important differences in client characteristics were noted by gender. Women clients were less likely to exchange gifts or money for sex (30% of women versus 40% of men, P = 0.003), or to report multiple sexual partners (6% of women versus 18% of men, P < 0.001). Women were more likely than men to cite a reason for testing as ‘unfaithful sex partner’ (20% versus 9%, P < 0.001) and to report perceived symptoms of HIV (19 versus 9%, P < 0.001). An examination of the HIV-infected clients by gender showed that men who tested positive were more likely than women to report having more than one sexual partner in the past year (27 versus 6%, P = 0.001), being married (43 versus 24%, P = 0.04), and having used condoms (53 versus 29%, P = 0.01). There were no significant gender differences in the other factors associated with seropositivity.

### Discussion

In regions of the world where HIV prevalence is high, one of the goals for prevention is to equip sexually active individuals with knowledge of their serostatus, thereby encouraging personal responsibility for their potential acquisition and transmission of HIV. The benefits of knowledge of serostatus extend to both infected and uninfected individuals. Those who are infected can access medical care and social support and can learn how to prevent further transmission. Those who are uninfected receive reinforcement of health information and risk behaviour reduction strategies. Balanced against these potential benefits, however, is the risk of stigmatization among clients presenting for testing by spouses, families, and community, as well as the barriers of cost, access, and availability of testing.

Forty-eight per cent of those found to be HIV infected presented with symptoms they perceived to be a manifestation of HIV infection. Previous research has not shown a significant association of reporting ‘HIV-related symptoms’ with HIV seropositivity, although repeated illness and suspicion of HIV was a common reason for testing. Nyblade et al. found no association of reporting illness and possible symptoms of HIV (e.g., weight loss, diarrhoea, TB, herpes zoster) with uptake of VCT in men, although the presence of symptoms was associated with greater uptake of services in women. This observation is confirmed by our data, which shows that there are more symptomatic women than men presenting for testing. It is possible that clients who are symptomatic feel encouraged to present to KIWAKKU for HIV testing because of the range of services that are offered to those who are HIV infected in the form of home-based care, opportunistic.
infection prophylaxis, and membership in support groups.

Given the high rate of symptoms, in particular fever, cough, and weight loss, in this client population, our VCT centre could also potentially serve as an entry point for TB prevention, identification, and care for both seropositive and seronegative clients. All clients presenting to our VCT centre could be screened for TB. Both HIV-infected and HIV-uninfected individuals with active TB could be referred for TB treatment, while those who are HIV infected with a positive tuberculin skin test and without active TB could be initiated on isoniazid for prophylaxis in accordance with Tanzania Ministry of Health Guidelines. A sizeable proportion of the HIV-positive clients would also likely qualify for trimethoprim-sulfamethoxazole prophylaxis for prevention of various bacterial and parasitic infections. The offer of TB treatment, as well as home-based support services and opportunistic infection prophylaxis, would then serve as a greater incentive to present for VCT.

To be most useful as a prevention strategy, VCT should ideally identify HIV-infected individuals early in infection when risk behaviour reduction and drug treatment are most effective. HIV seroprevalence in this population is 16.7%, compared with a national average of 11%, and a regional prevalence rate of 6.3% in the antenatal clinic population and 6.8% among blood donors. Among bar and hotel workers in the Kilimanjaro Region, the seroprevalence rate is 26.1%. By comparing our results with these populations, we can assume that we are attracting an at-risk population that is not necessarily representative of the general population, nor representative of traditionally high-risk groups. In this group, which may benefit from more specific messages than generalized advertising campaigns, the VCT strategy of risk behaviour identification and reduction may be particularly effective.

In our setting, only 40% of the seropositive clients were asymptomatic. This is likely to remain the case in an environment where stigma associated with HIV-infection coupled with the relative lack of drug treatment likely outweighs the possible benefits of early awareness of serostatus. If antiretroviral therapy were available, we believe VCT testing uptake would increase substantially, as lack of available care is a frequently cited barrier to testing.

The increased seroprevalence in women presenting to this VCT centre compared with men may reflect a referral bias. As KIWAKKUKI is a women’s organization, women suspecting HIV infection may have found this to be a more accepting environment in which to present for VCT. However, the high rate of infection in women in our population mirrors a trend seen across sub-Saharan Africa. Although women have increased biological susceptibility to HIV infection, this alone does not explain the striking difference in HIV infection rates. The high rate of infection in women is also likely to be attributable to the gender inequality that does not permit women to negotiate sexual relationships. Women may not be empowered to insist that their partners use condoms or abstain from sex with other partners. In our study, the characteristics of the seropositive client included being women, having a partner with other partners, and having an ill sexual partner. This suggests that until women are able to reduce risk of HIV acquisition from their infected-sexual partners, the trend of an increasing rate of infections among women is unlikely to change.

The initiation of VCT at KIWAKKUKI has demonstrated that people who have symptoms and perceive themselves to be symptomatic from HIV are willing to present for VCT despite continued economic barriers to universal antiretroviral therapy. The counselling service has
provided clients with risk behaviour knowledge and risk reduction strategies. It has linked HIV-infected clients to a package of care, including home-based visits, opportunistic infection management, nutritional support, and associations of people living with HIV/AIDS. It will, in the future, help provide the framework necessary for the delivery of antiretroviral therapy.

Acknowledgements: We are very grateful to the staff of KIWAKKUKI AIDS Information Centre for their collaboration, and in particular to the VCT counsellors Beatrice Mandao, Eliakiesh Shangali, Anna Msuya, Anna Mchaki, Agatha Chuwa, Alexia Mella, Awaichi Malle, B Haule, E Kiwla, Grace Gumbo, Lillian Mtui, Naomi Ringo, Magdalena Lyimo, Sylvia Mlay, and Yesusia Mariki. This study was supported in part by Roche Laboratories. Additional investigator support was obtained from AIDS Clinical Trials Group (U01 AI-39156, Drs Bartlett and Thielman) and Mid-career Investigator (K24 AI-0744-01, Dr Bartlett) awards from the National Institutes of Allergy and Infectious Diseases and from the US Department of State Fullbright Programme (Drs Thielman and Chu).

References


(Accepted 2 July 2004)
5.2. Validation, performance under field conditions, and cost-effectiveness of Capillus HIV-1/HIV-2 and Determine HIV-1/2 rapid HIV antibody assays using sequential and parallel testing algorithms in Tanzania


CONTRIBUTION

My position as last author reflects my role as senior, supervising author on the paper. I conceived the research idea, sought and obtained funding, designed the study, and supervised the research including the study team members responsible for participant enrollment, data collection, data analysis, and write up. I mentored the medical student, Ms. Mayhood. Afwamba, Odhiambo, and Ndanu worked with Mayhood on the project, jointly gaining experience in research, data analysis, and scientific writing. Thielman assisted with the supervision of these trainees and liaison with the HIV counseling and testing site. Morrissey oversaw day-to-day operations in the laboratory. Shao managed interactions with personnel at study sites and partner institutions. Pence contributed to statistical and economic analyses. All authors contributed to revisions of the manuscript.

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Validation, Performance under Field Conditions, and Cost-Effectiveness of Capillus HIV-1/HIV-2 and Determine HIV-1/2 Rapid Human Immunodeficiency Virus Antibody Assays Using Sequential and Parallel Testing Algorithms in Tanzania

Meghan K. Mayhood,1 Isaac A. Afwamba,2 Christopher O. Odhiambo,2 Epimack Ndanu,3 Nathan M. Thielenman,1,4 Anne B. Mcrissreyse,1,2 John F. Shao,2,5 Brian Wells Pence,1,4,6,7 and John A. Crump1,2,4,5,*

Duke University Medical Center, Durham, North Carolina; Kilimanjaro Christian Medical Centre, Moshi, Tanzania; Kåndi cha Wanawake Kilimanjaro Kapambana na UKIMWI (KIWKUKI, Women Against AIDS in Kilimanjaro), Moshi, Tanzania; Duke Global Health Institute, Duke University, Durham, North Carolina; Kilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania; Sanford Institute of Public Policy, Duke University, Durham, North Carolina; and Center for Health Policy, Duke University, Durham, North Carolina

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Rapid human immunodeficiency virus (HIV) antibody tests support the effort to expand access to HIV testing and counseling services in remote, rural, and poor parts of the world. We validated the Capillus HIV-1/HIV-2 (Trinity Biotech PLC, Bray, County Wicklow, Ireland) and Determine HIV-1/2 (Abbott Laboratories, Abbott Park, IL) rapid tests in a reference laboratory using patient samples from Tanzania and evaluated the performance of the tests under field conditions in northern Tanzania. We used the resulting data to study sequential and parallel testing algorithms. In the validation study, sensitivity, specificity, the predictive value of a positive test (PV+), and the predictive value of a negative test (PV−) were all 100% for Capillus and Determine. In the field evaluation among 12,737 clients, sensitivity, specificity, PV+, and PV− were 99.7%, 99.8%, 96.8%, and 99.9%, respectively, for Capillus and 99.6%, 99.9%, 99.5%, and 99.9%, respectively, for Determine. A sequential testing algorithm that did not confirm a negative initial Capillus result with a Determine result cost $7.77 per HIV diagnosis but missed 0.3% of HIV infections. A sequential testing algorithm that did not confirm a negative initial Determine result with a Capillus result cost $7.64 per HIV diagnosis but missed 0.4% of HIV infections. A parallel testing algorithm cost $13.46 per HIV diagnosis but detected more HIV-infected clients.

Human immunodeficiency virus (HIV) voluntary counseling and testing (VCT) is an important tool for both HIV prevention and care in sub-Saharan Africa. VCT use has been associated with a reduction in high-risk sexual behavior and of risk for HIV transmission (6, 30). VCT also provides the means for persons to learn their HIV status in order to access treatment and care services (5, 9). However, these benefits hinge on the accurate diagnosis of HIV infection. False-negative results lead to delayed entry into care or failure to enter care, while false-positive results place economic, social, and emotional burdens on patients. As efforts to expand HIV testing services to reach the entire populations of countries with generalized HIV epidemics gain momentum (5), so the number of tests conducted increases. Increasing the amount of HIV testing will mean that even small shortcomings in the sensitivity and specificity of assays or testing algorithms may lead to false-positive or false-negative results for large numbers of people.

The development and deployment of rapid HIV antibody tests have allowed the expansion of access to HIV testing and counseling services in remote, rural, and poor areas where more complex and expensive means of diagnosing HIV infection are difficult to implement (7). The use of rapid HIV antibody tests has been endorsed by the World Health Organization, and they have been adopted into national guidelines for HIV VCT in many countries in sub-Saharan Africa (1, 18). Capillus HIV-1/HIV-2 (Trinity Biotech PLC, Bray, County Wicklow, Ireland), Determine HIV-1/2 (Abbott Laboratories, Abbott Park, IL), and other rapid HIV antibody tests have been evaluated in several studies and have been found to have sensitivities and specificities exceeding 99.5% (8, 12, 14, 16, 19, 20, 22, 27, 28, 31, 32). These rapid HIV antibody tests may be implemented in several types of diagnostic algorithms employing one or more rapid tests used in sequence or in parallel (1). Understanding the performance of rapid HIV antibody tests in different testing algorithms is critical to inform local and national HIV testing policies. In Tanzania, as in many other resource-poor settings, national VCT guidelines recommend a sequential testing approach in which a single rapid test, if negative, is not confirmed with a second test but is reported as a negative result. If the first test is positive, it is confirmed with a second, different rapid test, with discordant results resolved with an enzyme-linked immunosorbent assay (ELISA) (1, 18).

Since negative results for the first rapid test are not confirmed, the sequential approach has the potential to miss some cases of HIV infection.
We validated the Capillus and Determine rapid HIV antibody tests and evaluated their performance under field conditions in northern Tanzania in a large cohort over a 5-year time period. We assessed the performance of a sequential testing approach and compared the diagnostic accuracy of the sequential algorithm to an alternative parallel testing algorithm in which all samples were tested with two rapid tests. We further considered the incremental cost of implementing the parallel algorithm and the cost per case of HIV infection identified for each algorithm.

**MATERIALS AND METHODS**

**Validation study.** Blood samples were collected from medical inpatients at Kilimanjaro Christian Medical Centre (KCMC) and clients of Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWA KKUKI; Women Against AIDS in Kilimanjaro). The medical inpatients were unselected and had symptoms expected among patients admitted to the hospital. Whole blood was tested using both the Capillus HIV-1/HIV-2 (Trinity Biotech PLC, Bray, County Wicklow, Ireland) and Determine HIV-1/2 (Abbott Laboratories, Abbott Park, IL) rapid HIV antibody tests. If both rapid tests were negative, the sample was tested with an ELISA (Vironostika Uni-Form II plus O Ab; bioMérieux, Durham, NC). If the ELISA was negative, no further testing was done. If the ELISA was positive, a Western blot (Genetic Systems HIV-1 Western blot kit; Bio-Rad, Hercules, CA) was planned to be done (Fig. 1). If both rapid tests were positive or were discordant, the sample was tested with an ELISA and a Western blot. Sample collection was planned to continue until a minimum of 100 HIV-infected and 100 HIV-uninfected subjects was reached. Laboratory technologists were blinded to the results of other HIV antibody tests performed on the same sample.

**Field evaluation.** KIWA KKUKI began offering HIV VCT services on 13 March 2003 at its AIDS Information Centre in downtown Moshi. The characteristics of KIWA KKUKI VCT clients have been described in detail elsewhere (3, 24). All counseling procedures were performed in accordance with guidelines provided by the Tanzanian Ministry of Health’s National AIDS Control Programme (18). A 2-mL blood sample was drawn from each patient and tested using both the Capillus and Determine rapid HIV antibody tests. Concordant results were reported, whereas for discordant results, sequential testing on the sample was performed at the KCMC clinical laboratory using an ELISA (Vironostika HIV Uni-Form II plus O Ab; bioMérieux, Durham, NC). For quality-control purposes, an ELISA was also performed on every 10th sample at the KCMC laboratory regardless of the Capillus and Determine results.

**Sequential and parallel testing algorithms.** In the field evaluation, we implemented a strategy of parallel rapid testing, in which all samples were tested in parallel using both the Capillus and Determine assays; discordant rapid test results were accepted, while discordant results were resolved with ELISA (see Fig. 3). We compared the parallel testing strategy with the performance that would have been observed with four alternative testing strategies for determining HIV infection status: strategy 1, testing with Capillus alone with no second test; strategy 2, testing with Determine alone with no second test; strategy 3, initial testing with Capillus with positive results confirmed by Determine and no additional testing for negative results (Fig. 2a); and strategy 4, initial testing with Determine with positive results confirmed by Capillus and no additional testing for negative results (Fig. 2b). Strategy 3 reflected the Tanzanian national testing guideline at the time. We calculated the sensitivity, specificity, positive predictive value (PV+), and negative predictive value (PV−), along with the exact 95% confidence interval, of each alternative testing strategy relative to the parallel testing strategy in determining the HIV infection status. To examine the clinical relevance of discordant rapid HIV antibody test results, we reviewed the results of subsequent rapid HIV antibody tests among the group of patients with initial discordant results who returned subsequently for repeat testing.

**Cost-effectiveness analyses.** We calculated the cost-effectiveness of the parallel testing strategy and each of the four alternative strategies. Cost-effectiveness was defined as the cost per case of HIV infection correctly identified. For inputs, we assumed a cost of $1.00 per Capillus assay, $1.00 per Determine assay, and $17.00 per ELISA, including labor (approximate prices in Tanzania at the time of writing).

**Statistical analyses.** Study data were entered into an Excel database (Microsoft Corporation, Seattle, WA) and analyzed using SAS version 9.1 (SAS Institute, Inc., Cary, NC). Sensitivity and specificity were calculated in the validation study using an ELISA (for concordant results) and Western blotting (for discordant results) as the gold standard. Confidence intervals were calculated in the field evaluation using two rapid tests (for concordant results) and an ELISA (for discordant results) as the gold standard. Confidence intervals were calculated using exact methods.

**Research ethics.** The validation study was part of a protocol approved by the KCMC Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an institutional review board (IRB) of Duke University Medical Center. The field evaluation used on-shell data obtained from KIWA KKUKI records. All links to personal identifiers were removed and held by a privacy officer not involved in the study. A Duke University Health System IRB exempted this part of the study from IRB review.

**RESULTS**

**Validation study.** In order to meet the enrollment targets for HIV-infected and HIV-uninfected subjects, samples were collected from 206 subjects of whom 105 (51%) were ultimately
shown to be HIV infected and 101 (49%) were ultimately shown to be HIV uninfected. For all patients, there was 100% concordance between the Capillus, Determine, ELISA, and Western blot results (Fig. 1). The sensitivity, specificity, PV+, and PV− were 100% for Capillus and Determine, both when used individually and in combination.

Field evaluation. Between 13 March 2003 and 14 December 2007, a total of 12,737 clients received VCT at KIWAKKUKI and had the results of both the Capillus and Determine rapid tests available. Of these, 1,938 (15.2%) had concordant positive HIV rapid tests and 10,736 (84.3%) had concordant negative HIV rapid tests. Of the 63 (0.5%) clients with discordant rapid HIV test results, 43 (68%) clients had a positive Capillus result and a negative Determine result and 20 (31.7%) clients had a negative Capillus result and a positive Determine result. The results of the ELISA testing of samples with discordant rapid HIV antibody tests are shown in Fig. 2a and b. Based on these findings, for Capillus, sensitivity in the field evaluation was 99.7% (exact 95% confidence interval, 99.3 to 99.9%), specificity 99.8% (99.7 to 99.9%), PV+ 98.7% (98.1 to 99.2%), and PV− 99.9% (99.9 to 100.0%). For Determine, sensitivity in the field evaluation was 99.6% (99.2 to 99.8%), specificity 99.9% (99.8 to 100.0%), PV+ 99.5% (99.1 to 99.8%), and PV− 99.9% (99.1 to 100.0%) (Table 1). Of the 63 clients with discordant rapid HIV antibody test results, 5 (7.9%) returned subsequently for repeat testing and had results available. Of these 5, 4 (80.0%) tested concordant negative on the repeat test, and one had persistently discordant results. ELISA testing of every 10th sample identified 10 instances of discordant rapid test results with discordant ELISA. These results were resolved with repeat testing in accordance with national guidelines.

Sequential versus parallel testing algorithms. The parallel testing algorithm with ELISA resolution of discordant results (Fig. 3) identified 1,952 cases of HIV infection among the 12,737 clients in the cohort. The same data set and assay results were used to study each algorithm. In a sequential testing algorithm with an initial Capillus test where negative Capillus results were not confirmed and only positive Capillus results were confirmed by Determine (Fig. 2a), 1,946 cases would have been identified. This algorithm would have failed to identify six (0.3%) HIV-infected clients (Table 2). An alternative sequential algorithm that relied on an initial Determine test that was not confirmed if negative and was confirmed by Capillus (Fig. 2b) if positive would have identified 1,944 cases and would have missed 8 (0.4%) HIV-infected clients (Table 2).

Cost-effectiveness of sequential versus parallel testing algorithms. The cost-effectiveness of sequential versus parallel testing algorithms is shown in Table 3. The cost per case identified using the Capillus or Determine tests alone are each $6.54. The cost per case identified in a sequential testing algorithm using an initial Capillus test or an initial Determine test are $7.77 and $7.64, respectively. A parallel testing algorithm costs $13.46 per case identified.

**DISCUSSION**

We demonstrate that the Capillus and Determine rapid HIV antibody tests are accurate diagnostic tests both when validated in a central laboratory against ELISA and Western blotting and when implemented under field conditions in a large cohort, although sensitivity, specificity, PV+, and PV− were less than 100% under field conditions. Furthermore, we show that a commonly used sequential testing algorithm, for which a negative initial rapid test result is not confirmed with a second rapid test, identifies 0.3 to 0.4% fewer HIV infections than a
TABLE 1. Sensitivity and specificity of Capillus and Determine rapid HIV tests

<table>
<thead>
<tr>
<th>Test(s)</th>
<th>Result</th>
<th>HIV infected</th>
<th>HIV uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Second rapid test</td>
<td>ELISA</td>
<td>Second rapid test and/or ELISA*</td>
</tr>
<tr>
<td>Capillus</td>
<td>Positive</td>
<td>1,938</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1,952</td>
<td></td>
</tr>
<tr>
<td>Determine</td>
<td>Positive</td>
<td>1,938</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1,952</td>
<td></td>
</tr>
</tbody>
</table>

* Fourteen ELISA results were not available.

A testing algorithm that uses the two rapid tests in parallel on all samples.

Although the sensitivity, specificity, PV+, and PV- for Capillus and Determine were slightly lower under field conditions than in the validation study, the performance of the two tests did not differ substantially and was consistent with that reported by other investigators (8, 12, 14, 16, 19, 20, 22, 27, 28, 31, 32). There are several possible explanations for sensitivity and specificity levels below 100% for Capillus and Determine. First, it is known that standard antibody-based HIV diagnostic tests are not 100% sensitive with patients with early or acute HIV infection. When the performance of Capillus and Determine has been studied in acute HIV infection panels, the Determine assay appears to be more sensitive than the Capillus assay (8, 15, 23). The HIV seroincidence in the KIWAKKUKI VCT cohort has been estimated to be approximately 2 per 100 person-years (26). Despite the likelihood that some VCT clients included in this study were experiencing acute HIV infection at the time of HIV testing, among clients with discordant HIV rapid test results the combination of positive Determine and negative Capillus results was not more common than positive Capillus and negative Determine results. Furthermore, in the small group of clients who had initially discordant rapid HIV antibody test results and returned for repeat testing, none moved from having discordant to concordant positive results. Thus, it is unlikely that acute HIV infection played a major role in the performance of the rapid HIV antibody tests in our study. HIV-1 subtype diversity in the testing population has been shown to affect the performance of antibody-based HIV diagnostic tests and has led to recommendations that rapid HIV antibody tests be evaluated in the local population prior to widespread implementation (19, 25). In Tanzania, where HIV-1 subtypes A, C, and D and their recombinants predominate (2, 10, 11, 21, 27, 29), it is possible that subtype diversity plays a role in the performance of rapid HIV antibody tests. Although we did not evaluate HIV subtypes among HIV-infected clients, the accuracy of both the Capillus and Determine tests in our study suggests that the role of subtype diversity is likely to be minimal. Finally, sample collection, labeling, transport, and testing conditions may play
a role in the performance of any diagnostic test under field conditions. Each of these steps may be challenged in resource-constrained settings (13, 17).

Sequential HIV testing algorithms that do not confirm the result of an initial negative rapid test with a second rapid test are widely employed (1, 18). Because our field evaluation was done in a setting where a parallel testing strategy with two rapid tests was used, the availability of both rapid test results and ELISA results for discordant samples allowed us to examine how such a sequential strategy would have performed in the same population. We showed that a sequential testing algorithm that did not confirm a negative Capillus result with a Determine test would fail to detect 0.3% of HIV-infected clients and that a sequential testing algorithm that did not confirm a negative Determine result with a Capillus test would fail to detect 0.4% of HIV-infected clients. Although such sequential algorithms have been recommended and widely adopted (1, 18, 31), our study suggests that this strategy will fail to detect some HIV-infected clients. While the proportion of missed infections is small, when applied to the large testing populations that the serostatus approach to HIV prevention and care demands, the numbers of missed HIV diagnoses can become substantial (4). Indeed, in a hypothetical country with a population of 30 million and an HIV seroprevalence of 7% with universal HIV testing, our data suggest that a testing strategy that does not confirm the negative result of the initial rapid HIV antibody test would fail to detect 6,300 to 8,400 HIV-infected persons. Both assays yielded false-positive results, but because both sequential and parallel algorithms call for the use of a second rapid HIV antibody test, neither strategy offered an advantage over the other in identifying false-positive results.

We determined that a parallel testing strategy would cost $134.46 per HIV-infected client identified, compared with $7.77 for a sequential testing strategy using an initial Capillus test and $7.64 for a sequential testing strategy using an initial Determine test. Policy makers must decide if the additional cost of $5.69 to $5.82 per HIV diagnosis for a parallel testing strategy is offset by the prevention and treatment benefits to those HIV-infected persons who would be missed by using a sequential testing algorithm that did not confirm negative results of an initial rapid HIV antibody test.

We demonstrate that both the Capillus and Determine rapid HIV antibody tests perform well in both the reference laboratory and under field conditions in Tanzania in a cohort of >12,000 VCT clients. We suggest that testing algorithms that do not confirm the negative result of an initial test with a second rapid test miss a small proportion of HIV-infected clients. While the proportion of missed HIV infections is small, the absolute number of undetected HIV infections is substantial when applied to a large or national VCT program. Policy makers must decide whether the additional cost of adopting a parallel rapid HIV antibody testing strategy is offset by the prevention and treatment benefits to those additional HIV-infected persons detected by this testing strategy.

ACKNOWLEDGMENTS

VCT services at KIWANKUKI were funded by Roche Laboratories, and initial analyses were supported by a global health research training grant from the North Carolina GlaxoSmithKline Foundation. Investigator support was obtained from the Fogarty International Center (D43 PA-03-018 to N.M.T. and J.A.C.), the Duke Clinical Trials Unit and Clinical Research Sites (U01 AI069484-01 to N.M.T. and J.A.C.), the International Studies on AIDS-Associated Co-Infections award (ISAAC) (U01 AI-03-036 to I.A.A., C.O.O., E.N., N.M.T., A.B.M., J.F.S., and J.A.C.), and a Duke University School of Medicine (to M.K.M.).

REFERENCES

TESTS OF RAPID HIV ANTIBODY TESTS IN TANZANIA


5.3. Cost-effectiveness of free HIV voluntary counseling and testing through a community-based AIDS service organization in northern Tanzania


CONTRIBUTION

My position as last author reflects my role as senior, supervising author on the paper. Thielman, Itemba, and I conceived the research idea. Thielman and I sought and obtained funding, designed the study, and supervised the research including the study team members responsible for participant enrollment, data collection, data analysis, and write up. We mentored the medical student, Ms. Chu. Ostermann led statistical and economic analyses. Itemba, Mgonja, and Mtweve ensured that research activities were coordinated with HIV counseling and testing service delivery. Bartlett and Shao managed interactions with personnel at study sites and partner institutions and assisted with obtaining funding. All authors contributed to revisions of the manuscript.

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Cost-Effectiveness of Free HIV Voluntary Counseling and Testing Through a Community-Based AIDS Service Organization in Northern Tanzania

Nathan M. Thielman, MD, MPH, Helen Y. Chu, MD, Jan Ostermann, PhD, Dafrosa K. Itemba, BA, Anna Mgonja, Sabina Mtweve, MD, MPH, John A. Bartlett, MD, John F. Shao, MD, PhD, and John A. Crump, MB, CHB, DTM&H

In sub-Saharan Africa, HIV voluntary counseling and testing (VCT) is a cost-effective method of reducing high-risk sexual behavior and preventing HIV transmission. A large multicenter study conducted in Kenya, Trinidad, and Tanzania demonstrated that VCT reduced unprotected sexual contact with a nonprimary partner by 35% among men and 39% among women (vs 13% and 17% reductions, respectively, among those who received health information only). It has been estimated that VCT offered to 10,000 Tanzanians would avert 895 HIV infections at a cost of $346 per infection averted and $17.78 per disability-adjusted life year (DALY) saved.

Universal voluntary testing with individual informed consent and confidentiality protection in Africa has been advocated. The World Health Organization and Joint United Nations Programme on HIV/AIDS have recently endorsed moving from client-initiated requests for VCT to provider-initiated approaches. In addition to promoting behavior change, VCT can serve as a point of referral for preventive services, including the prevention of mother-to-child transmission and as an entry point for treatment programs for sexually transmitted infections, prophylaxis of opportunistic infections, diagnosis and treatment of tuberculosis, and, increasingly, initiation of highly active antiretroviral therapy, thereby further enhancing its cost-effectiveness. Greater access to VCT has been facilitated through cheaper, rapid, and simple HIV testing kits, which reduce the cost per test performed.

Despite these considerations, VCT is vastly underutilized, particularly in poor countries, where the current overall coverage is estimated to be less than 1% to 10% of those at risk for HIV infection. In a population-based nationally representative survey in Tanzania, approximately 7% of women and 12% of men reported ever having received an HIV test. In the Kilimanjaro Region, even in a hospital setting, 44% of those found to be HIV infected in a systematic serosurvey were previously unaware of their infection.

Barriers to accessing VCT services include stigmatization (with abandonment and being common, particularly among women who test positive), geographic accessibility, lack of social promotion, inefficient counseling and testing practices, and cost. We describe a newly established VCT program in Moshi, Tanzania, designed to overcome many of these barriers and in particular focus on testing uptake before, during, and after a free VCT campaign.

METHODS

Location and Context

A new VCT program was established in a well-established HIV service organization, Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI), Women Against AIDS in Kilimanjaro) at their easily accessible AIDS Information Centre in downtown Moshi, Tanzania. This nongovernmental organization, established in 1990 with strong community ties, supports persons living with HIV/AIDS by providing home-based care, counseling, and information about HIV infection, and orphan care and assistance. The KIWAKKUKI VCT program set charges of 1000 Tanzania shillings (TSh; US$0.95 at the 2003 exchange rate) for VCT, except for clients aged 24 years or younger and KIWAKKUKI members (the latter estimated to receive less than 5% of all tests).

The VCT program was initiated in March 2003, and data collection to analyze sociodemographic and clinical characteristics of clients began on May 19, 2003. These characteristics are described in detail elsewhere. Women were female, and the median age was 29 years (13 to 80 years). A stable number of clients testing per day of 4.1 was observed.
for 1 month before initiating a free VCT campaign from July 8 through July 21, 2003, during which KIWAKKUKI waived all VCT costs. The free VCT campaign was advertised on national radio in a series of 4 announcements. Posters advertising the availability of free testing were posted in Moshi municipality, and public announcements were made throughout the district.

VCT Procedures and Costs

Clients presenting for VCT received confidential pre- and post-test counseling with a trained counselor according to Tanzanian Ministry of Health Guidelines. The protocol and prevention messages of the KIWAKKUKI VCT service have been outlined previously. Test results were typically received within 30 to 40 minutes. Those testing positive were invited to participate in a KIWAKKUKI-sponsored peer support group and to join the KIWAKKUKI home-based care program that provided trimethoprim-sulfamethoxazole prophylaxis, weekly visits by a trained home care worker, food supplements, and treatment of some opportunistic infections. In addition, all such patients were referred to the zonal hospital HIV clinic, where antiretroviral therapy was available. Sociodemographic data, risk behaviors, and general medical information were recorded on standard questionnaires, and daily numbers of persons testing were tabulated.

Trained counselors were paid approximately $3 (all dollar amounts are in US dollars) per day. HIV testing was accomplished using Capillus (Trinity Biotech PLC, Bray, County Wicklow, Ireland) and Determine (Abbott Laboratories, Abbott Park, Ill) HIV1/2 rapid antibody tests. Every 20th blood sample or any blood sample yielding discrepant rapid testing results was sent to the zonal referral hospital for confirmatory testing using Vironostika HIV (Organon Teknika, Charlotte, NC).

The costs associated with testing were estimated to be $2700 per year for a laboratory technician, $1 per person for each rapid test, $5 for each validation sample sent for confirmatory testing, and $360 per year for laboratory consumables. Additional costs for the program included $500 per year for building space for the program, $600 per year for telephone, $150 per year for electricity consumption, $500 for consumables such as paper forms and copying fees, and $3700 per year for program coordination. During the free VCT campaign, additional costs included hiring a second counselor, advertising (estimated at $40), and proportional increases in consumables, including laboratory supplies and HIV test kits. No additional rental, telephone, or electricity costs were incurred.

Analysis

Data from questionnaires were entered into an electronic database constructed with Epi-Info 2002 software (Centers for Disease Control and Prevention, Atlanta, Ga). Data were validated by randomly sampling 10% of the questionnaires, with an acceptable error rate being less than 1 error per 5 forms. Data were analyzed with EpiInfo 2002 and Stata 8.0 (Stata Corp, College Station, Tex). Differences in daily number of persons testing during and after the free testing period relative to the prefree testing period were analyzed with t tests. Rates of seropositivity in the tested population, published estimates of the effectiveness of VCT in preventing HIV infections, and estimates of DALYs saved/gained from prevention and treatment were combined with KIWAKKUKI cost data to estimate the cost per DALY saved/gained because of free testing, with and without subsequent treatment of those testing positive.

RESULTS

Observed Number of Clients Testing per Day in Relation to the Free VCT Campaign

More than 99% of the 813 individuals presenting to KIWA KKUKI for testing from May 19 to November 23, 2003, consented to participate in a study describing the sociodemographic and clinical characteristics of such clients. The number of clients testing per day was considered in relation to the free testing campaign and divided into the prefree testing period from May 19 to July 7, 2003, during which a modest fee (1000 Tsh, approximately $0.05 at the 2003 exchange rate) was charged for clients older than 24 years; the free testing period from July 8 to July 21, 2003; and the postfree testing period from July 22 to November 13, 2003, during which the usual fee schedule was resumed. The secular trends in the number of clients testing per day for all age groups are shown in Figure 1. The periods of peak attendance corresponded to the days when testing was offered free, increasing from mean ± SD of 4.1 ± 2.5 clients per day in the prefree testing period to 15.0 ± 4.8 and 7.1 ± 2.6 clients per day, respectively, during free and postfree testing periods (P < .0001 compared with prefree period for each).

Because fees are usually waived for clients younger than 25 years, we considered the number of clients testing per day by age strata as well. The mean daily number of tested persons aged 25 years and older quadrupled during the free testing period relative to the period before free testing (11.4 ± 3.5 vs 2.7 ± 2.1 clients per day; P < .0001). After free testing ended, the number of persons testing declined to 4.6 ± 2.2 clients per day but remained significantly higher than before free testing (P = .0004). During the free testing period, the daily number of clients testing increased for persons younger than 25 years who received free testing throughout the study period (3.6 ± 1.8 vs 1.4 ± 1.2 clients per day; P = .0003). As with the older group, the daily number of clients testing per day remained at a higher level after the free VCT campaign than during the prefree testing period (2.5 ± 1.8 clients per day; P = .0091). The magnitude of the differences between the 3 periods was smaller for this group of clients than for the older group. There were no significant differences among study subjects in sociodemographic characteristics, knowledge of HIV/AIDS, prevalence of symptoms, or seropositivity between any of the 3 periods.

Annualized Models of Cost-Effectiveness

During the free VCT campaign, 109 excess clients than would have been predicted (based on the prefree testing rate of 4.1 clients per day) presented for testing, and in the following 80 days, 238 excess clients were tested. We used the HIV seroprevalence and postfree period number of persons testing per day seen over the latter 26 weeks of observation to develop a model annualizing these data in order to perform cost-effectiveness analyses. Over 1 year, without a free VCT campaign, 966 individuals would...
be tested at a net cost of $11,518 ($11.92 per client tested) (Table 1). With the addition of a 2-week free VCT campaign, 1864 persons would be tested for $13,771 ($7.38 per client tested) over 1 year, assuming no increases in fixed costs since the number of persons testing per day in our circumstance had not reached the capacity of the VCT center. A third scenario applied the free VCT daily client testing number to the entire year with appropriate cost increases in rent, telephone, power, testing supplies, and consumables, including adding 2 additional counselors for $6.60 per day. Under these conditions, the cost of sustained free VCT over 1 year would be reduced further to $6.45 per client.

We applied the previous estimates of Sweat et al.2 stratified by gender and serostatus, for HIV infections averted by VCT among individuals in Tanzania. Without free VCT, we estimated that, over 1 year, 68 HIV infections would be prevented at a cost of $169.69 per infection averted and $8.72 per DALY gained (Table 2). The increased daily testing number and cost-efficiency of testing afforded by the addition of a free VCT campaign would avert 63 additional HIV infections, at a cost of $105.12 per infection averted, reducing the cost per DALY saved to $5.40, and a model of sustained free VCT program would reduce the cost per infection averted further to nearly $92 and a cost per DALY saved of $4.72.

Assuming that 30% of those presenting for testing would receive antiretroviral therapy at a cost of $420 per year (on the basis of current retail pricing for fixed dose combination stavudine/lamivudine/nevirapine in retail pharmacies in the Kilimanjaro Region) and that 50% would receive tuberculosis prophylaxis with isoniazid for 6 months at a cost of $25 (on the basis of estimates from Creese et al.15), we calculated that a total of 1381 DALYS would be gained at a cost of $24.52 per DALY without free VCT. With the free VCT campaign, 2666 DALYS would be gained at a cost per DALY of $21.34, and with the sustained free VCT program, 5597 DALYS would be gained at a cost per DALY of $20.69. Sensitivity analyses included in Table 2 varied assumptions of HIV seroprevalence and rates of treatment. At an HIV seroprevalence of 25%, there is marked improvement in the cost-effectiveness of VCT, particularly with a free VCT campaign. Holding HIV seroprevalence constant and varying rates of treatment with antiretrovirals and tuberculosis prophylaxis shows small improvements in cost-effectiveness.

**DISCUSSION**

We have demonstrated that a period of free VCT significantly increases the number of persons testing per day and enhances cost-effectiveness of VCT when offered as an integrated program within an existing AIDS service-oriented nongovernmental organization. Modeled over a 1-year time horizon, a policy of sustained free VCT would likely further enhance the cost-effectiveness of this intervention.

Previous work in Tanzania estimated the cost of VCT per infection prevented to be $346, and that of per DALY saved, $17.78.2 Even without a free VCT campaign or sustained free services for all clients, we have...
shown standard VCT practices in our setting to be nearly twice as cost-effective as these estimates.

Several factors likely explain these differences. First, our VCT program was integrated into an existing, community-based, volunteer AIDS service organization, minimizing startup costs and costs for counselors (who were motivated women from the community paid approximately $3.30/day). Second, testing costs were reduced by the use of onsite rapid HIV antibody testing, which is the method of choice in this region. Others have shown this approach to increase the number of patients receiving their results, making it both more convenient and economical in comparison with conventional enzyme-linked immunosorbent assay testing.8 Third, operating costs may be lower in Moshi municipality, compared with larger urban settings in Tanzania.

Surprisingly little research has focused on strategies to enhance testing uptake and cost-effectiveness at community-based VCT programs. Greenguatt et al.9 described increased testing volumes in Bangui, Central African Republic, at a VCT site during annual AIDS day free testing events, which attracted mostly asymptomatic students, but they did not note the effect of this campaign on subsequent testing.

A survey assessing willingness to pay among VCT clients in Kenya suggested that more people are reluctant to access VCT services as the costs approach $1 to $2.10 In our study, after the introduction of a free VCT campaign, increases in the number of clients testing per day were sustained and cost increases were limited primarily to excess testing supplies. The costs per infection averted and per DALY gained decreased by 38% to $105 and $5.40, respectively. If free VCT were offered year-round, assuring sustained client testing numbers of around 15 clients per day, the costs per infection averted and per DALY gained would decrease by 46%.

The provision of free VCT, only if for a brief campaign, thus renders VCT an even more effective intervention, on the order of single-dose nevirapine for the prevention of mother-to-child transmission.25 When VCT is considered as an entry point for care of the HIV-infected and is included in the summary cost per DALY calculation, it contributes around 96% of the DALYS gained and substantially reduces the cost per DALY.

Why did more clients present for testing in the interval following the free testing campaign? The free VCT testing program was promoted soon after the initiation of VCT at KIWAKKUKI, raising the possibility that the increase in daily testing numbers reflected growing awareness of this new service by the community. Against this, the VCT program was initiated 2 months before data collection for this study, and a stable daily testing pattern was observed before the free VCT campaign. It is possible that some clients presented thinking VCT was still offered free at KIWAKKUKI, but this seems unlikely several months after the campaign, when daily testing numbers remained sustained and clients were asked to pay before testing.

It is likely that the combination of a simple, inexpensive advertising campaign helped to increase the social acceptability and awareness of VCT. Further, the provision of free testing removed a cost barrier to VCT, making it accessible to less wealthy clients. From a health policy point of view, including an inexpensive promotional campaign is necessary to create awareness of the free service.

There were limitations to this study. Although the calculations for the numbers of HIV infections averted and cost-effectiveness of this intervention were based on data from Tanzania, these data may not necessarily be representative of our population. In addition, our study was observational. Without formal hypothesis testing in which clinics are randomized to free VCT campaigns or their standard fee schedule, we cannot rigorously infer that free VCT campaigns lead to increased participation. Clearly a large, multisite, longer-term, randomized trial would help to refine the magnitude of the impact and cost-effectiveness of free VCT campaigns, but only after several years.

We used daily testing numbers obtained over a 2-week free testing period to estimate the cost-effectiveness of free VCT offered over a 1-year time horizon but did not demonstrate whether such daily testing numbers can be sustained. In response to these analyses, KIWAKKUKI has implemented a free VCT service and has sustained daily testing.

<table>
<thead>
<tr>
<th>TABLE 1—One-Year Estimated Testing Volumes and Costs in US Dollars Without Free VCT, With a Free VCT Campaign, and With Sustained Free VCT: Moshi, Tanzania, May through November, 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Economic parameters</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Building rent</td>
</tr>
<tr>
<td>Telephone</td>
</tr>
<tr>
<td>Power</td>
</tr>
<tr>
<td>Advertising</td>
</tr>
<tr>
<td>Labor</td>
</tr>
<tr>
<td>Lab supplies</td>
</tr>
<tr>
<td>HIV test kits</td>
</tr>
<tr>
<td>Other consumables</td>
</tr>
<tr>
<td>Total cost</td>
</tr>
<tr>
<td>Estimated income from VCT charges</td>
</tr>
<tr>
<td>Net cost</td>
</tr>
</tbody>
</table>

Note: VCT = voluntary counseling and testing.  
1Applies free VCT daily client volumes to 261 testing days in a calendar year.  
2Applies free VCT daily client volumes to 10 days and postfree VCT daily client volumes to 251 testing days in calendar year.  
3Applies free VCT daily client volumes to 251 testing days in a calendar year.  
4Radio advertisements, gasoline for car during free VCT campaign.  
5Laboratory technician, VCT program director, counselors.  
6Assumes fees are waived for those aged <25 years per Tanzanian Ministry of Health guidelines.
## Table 2—Cost-Effectiveness and Sensitivity Analyses Without Free VCT, With a Free VCT Campaign, and With Sustained Free VCT

<table>
<thead>
<tr>
<th>Prevention</th>
<th>Without Free VCT</th>
<th>Free VCT Campaign</th>
<th>Sustained Free VCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Treated</td>
<td>New Diagnoses</td>
<td>Infections Averted</td>
<td>Cost Per DALY, US$</td>
</tr>
<tr>
<td>HIV prevalence</td>
<td>Observed (16.7%)</td>
<td>67.9</td>
<td>169.69</td>
</tr>
<tr>
<td></td>
<td>Low (8%)</td>
<td>40.0</td>
<td>288.04</td>
</tr>
<tr>
<td></td>
<td>High (25%)</td>
<td>96.0</td>
<td>121.24</td>
</tr>
</tbody>
</table>

### New diagnoses

<table>
<thead>
<tr>
<th>Antiretroviral treatment*</th>
<th>Without Free VCT</th>
<th>Free VCT Campaign</th>
<th>Sustained Free VCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of treatment</td>
<td>Medium (30%)</td>
<td>48.4</td>
<td>420.00*</td>
</tr>
<tr>
<td>Low (10%)</td>
<td>16.1</td>
<td>420.00*</td>
<td>31.1</td>
</tr>
<tr>
<td>High (50%)</td>
<td>80.7</td>
<td>420.00*</td>
<td>155.6</td>
</tr>
</tbody>
</table>

### Tuberculosis prophylaxis*

<table>
<thead>
<tr>
<th>Rates of treatment</th>
<th>Without Free VCT</th>
<th>Free VCT Campaign</th>
<th>Sustained Free VCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of treatment</td>
<td>Medium (50%)</td>
<td>80.7</td>
<td>166.67*</td>
</tr>
<tr>
<td>Low (30%)</td>
<td>48.4</td>
<td>166.67*</td>
<td>93.4</td>
</tr>
<tr>
<td>High (70%)</td>
<td>112.9</td>
<td>166.67*</td>
<td>217.9</td>
</tr>
</tbody>
</table>

### Summary

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Without Free VCT</th>
<th>Free VCT Campaign</th>
<th>Sustained Free VCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium treatment</td>
<td>1381.4</td>
<td>24.52</td>
<td>...</td>
</tr>
<tr>
<td>Low treatment</td>
<td>1344.3</td>
<td>14.51</td>
<td>...</td>
</tr>
<tr>
<td>High treatment</td>
<td>1418.5</td>
<td>34.00</td>
<td>...</td>
</tr>
</tbody>
</table>

Note: VCT = voluntary counseling and testing; DALY = disability-adjusted life year.
*Based on previous estimates in Tanzania.
1* With an observed HIV infection prevalence of 16.7%.
2Assumes cost for antiretroviral therapy of $35 per month and does not include cost for monitoring and care.
3Based on an estimated cost of $25 for 6 months of preventive therapy.

---

**Table 2—Cost-Effectiveness and Sensitivity Analyses Without Free VCT, With a Free VCT Campaign, and With Sustained Free VCT**

<table>
<thead>
<tr>
<th>Prevention</th>
<th>Without Free VCT</th>
<th>Free VCT Campaign</th>
<th>Sustained Free VCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Treated</td>
<td>New Diagnoses</td>
<td>Infections Averted</td>
<td>Cost Per DALY, US$</td>
</tr>
<tr>
<td>HIV prevalence</td>
<td>Observed (16.7%)</td>
<td>67.9</td>
<td>169.69</td>
</tr>
<tr>
<td></td>
<td>Low (8%)</td>
<td>40.0</td>
<td>288.04</td>
</tr>
<tr>
<td></td>
<td>High (25%)</td>
<td>96.0</td>
<td>121.24</td>
</tr>
</tbody>
</table>

### New diagnoses

<table>
<thead>
<tr>
<th>Antiretroviral treatment*</th>
<th>Without Free VCT</th>
<th>Free VCT Campaign</th>
<th>Sustained Free VCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of treatment</td>
<td>Medium (30%)</td>
<td>48.4</td>
<td>420.00*</td>
</tr>
<tr>
<td>Low (10%)</td>
<td>16.1</td>
<td>420.00*</td>
<td>31.1</td>
</tr>
<tr>
<td>High (50%)</td>
<td>80.7</td>
<td>420.00*</td>
<td>155.6</td>
</tr>
</tbody>
</table>

### Tuberculosis prophylaxis*

<table>
<thead>
<tr>
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<th>Free VCT Campaign</th>
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<td>1344.3</td>
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<td>...</td>
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<tr>
<td>High treatment</td>
<td>1418.5</td>
<td>34.00</td>
<td>...</td>
</tr>
</tbody>
</table>

Note: VCT = voluntary counseling and testing; DALY = disability-adjusted life year.
*Based on previous estimates in Tanzania.
1* With an observed HIV infection prevalence of 16.7%.
2Assumes cost for antiretroviral therapy of $35 per month and does not include cost for monitoring and care.
3Based on an estimated cost of $25 for 6 months of preventive therapy.
of 13 persons per day over 6 months. Furthermore, there was no change in HIV seroprevalence among VCT clients over the same 6-month period. Our data suggest that VCT with free campaigns or the provision of sustained free VCT should be adopted into national HIV control policies.

In some instances, governments and nongovernmental organizations offer free VCT, but at least partial cost recovery through client charges is more typical. In resource-limited settings, sustained provision of universal free VCT may be unrealistic given the multiple demands on the resources allocated for HIV/AIDS prevention and care services.

An approach of strengthening existing health infrastructure by investing enough funds to maximize testing capacity within stable community-based organizations rather than the creation of new services would also be beneficial in terms of the relative ease of implementation. Similarly, costs could be reduced by integrating VCT into other health services, such as those for tuberculosis, sexually transmitted infections, hospital inpatient and outpatient services, and antenatal care.

The integration of this VCT program within a volunteer-based AIDS service organization in Moshi, Tanzania, was highly cost-effective. Our study suggests that the provision of free VCT not only results in a prolonged increase in daily testing number but also optimizes testing throughput and efficiency. The enhanced cost-effectiveness of this intervention was reflected in potential disability averted by preventing new HIV infections and facilitating access to expanding HIV treatment programs. In addition to further operational research aimed at optimizing VCT in other settings, policymakers should support the small investment necessary to underway free VCT, particularly when integrated into existing community-based AIDS service organizations.

About the Authors
At the time of the study, Nathan M. Thielman, John A. Bartlett, John A. Crump, and Helen Y. Chu were with the Division of Infectious Diseases and International Health at Duke University Medical Center, Durham, NC. Jan Ostermann is with the Health Inequalities Program, Terry Sanford Institute of Public Policy, Duke University, Durham NC. Anna M. Magassa and Sara Magassa are with KIAWAKIWI (Kihondo cha Watinashe Kilimanjaro)

Kapambanza USKIMEWE Women Against AIDS in Kilimanjaro. John F. Shao is with Tumaini University, Moshi, Tanzania. Sabine Mwana and John A. Crump are also with Kilimanjaro Christian Medical Centre and Kilimanjaro Christian Medical College of Tumaini University, Moshi.

Requests for reprints should be sent to John A. Crump, Division of Infectious Diseases and International Health, Duke University Medical Center, Box 3877, Durham, NC 27711 (e-mail: crump017@mc.duke.edu).

This article was accepted March 5, 2005.

Contributors
N.M. Thielman and J.A. Bartlett originated the study. N.M. Thielman wrote the final article. They received assistance in designing the survey instrument from S. Mtwewe and D. Etuka. A. Ng'asonga enrolled clients at KIAWAKIWI, H.Y. Chu performed initial data analysis and wrote an early draft of the article. J. Ostermann oversaw statistical analyses and performed the final cost-effectiveness calculations. J.A. Crump contributed to study implementation, data management and analysis, interpretation, and article editing. J.F. Shao faciltated critical administrative support. All authors contributed to the final version of the article.

Acknowledgments
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We thank the clients of the KIAWAKIWI VCT program for their participation.

Human Participant Protection
Ethical approval for the study was granted by the Kilimanjaro Christian Medical Centre Research Ethics Committee, the institutional review board of Duke University Medical Center, and the Tanzania National Institute of Medical Research National Medical Research Coordinating Committee.

References

5.4. Characteristics of HIV voluntary counseling and testing clients before and during care and treatment scale-up in Moshi, Tanzania


CONTRIBUTION

My position as third author reflects my role providing on-site, day-to-day guidance and mentorship to Ms. Shorter and Ms. Tribble, medical students who worked on this project. I co-developed the analytic plan with Thielman and Ostermann and helped Shorter to write the first draft of the manuscript. Thielman sought and obtained funding, designed the project, and co-supervised the research with me, including the study team members responsible for participant enrollment, data collection, and data management. Ostermann led the analysis, with input from Thielman and me. Itemba, Mgonja, and Mtalo ensured that research activities were coordinated with HIV counseling and testing service provision. Bartlett, Shao, and Schimana assisted with institutional relationships and implementation of the research in inpatient areas. All authors contributed to revisions of the manuscript.

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Characteristics of HIV Voluntary Counseling and Testing Clients Before and During Care and Treatment Scale-Up in Moshi, Tanzania

Meghan M. Shorter, MD,* Jan Ostermann, PhD,†‡ John A. Crump, MBChB,*‡§‖ Alison C. Tribble, MD,* Dafrosa K. Itemba, BA,¶ Anna Mgonja,¶ Antipas Malo,¶ John A. Bartlett, MD,*‡§‖ John F. Shao, MD, PhD,§‖ Werner Schimana, MD,# and Nathan M. Thielman, MD, MPH*†

Objectives: We evaluated changes in characteristics of clients presenting for voluntary counseling and testing (VCT) before and during care and treatment center (CTC) scale-up activities in Moshi, Tanzania, between November 2003 and December 2007.

Methods: Consecutive clients were surveyed after pretest counseling, and rapid HIV antibody testing was performed. Trend tests were used to assess changes in seroprevalence and client characteristics over time. Multivariable logistic regression models were used to estimate the contribution of changes in sociodemographic and behavioral risk characteristics, and symptoms, to changes in seroprevalence before and during CTC scale-up.

Results: Data from 4391 first-time VCT clients were analyzed. HIV seroprevalence decreased from 26.2% to 18.9% after the availability of free antiretroviral therapy and expansion of CTCs beyond regional and referral hospitals. Seroprevalence decreased by 27% for females (P = 0.0002) and 34% for males (P = 0.0125). Declines in seropositivity coincided with decreases in symptoms among males and females (P < 0.0001) and a more favorable distribution of sociodemographic risks among females (P = 0.002). No changes in behavioral risk characteristics were observed.

Conclusions: Concurrent with the scale-up of CTCs, HIV seroprevalence and rates of symptoms declined sharply at an established freestanding VCT site in Moshi, Tanzania. If more HIV-infected persons access VCT at sites where antiretrovirals are offered, freestanding VCT sites may become a less cost-effective means for HIV case finding.

Key Words: antiretroviral therapy, care and treatment, HIV seroprevalence, risk factors, sub-Saharan Africa

INTRODUCTION

Despite substantial advances in HIV diagnostics and therapeutics over the past decade, approximately 2.5 million people were newly infected in the past year, bringing the global count of HIV-infected people to 33.2 million. Although many resources have been allocated to address the epidemic in sub-Saharan Africa, this region remains disproportionately affected, accounting for 68% of HIV-infected adults and 76% of adult and child deaths from AIDS worldwide.1 Continuing efforts to focus on prevention and treatment remains critical to containing the HIV/AIDS epidemic.2,3

Voluntary counseling and testing (VCT) has long been seen as an important intervention in sub-Saharan Africa, offering an individualized, client-centered approach that addresses prevention of transmission between partners and between mother and child.4 VCT also provides opportunities for early identification of infection,5 allowing for more effective treatment of HIV/AIDS and its coinfections,6,7 especially as access to antiretroviral therapy (ART) continues to expand.8 In Tanzania, free access to ART began in September of 2004 under the HIV/AIDS Treatment and Care Plan 2003–2008.9 With a goal of treating 400,000 HIV-infected Tanzanian residents by the year 2008, this plan also emphasized the need to expand VCT services as the primary entry point for care and treatment.

The interaction between prevention efforts and treatment rollout has been highlighted as an important strategy for effectively fighting the HIV epidemic.10,11 In developing
countries where access to ART may be limited, it has been postulated that synergistic effects between the two could lead to 29 million fewer infections and 10 million deaths averted, whereas unsuccessful integration or sole focus on treatment would be insufficient to control the epidemic. 

 Several studies have evaluated interactions between ART access and VCT, finding more favorable attitudes toward testing, expansion of education activities, and changing community attitudes toward HIV and testing after the availability of drugs. In Haiti, South Africa, and Botswana, VCT uptake increased after ART became accessible. However, it has yet to be determined how VCT client characteristics, including risk behaviors, may change after the regional scale-up of access to free care and treatment. Greater access to care and treatment centers (CTCs) might influence the characteristics of populations accessing freestanding VCT centers. Understanding such changes may help shape policy and practice around the optimal means for HIV case finding and care delivery. Using data from a cohort of clients presenting at an established stand-alone VCT Centre in Moshi, Tanzania, we examined changes in seropositivity, sociodemographic characteristics, and HIV risk behaviors and symptoms of clients from 2003 through 2007, a period intersected by the introduction of free ART in September 2004 and the rapid scale-up of multiple CTCs.

METHODS

Location and Context

Subjects were recruited from the AIDS Information Centre in downtown Moshi, Tanzania, an established stand-alone VCT site operated by Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI; Women Against AIDS in Kilimanjaro). VCT services have been offered since March of 2003, in conjunction with other services that have been incorporated since the founding of KIWAKKUKI in 1990 such as home-based care, education and outreach, and orphan care and assistance services. Initially, each test cost 1000 Tanzanian shillings (US $0.95 at 2003 exchange rates), but testing for clients became free in May 2004. In September 2004, the Tanzanian government began offering access to free ART; hence therapy became available locally at Kilimanjaro Christian Medical Centre (KCMC), though initially in limited supply. Scale-up of regional HIV CTCs through which patients could receive free ART progressed rapidly. Data collection for this report began November 21, 2003, and ended December 31, 2007.

Voluntary HIV Counseling and Testing Procedures

All counseling and testing was performed in accordance with guidelines provided by the Tanzanian Ministry of Health National AIDS Control Programme. Clients presenting for testing received confidential pretest counseling, including risk assessment and risk reduction planning with a trained counselor, after which informed consent was obtained for clients aged 18 and above. Clients younger than 18 were tested with a parent’s permission but not enrolled in this study. Counselors then administered a structured questionnaire designed to obtain information on sociodemographic characteristics, reasons for testing, past and current sexual behavior, partner relationship status, HIV testing history, potential HIV-related symptoms (fever, cough, bloody cough, diarrhea, rash, night sweats, genital ulcers, and weight loss), ART knowledge, and planned changes in behavior after testing. For marital status specifically, clients were given the option to choose cohabitating, divorced/separated, widowed, married (polygamous or monogamous), or single (including never married, have girlfriend or boyfriend, or engaged). The provision of VCT services was not contingent upon participation in the study.

After pretest counseling and administration of the questionnaire, a 2 mL blood sample was drawn and tested using both Capillus (Trinity Biotech PLC, Bray, County Wicklow, Ireland) and Determine (Abbott Laboratories, Abbott Park, IL) rapid HIV1/2 antibody tests. If the two test results did not match, the blood samples were sent to the referral laboratory at KCMC for confirmatory testing using the enzyme-linked immunosorbent assay method. For quality control purposes, every 10th sample also underwent repeat testing using the enzyme-linked immunosorbent assay method at the KCMC laboratory. Clients who tested positive were referred to the local CTCs, offered to join peer support groups and the home-based care program and encouraged to have sexual partners and children tested. For clients who tested negative, post-test counseling focused on prevention of infection with HIV, and clients were encouraged to return for repeat testing at the time interval recommended by the Ministry of Health. Regular testing of the sexual partner was also emphasized. Clients who screened positive for possible sexually transmitted diseases, domestic violence, and/or tuberculosis were referred to the appropriate care centers.

Ethical Considerations

Ethical approval for this study was obtained from the KCMC Research Ethics Committee, the Duke University Health System Institutional Review Board, and the Tanzanian National Institute of Medical Research National Medical Research Coordinating Committee. All study subjects were provided with Kiswahili versions of the written consent, and only consenting adults were enrolled in the study.

Analysis

Data were entered using Epi Info 2002 or Epi Info 3.3 software (Centers for Disease Control and Prevention, Atlanta, GA) or Teleform 9.0 (Cardiff, Visa, CA). Data were validated by randomly sampling 10% of the questionnaires, with an acceptable error rate of <1 error per 5 forms. Data were compiled into one database using JMP 6.0 (SAS Institute, Inc, Cary, NC), and all analyses were performed using STATA 10 (StataCorp, College Station, TX).

Differences in client characteristics by gender were assessed using $\chi^2$ and Student t tests. Nonparametric trend tests were used to descriptively assess changes in the characteristics of the client population over time. For analyses of changes in seropositivity and correlates of seropositivity, the study period was divided into 3 time intervals: pre-CTC...
period (before October 1, 2004); a transitional early CTC period (October 1, 2004 to June 30, 2006); and a CTC scale-up period (July 1, 2006 or later). The early CTC period was defined by the time of free-ART introduction at only the zonal and regional referral hospitals until decentralization to district hospitals and health centers.

Generalized Hausman tests were used to evaluate whether correlates of seropositivity, identified using gender-specific multivariable logistic regression models, differed significantly between the pre-CTC and the scale-up CTC periods. All models included the following variable domains as covariates: (1) sociodemographic characteristics (age, education, rural versus urban residence, and marital status), (2) behavioral risk (number of lifetime partners, 3 or more lifetime partners), and (3) presence of any HIV-related symptoms and the number of symptoms. All analyses were stratified by gender.

Combined models, stratified by gender and covering the entire study period, were estimated to identify the effects of changes in the client population on rates of seropositivity and to identify the relative effects of changes in the distribution of each of the 3 explanatory domains: sociodemographic characteristics, behavioral risk characteristics, and symptoms. Parameter estimates from the combined models were used to predict each client’s probability of seropositivity. To determine domain-specific contributions to changes in seropositivity, we iteratively analyzed changes in the predicted probabilities over time, holding all characteristics in the respective other domains constant at the sample mean. Time effects were analyzed using logistic regression models with predicted probabilities as dependent variables and time as the sole explanatory variable. The time variable was specified in 5 segments: the pre-CTC period was used as the reference group, the transitional (early CTC) period was described by a spline ranging from October 1, 2004 to June 30, 2006), and the CTC scale-up period was specified as a binary indicator variable. Significance was assessed by linear combinations of the spline and the CTC scale-up parameters. The process was repeated for each domain, separately for males and females.

RESULTS

Between November 21, 2003, and December 31, 2007, 8647 consecutive clients presented for VCT services at KIWAKKUKI, and of these, 4391 (50.8%) were first-time testers. Seropositivity rates among first-time testers declined significantly in the CTC scale-up period (Fig. 1), with a decrease from 14.9% to 9.9% (P = 0.0124) in males and from 35.4% to 25.9% (P = 0.0002) in females, compared with the pre-CTC period. For comparison, also shown in Figure 1 are available Kilimanjaro region-specific antenatal clinic and demographic surveillance data from this period.20-22 Sociodemographic characteristics, behavioral risk characteristics, and client-reported symptoms in the pre-CTC period and the CTC scale-up period, along with trends across the entire study period, are summarized in Table 1. Age distribution did not change significantly across time. The percent of female clients reporting a primary school education or less decreased significantly (P < 0.0001) as did the proportion residing in rural areas for both males and females (P < 0.0001).

Significantly, more men and women reported that they were divorced/separated during the later period (P = 0.0001 and P = 0.0058, respectively), and fewer women reported being widowed (P = 0.0090). The proportion of clients reporting 3 or more lifetime sexual partners increased over time from 55.6% to 66.7% for men (P = 0.0172) and from 26.4% to 31.4% for women (P = 0.0951). The number of lifetime sexual partners increased significantly for males (P = 0.0199) but not for females. Over time, the proportion of clients reporting any of the 8 listed HIV-related symptoms decreased by more than half for men and by more than a third for women (P < 0.0001); the mean number of symptoms did not change.

Summarized in Table 2 are client reasons for testing during the pre-CTC and CTC scale-up periods with trend tests for all clients across the study period, by gender. Over time, a greater percentage of men and women cited having multiple sexual partners (P < 0.0001 for each), suspicion of unfaithfulness in their partner (P < 0.0001 for each), and premarital planning (P = 0.0167 for males; P = 0.0002 for females). Women citing preconception planning as a reason for testing increased significantly (P = 0.0068). Multivariate analysis of correlates of seropositivity from 2003 to 2007 revealed no significant changes over time (Table 3). Among males, receiving less education [odds ratio (OR): 1.45, 95% confidence interval (CI): 1.07 to 2.07, P < 0.05], residing in a rural area (OR: 1.49, CI: 1.07 to 2.09, P < 0.05), cohabitating (OR: 5.76, CI: 3.49 to 9.51, P < 0.0001), being divorced/separated (OR: 2.99, CI: 1.67 to 5.34, P < 0.0001), being widowed (OR: 6.43, CI: 3.11 to 13.31, P < 0.0001), being married (OR: 1.80, CI: 1.11 to 2.90, P < 0.05), having more than 3 lifetime sexual partners (OR: 1.53, CI: 1.04 to 2.25, P < 0.05), and having a higher number of symptoms (OR: 1.81, CI: 1.50 to 2.19, P < 0.0001) were associated with increased risk. Among females, cohabitating (OR: 2.73, CI: 1.94 to 3.86, P < 0.0001), being divorced/separated (OR: 2.13, CI: 1.50 to 3.03, P < 0.0001), being widowed (OR: 4.57, CI: 3.21 to 6.49, P < 0.0001), having more than 3 lifetime sexual partners (OR: 1.97, CI: 1.55 to 2.51, P < 0.0001), having any symptoms (OR: 2.07, CI: 1.44 to 2.97, P < 0.0001), and having a higher number of symptoms (OR: 1.76, CI: 1.52 to 2.04, P < 0.0001) were associated with increased risk.

The differential effects of the 3 explanatory domains (sociodemographic characteristics, behavioral risk characteristics, and symptoms) on seroprevalence are summarized in Table 4. For females, changes in sociodemographic characteristics and symptoms contributed to decreased seroprevalence (P = 0.002 and P < 0.0001, respectively). For males, symptom-related decreases in seropositivity (P < 0.0001) were partially offset by small increases in behavioral risk characteristics (P = 0.0590).

DISCUSSION

In this analysis of over 4300 consecutive clients presenting for first-time VCT over 50 months and including intervals before and after CTC expansion in Moshi, Tanzania, we describe a significant decrease in HIV seroprevalence for both women and men. Reductions in symptoms for both men and women and a more favorable distribution of
Panel A  
Rates of seropositivity among first-time testers, by period

<table>
<thead>
<tr>
<th>Period</th>
<th>Pre-CTC</th>
<th>Early CTC</th>
<th>CTC Scale-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>HIV Seroprevalence (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4 2003</td>
<td>45%</td>
<td>40%</td>
<td>45%</td>
</tr>
<tr>
<td>Q1 2004</td>
<td>40%</td>
<td>35%</td>
<td>35%</td>
</tr>
<tr>
<td>Q2 2004</td>
<td>35%</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Q3 2004</td>
<td>30%</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>Q4 2004</td>
<td>25%</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>Q1 2005</td>
<td>20%</td>
<td>15%</td>
<td>15%</td>
</tr>
<tr>
<td>Q2 2005</td>
<td>15%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Q3 2005</td>
<td>10%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Q4 2005</td>
<td>5%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Panel B  
HIV seroprevalence reported in national and regional data

<table>
<thead>
<tr>
<th>Period</th>
<th>HIV Seroprevalence (%)</th>
</tr>
</thead>
</table>
| Q4 2003 | 8%  
| Q1 2004 | 6%  
| Q2 2004 | 4%  
| Q3 2004 | 2%  
| Q4 2004 | 0%  
| Q1 2005 | 8%  
| Q2 2005 | 6%  
| Q3 2005 | 4%  
| Q4 2005 | 2%  
| Q1 2006 | 8%  
| Q2 2006 | 6%  
| Q3 2006 | 4%  
| Q4 2006 | 2%  
| Q1 2007 | 8%  
| Q2 2007 | 6%  
| Q3 2007 | 4%  
| Q4 2007 | 2%  

Panel C  
CTC scale-up and number of clients in treatment

TABLE 1. Client Characteristics at a VCT Center in Moshi, Tanzania, Pre-CTC and During CTC Scale-Up, N = 4391

<table>
<thead>
<tr>
<th></th>
<th>Males (N = 1887)</th>
<th>Females (N = 2594)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-CTC</td>
<td>CTC Scale-Up</td>
</tr>
<tr>
<td>Age</td>
<td>31.5</td>
<td>32.4</td>
</tr>
<tr>
<td>Primary school education or less</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Rural residence</td>
<td>59.2%</td>
<td>44.2%</td>
</tr>
<tr>
<td>Seropositivity</td>
<td>14.9%</td>
<td>9.9%</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohabiting</td>
<td>5.7%</td>
<td>5.4%</td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>4.3%</td>
<td>9.7%</td>
</tr>
<tr>
<td>Widowed</td>
<td>3.7%</td>
<td>3.1%</td>
</tr>
<tr>
<td>Married</td>
<td>25.3%</td>
<td>23.0%</td>
</tr>
<tr>
<td>Single</td>
<td>61.1%</td>
<td>58.8%</td>
</tr>
<tr>
<td>Behavioral risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 or more lifetime partners</td>
<td>55.6%</td>
<td>66.7%</td>
</tr>
<tr>
<td>No. lifetime partners</td>
<td>4.47</td>
<td>5.88</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any symptoms</td>
<td>26.1%</td>
<td>12.9%</td>
</tr>
<tr>
<td>No. symptoms*</td>
<td>2.40</td>
<td>2.01</td>
</tr>
</tbody>
</table>

The P value denotes significance of trend among all clients (November 2003 to December 2005).

*Mean number of symptoms for clients with any symptoms.

sociodemographic risk factors among women contributed to this decrease.

Although causality between the scale-up of CTCs and changes in VCT client characteristics cannot be assessed from this study, it is evident that the proportion of clients found to be HIV seropositive decreased significantly in this region with the addition of more CTCs. Several reasons could underlie the observed results. Persons who suspect that they are HIV infected (either because they have a family member being cared for at a CTC or because they are symptomatic) may prefer to be tested at sites where treatment is known to be available. It is also possible that because treatment availability may reduce community stigma, community members may be more inclined to accept testing even when well. It has been shown in South Africa that VCT coupled with ART availability changes community perceptions favorably. Similarly, educational campaigns concurrent with CTC scale-up may have resulted in a broader population accessing VCT services. This is supported by the changing sociodemographic characteristics of women. Another possibility is that seroprevalence is decreasing in the general population. Nationally, Tanzania has seen a decreasing trend over the past few years, with seropositivity rates dropping from 9% in 2003 to 7% in 2004 to 6.5% in 2005 to 6% in 2007–2008. As shown in Figure 1, population-based survey estimates of HIV seroprevalence in the Kilimanjaro Region were 7.3% in 2003–2004 and 1.9% in 2007–2008. Even though prevalence decreases of such magnitude seem unlikely, it is quite possible that seroprevalence is actually decreasing in this region; additional data are needed to corroborate these observations. Our data and those from other similar cohorts suggest that all of the explanations may have contributed to these changes.

We also note persisting differences among VCT clients by gender. Disparity of power within relationships and in society are prominent within sub-Saharan culture and are often cited as contributing factors to higher HIV risk among women in this region. It is possible that greater access to care and treatment may attenuate gender disparities by providing more personal control of health to men and women equally. Despite this hypothesis, our results show a continuing gender differential in HIV seroprevalence, suggesting that, for females, testing may be viewed less as a means of prevention and more as an entry point to care, plausibly leading to gender-specific responses to the increased access to CTCs.

TABLE 2. Reasons for Testing Cited by Clients Presenting for VCT in Moshi, Tanzania

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-CTC, %</td>
<td>CTC Scale-Up, %</td>
</tr>
<tr>
<td>Personal illness</td>
<td>17.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Sexual partner ill or dead</td>
<td>5.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Multiple sexual partners</td>
<td>20.1</td>
<td>50.4</td>
</tr>
<tr>
<td>Suspect an unfaithful partner</td>
<td>23.9</td>
<td>34.7</td>
</tr>
<tr>
<td>Pre-marital</td>
<td>22.1</td>
<td>26.6</td>
</tr>
<tr>
<td>Pre-conception</td>
<td>3.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>All Clients</th>
<th>Pre-CTC</th>
<th>CTC Scale-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td>1.01</td>
<td>(1.00 to 1.03)</td>
<td>0.0770</td>
</tr>
<tr>
<td>Primary school education or less</td>
<td>1.45</td>
<td>(1.01 to 2.07)</td>
<td>0.0430</td>
</tr>
<tr>
<td>Rural residence</td>
<td>1.49</td>
<td>(1.07 to 2.09)</td>
<td>0.0190</td>
</tr>
<tr>
<td>Cohabiting</td>
<td>5.76</td>
<td>(3.49 to 9.51)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>2.99</td>
<td>(1.67 to 5.34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Widowed</td>
<td>6.43</td>
<td>(3.11 to 13.31)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Married</td>
<td>1.80</td>
<td>(1.11 to 2.90)</td>
<td>0.0170</td>
</tr>
<tr>
<td>More than 3 lifetime partners</td>
<td>1.53</td>
<td>(1.04 to 2.25)</td>
<td>0.0330</td>
</tr>
<tr>
<td>Number of lifetime partners</td>
<td>1.00</td>
<td>(0.99 to 1.02)</td>
<td>0.5260</td>
</tr>
<tr>
<td>Any symptoms</td>
<td>1.66</td>
<td>(0.97 to 2.84)</td>
<td>0.0650</td>
</tr>
<tr>
<td>Number of symptoms*</td>
<td>1.81</td>
<td>(1.50 to 2.19)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Mean number of symptoms for clients with any symptoms.
Test for equality: pre-CTC vs. CTC scale-up—p = 0.7401 for males and p = 0.3729 for females.

We note several limitations to this study. All results were self-reported by clients; underestimation of current sexual behaviors may have occurred due to social desirability bias. 27 Similarly, clients are a self-selected group who attend counseling and testing services voluntarily and cannot be considered a sample of the general population. Also, our pre-CTC period estimates are limited to a relatively short period, and thus we cannot fully distinguish what occurred during this time frame; there may have been changes in anticipation of the provision of free ART that we could not detect. Finally, other correlates of seropositivity, not included in our analyses, may have contributed to the observed changes in seropositivity over time. It is not possible to ascertain the contribution of other characteristics from our data.

Collectively, these findings hold several important policy-level and programmatic implications for VCT programs. First, as CTC sites increase in number and become more decentralized, more symptomatic clients may access HIV testing services at sites where antiretrovirals are offered. Freestanding VCT sites may thus become a less cost-effective means for HIV case finding. Given the rationale and mandate for universal testing in this region and anticipated worsening fiscal constraints, alternative methods for identifying HIV-infected persons, such as mobile VCT, provider-initiated testing and counseling, home-based testing, and CTC-based testing campaigns and their relative cost-effectiveness should be explored. Second, because the declines in seroprevalence among first-time testers at this VCT site seem to mirror those of population-based serosurveys for the Kilimanjaro region, seroprevalence data from stable VCT sites, such as this one, may be useful for ascertaining trends reflective of the larger population, despite the inherent bias of this self-selected, self-referred sample. Third, we observed no appreciable changes in behavior risk characteristics over time; there remains a pressing need to sharpen and focus HIV risk behavior reduction messaging.


<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect on Seroprevalence, %</td>
<td>P</td>
<td>CI %</td>
<td>Effect on Seroprevalence, %</td>
<td>P</td>
<td>CI %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sociodemographics characteristics</td>
<td>-0.77</td>
<td>0.1350</td>
<td>-1.79 to 0.24</td>
<td>-2.12</td>
<td>0.0020</td>
<td>-3.48 to -0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behavioral risk characteristics</td>
<td>0.19</td>
<td>0.0590</td>
<td>-0.01 to 0.38</td>
<td>0.21</td>
<td>0.5990</td>
<td>-0.57 to 0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>-3.77</td>
<td>&lt;0.0001</td>
<td>-5.27 to -2.26</td>
<td>-6.51</td>
<td>&lt;0.0001</td>
<td>-8.80 to -4.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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In conclusion, we identified a decrease in seroprevalence in both genders. Declines in symptoms contributed to this overall finding in both men and women, as did a more favorable distribution of sociodemographic risks among women. Although this analysis cannot establish a causal relationship between these events and the scale-up of CTCs within this region, it is clear that the client population presenting for VCT has changed. It will be important for VCT programs to incorporate this new knowledge into their prevention programs if they are to appropriately target the changing needs of their client population.

ACKNOWLEDGMENTS

We would like to thank the study participants and the staff, volunteers, and counselors of KIWAKKUKI.

REFERENCES

5.5. A cost-effectiveness analysis of alternative HIV re-testing strategies in sub-Saharan Africa


CONTRIBUTION

My position as last author reflects my role as senior, supervising author on the paper. I conceived the research idea, sought and obtained funding, and brought together the research team. I led and supervised the research, including data collection, design of the analysis, and write up. I mentored the medical students, Mr. Waters and Mr. Reeves from inception to completion of the project. Ostermann and Masnick led building of mathematical models and cost-effectiveness analyses. Thielman and Bartlett contributed to study design and collation of input data, including expert opinion, for the model. All authors contributed to revisions of the manuscript.

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A Cost-Effectiveness Analysis of Alternative HIV Retesting Strategies in Sub-Saharan Africa

Richard C. Waters, MSc,† Jan Ostermann, PhD,** Travis D. Reeves, MD,‡ Max F. Masnick, BA,*,†† Nathan M. Thielman, MD, MPH,*‡‡ John A. Bartlett, MD,*†§§ and John A. Crump, MB, ChB, DTM&H*‡§§

Background: Guidelines in sub-Saharan Africa on when HIV-seronegative persons should retest range from never to annually for lower-risk populations and from annually to every 3 months for high-risk populations.

Methods: We designed a mathematical model to compare the cost-effectiveness of alternative HIV retesting frequencies. Cost of HIV counseling and testing, linkage to care, treatment costs, disease progression, and mortality, and HIV transmission are modeled for three hypothetical cohorts with postulated annual HIV incidence of 0.8%, 1.3%, and 4.0%, respectively. The model compared costs, quality-adjusted life-years gained, and secondary infections averted from testing intervals ranging from 3 months to 30 years. Input parameters from sub-Saharan Africa were used and explored in sensitivity analyses.

Results: Accounting for secondary infections averted, the most cost-effective testing frequency was every 7.5 years for 0.8% incidence, every 5 years for 1.3% incidence, and every 2 years for 4.0% incidence. Optimal testing strategies and their relative cost-effectiveness were most sensitive to assumptions about HIV counseling and testing and treatment costs, rates of CD4 decline, rates of HIV transmission, and whether tertiary infections averted were taken into account.

Conclusions: While higher risk populations merit more frequent HIV testing than low risk populations, regular retesting is beneficial even in low-risk populations. Our data demonstrate benefits of tailoring testing intervals to resource constraints and local HIV incidence rates.

Key Words: HIV counseling and testing, retesting, cost-effectiveness, guidelines, sub-Saharan Africa

(J Acquir Immune Defic Syndr 2011;56:443–452)

BACKGROUND

HIV counseling and testing (HCT) is promoted to increase serostatus awareness and entry into HIV care and treatment programs, particularly in low- and middle-income countries. Although uncertainty remains about its efficacy in producing behavior change, the role of HCT in linking HIV-infected persons to care and treatment services is undisputed.

Moreover, an increased understanding of the relationship between plasma HIV-1 RNA concentration and risk for HIV transmission has prompted consideration of antiretroviral therapy as an HIV prevention strategy. Further increasing the importance of HCT as an entry point into care.

The generalized nature of the HIV epidemic in sub-Saharan Africa has led to the promotion of universal HCT. Although many campaigns and strategies appropriately emphasize HCT for persons who have never tested, the risk for HIV infection for an individual typically persists beyond the initial HCT encounter, raising the question of when, if at all, seronegative testers should retest.

For nonpregnant HIV-seronegative testers, recommendations on when to retest for HIV are varied. Several national guidelines make no mention of the frequency with which a seronegative tester should continue to test. Other studies have suggested that a single test after 1 to 2 months in case the initial HIV antibody test was performed before development of HIV antibodies; some promote testing every 3 months or "periodically" for those who engage in high-risk behaviors and annual testing for the general population. The World Health Organization has recommended annual testing for those at risk.

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From the *Duke Global Health Institute, Duke University, Durham, NC; †Center for Health Policy and Inequalities Research, Duke University, Durham, NC; ‡Division of Infectious Diseases and International Health, Duke University Medical Center, Durham, NC; ¶Kilimanjaro Christian Medical Center, Moshi, Tanzania; and ‡Kilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania.

Funding provided by the US National Institutes of Health. Investigator support was obtained from the Fogarty International Center (D43 PA-03-018, J.A.B., N.M.T., J.A.C.), the Duke Clinical Trials Unit and Clinical Research Sites (U01 AI069452-01 JAB, N.M.T., J.A.C.), the International Studies on AIDS Associated Co-infestions award (U01 AI-03-036 J.A.B., N.M.T., J.A.C.), Center for HIV/AIDS Vaccine Immunology (U01 AI067854 J.A.B., N.M.T., J.A.C.), and the Duke University Center for AIDS Research (P30 AI 64518 J.O., J.A.B., N.M.T.).

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R.C.W. and J.O. contributed equally to this work.

J.A.C., J.O., T.D.R., and N.M.T. originated the work. J.O., T.D.R., and R.C.W. developed the analytic framework. T.D.R. and R.C.W. identified the background information and input parameters for the model. M.F.M. and R.C.W. streamlined the original analyses and implemented the model in Matlab. J.A.C., J.O., and R.C.W. wrote the final article. J.A.B. and N.M.T. contributed to study interpretation and article editing. All authors contributed to the final version of the article.

The authors have no conflicts of interest to disclose.

Correspondence to: John A. Crump, MB, ChB, DTM&H, Division of Infectious Diseases and International Health, Duke University Medical Center, Box 1022359, Durham, NC 27710 (email: john.crump@duke.edu).

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Health Organization recently released guidelines on retesting, advising annual testing for persons living in countries with generalized HIV epidemics who are at high risk for HIV, who do not know the HIV status of their partner, or who have any other ongoing risk behavior.28 Testing every 3 months is discouraged in these recommendations as it is retesting for individuals who have not had new potential exposures to HIV.

Mathematical models have been used previously to study the cost-effectiveness of one-time and repeated HIV screening in the United States, Russia, and South Africa.29-34 In simulating repeated screening for HIV, these models take into account long-standing undiagnosed prevalent cases, recent incident cases, and variable uptake of HCT, and the results have contributed to the formation of new guidelines for HIV screening.35,36 However, there has been little evaluation of the cost-effectiveness of different frequencies of retesting for persons who test HIV-seronegative, in which retesting concerns the detection of incident cases only. With national testing campaigns gradually raising rates of HIV serostatus awareness,37-41 cost-effectiveness analyses need to be extended to address the detection of incident HIV infections and the costs and benefits of alternative retesting frequencies.

To evaluate the question of when a seronegative tester should retest for HIV, we designed a mathematical model that compares the cost-effectiveness of alternative frequencies of HIV retesting using input parameters from sub-Saharan Africa when available.

METHODS

Overview
The model follows a cohort of individuals assumed to have initially tested HIV-seronegative; i.e., HIV prevalence at the start of the model is 0%. The cohort is followed for 45 years in the base-case scenario. Three different annual incidence rates mimic different HIV-risk environments comparable to those seen in previous studies in sub-Saharan Africa: 0.8% (low), 1.3% (medium), 4.0% (high).37-41 Twelve HCT strategies compare testing intervals ranging from every 3 months to once after 30 years. No further HIV risk or testing is assumed to occur during the final 15 years of the model. The base-case scenario uses a starting age of 20 years and age-specific mortality data for uninfected persons from South Africa.42 The primary outcome of interest was the cost per quality-adjusted life-year (QALY) gained from each testing strategy when compared with a scenario without HIV testing or treatment. Cost and QALY calculations account for secondary infections averted from effective antiretroviral therapy and behavior change (see subsequently). Incremental cost-effectiveness ratios for each strategy were calculated in comparison with the respective next longer retesting interval, with a single repeat test after 30 years compared with no retesting. The model is estimated iteratively in 3 month cycles; costs and benefits are discounted at 3% per year and expressed in year 2011 US dollars ($).43 Table 1 summarizes the base-case assumptions and sensitivity analysis ranges used in the model. Further discussion of the model and input parameters are presented in the Supplemental Appendix (see Supplemental Digital Content 1, http://links.lww.com/QAI/A144). The model was estimated in MATLAB Version R2009a (MathWorks Inc, Natick, MA); formulas are available from the authors on request.

HIV Infection and Disease Progression Without Treatment

Individuals become infected with HIV at the given incidence rate but remain undiagnosed until testing. HIV disease progression is modeled by changes in CD4 counts with associated changes in quality-of-life values and mortality rates over time. The base case scenario assumes a median time from seroconversion to AIDS of 10.3 years and a 10-year cumulative mortality of 39%.44-49

HIV Testing, Linkage to Care, and Treatment
Testing frequencies range from retesting every 3 months to one test after 30 years (Table 1). To avoid biases resulting from different lengths of follow-up after the last test, testing frequencies were chosen such that the last test for all strategies takes place 30 years after the start of the model. To compare the relative cost-effectiveness of each strategy, all individuals tested according to the given frequencies (see Appendix, Supplemental Digital Content 1, http://links.lww.com/QAI/A144). HIV tests were assumed to be rapid, point-of-care tests and have 100% sensitivity and specificity. Individuals testing seronegative continue to test at the specified frequency; individuals testing seropositive do not retest but are linked to care and then started on first-line highly active antiretroviral therapy (HAART) if the CD4 count is 350 cells/mm3 or less.49 After initiating HAART, a person may be lost to follow-up at a rate of 10% per year (range, 5-20% yearly in sensitivity analysis).51 Following HAART initiation and virologic suppression, a patient's CD4 count gradually increases as a function of the CD4 count at the start of HAART.29,52,53 Failure rates and mortality on first- and second-line HAART were assumed to be greatest immediately after initiation of HAART.54-58 It was assumed to take 6 months for virologic failure to be detected and patients to be switched to second-line HAART. To avoid unrealistic increases of CD4, CD4 counts were assumed to remain constant during effective second-line therapy in the base case; the effect of this assumption was explored in sensitivity analysis (see Appendix, Supplemental Digital Content 1, http://links.lww.com/QAI/A144). During nonsuppressive therapy, CD4 counts were assumed to drop again. Patients failing second-line therapy are kept on nonsuppressive therapy, consistent with guidelines.50,59,60

Costs
With the most substantial increase in persons on HAART likely to occur in South Africa,41 costs for HAART therapy were derived for the drug regimens indicated by the South Africa 2010 guidelines for patients newly starting therapy, tenofovir + emtricitabine/lamivudine + efavirenz/ nevirapine, and averaging the costs for the four possible regimens.51 Costs were similarly modeled for a second-line therapy of zidovudine + lamivudine + ritonavir-boosted lopinavir, consistent with the same guidelines. HCT cost per test, laboratory costs for prophylaxis for opportunistic infections, and cost per person for treatment of opportunistic infections were derived from studies in sub-Saharan
### TABLE 1. Input Parameters for the HIV Retesting Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base-Case Assumption</th>
<th>Sensitivity Analysis Range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population and testing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual incidence rates (%)</td>
<td>0.8, 1.3, 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of starting cohort (years)</td>
<td>20</td>
<td>20–30</td>
<td>Assumed</td>
</tr>
<tr>
<td>Years with constant incidence rate</td>
<td>30</td>
<td>5–10</td>
<td>Assumed</td>
</tr>
<tr>
<td>Mortality rates of HIV-uninfected persons (% per year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 20–24: 0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 25–34: 1.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 35–44: 1.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 45–54: 1.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 55–65: 2.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testing frequencies studied (years)</td>
<td>0.25, 0.5, 1, 2, 3</td>
<td>4, 5, 6, 7.5, 10,</td>
<td></td>
</tr>
<tr>
<td>15, 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle length (months)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discount rate (% per year)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HIV disease progression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) CD4 count at seroconversion (cells/mm³)</td>
<td>600 (240)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CD4 decline per year (cells/mm³ per year), untreated</td>
<td>39</td>
<td>22–75</td>
<td></td>
</tr>
<tr>
<td>Mortality rates (%), untreated HIV per CD4 count; per year</td>
<td>≥500: as HIV-uninfected</td>
<td>0.75–1.5 × base</td>
<td></td>
</tr>
<tr>
<td>HIV care and treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of linkage from diagnosis to care, per year post-test</td>
<td>70% in first year,</td>
<td>30–100%</td>
<td></td>
</tr>
<tr>
<td>post-test, 19% thereafter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Criteria for starting HAART</td>
<td>CD4 &lt;350 cells/mm³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss to follow-up from HIV care (% per year)</td>
<td>10.33</td>
<td>0–20</td>
<td></td>
</tr>
<tr>
<td>Failure rates on first-line HAART</td>
<td>27% first year, 7.8% thereafter</td>
<td>1–40% first year, 1–15% thereafter</td>
<td></td>
</tr>
<tr>
<td>Failure rates on second-line HAART</td>
<td>27% first year, 7.8% thereafter</td>
<td>1–40% first year, 1–15% thereafter</td>
<td></td>
</tr>
<tr>
<td>Mortality rates while on HAART, by CD4 count</td>
<td>$</td>
<td>0.5–2 × base</td>
<td></td>
</tr>
<tr>
<td>Time to detect virologic failure (months)</td>
<td>6</td>
<td>0–18</td>
<td>Assumed</td>
</tr>
<tr>
<td><strong>Transmission of HIV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV transmission per person-year, untreated HIV by CD4 count</td>
<td>Acute: 0.58, Subacute: 0.10</td>
<td>Acute: 0.23–0.64, Subacute: 0.04–0.12</td>
<td></td>
</tr>
<tr>
<td>Decline in HIV transmission rate from testing and counseling (%)</td>
<td>20</td>
<td>0–50</td>
<td></td>
</tr>
<tr>
<td>HIV transmission, on suppressive HAART, per person-year</td>
<td>0.0037</td>
<td>0.001–0.02</td>
<td></td>
</tr>
<tr>
<td>Reduction in transmission resulting from nonsuppressive HAART (%)</td>
<td>20</td>
<td>0–70</td>
<td></td>
</tr>
</tbody>
</table>

(continued on next page)
### TABLE 1. (continued) Input Parameters for the HIV Retesting Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base-Case Assumption</th>
<th>Sensitivity Analysis Range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Costs (2011 US dollars)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT cost ($ per person per test)</td>
<td>8.17</td>
<td>2-50</td>
<td>62</td>
</tr>
<tr>
<td>First-line therapy ($/person/year)</td>
<td>560</td>
<td>50-1000</td>
<td>61</td>
</tr>
<tr>
<td>Second-line therapy ($/person/year)</td>
<td>752</td>
<td>200-2000</td>
<td>61</td>
</tr>
<tr>
<td>Laboratory costs ($ per person per year)</td>
<td>254</td>
<td>50-600</td>
<td>63</td>
</tr>
<tr>
<td>Cost for treatment of opportunistic infections ($ per person per year)</td>
<td>519</td>
<td>190-1500</td>
<td>63</td>
</tr>
<tr>
<td>Quality-of-life values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quality-of-life value, HIV-uninfected persons</td>
<td>1</td>
<td></td>
<td>Assumed</td>
</tr>
<tr>
<td>Quality-of-life value, HIV-infected persons, by CD4 count</td>
<td>&gt;350: 0.94</td>
<td>&gt;350: 0.70-0.98</td>
<td>64</td>
</tr>
<tr>
<td>200-349: 0.82</td>
<td>200-349: 0.50-0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200: 0.70</td>
<td>&lt;200: 0.30-0.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Rise in CD4 occurs gradually over a period of 2 years, and treatment failure or death can prevent maximum rise from being reached. 
†The testing frequency of every 4 years is actually every 4.29 years (derived from seven equal intervals over 30 years). 
‡For further explanation on the role of acute HIV transmission in the model, see Appendix, Supplemental Digital Content 1, http://links.lww.com/QAII/A144. 
§See Appendix, Supplemental Digital Content 1, http://links.lww.com/QAII/A144.

**SO, standard deviation; HAART, highly active antiretroviral treatment; HCT, HIV counseling and testing; AZT, zidovudine; 3TC, lamivudine; LPV/r, lopinavir/ritonavir.**

### TABLE 2. Base-Case Results for Selected HIV Retesting Frequencies

<table>
<thead>
<tr>
<th>Selected HIV Retesting Frequencies</th>
<th>0.8% incidence</th>
<th>1.5% incidence</th>
<th>4.0% incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Months</td>
<td>1 Year</td>
<td>2 Years</td>
<td>3 Years</td>
</tr>
<tr>
<td>HCT cost per case identified ($)</td>
<td>2792</td>
<td>696</td>
<td>347</td>
</tr>
<tr>
<td>Total cost ($) per person</td>
<td>898</td>
<td>507</td>
<td>425</td>
</tr>
<tr>
<td>Percent cost from HCT (%)</td>
<td>56.8</td>
<td>24.7</td>
<td>14.1</td>
</tr>
<tr>
<td>QALYs gained per person</td>
<td>0.41</td>
<td>0.41</td>
<td>0.40</td>
</tr>
<tr>
<td>CE ratio ($/QALY)</td>
<td>2196</td>
<td>1251</td>
<td>1096</td>
</tr>
<tr>
<td>Reduction in transmission of HIV (%)</td>
<td>29.8</td>
<td>26.8</td>
<td>24.8</td>
</tr>
<tr>
<td>CE ratio ($/QALY), accounting for infections averted</td>
<td>1565</td>
<td>866</td>
<td>760</td>
</tr>
<tr>
<td>ICER ($/ΔQALY), accounting for infections averted</td>
<td>51604</td>
<td>4324</td>
<td>1958</td>
</tr>
</tbody>
</table>

*Denotes dominated testing strategy. 
HCT, HIV counseling and testing; QALY, quality-adjusted life-year; CE, cost-effectiveness; ICER, incremental cost-effectiveness ratio; Δ$/ΔQALY, change in cost in US dollars/ change in QALYs gained. 
All cost and benefits have been discounted at a rate of 3% per year. ICERs are compared with the next most frequent testing interval, some of which are not shown in the table. 
(Testing every 6 months, 4 years, 6 years, and 15 years are not shown.)
Africa. Costs for healthcare facilities overhead, salaries of healthcare workers, and costs to the patient for time spent obtaining care are not explicitly included in the model, although significantly higher costs for HAART—where overhead costs can be implicit—were explored in the sensitivity analysis.

Quality-of-Life Estimates

The quality-of-life value for HIV-uninfected persons was assumed to be 1. Quality-of-life values (Table 1) for an HIV-infected individual were assumed to be dependent on CD4 counts: CD4 <200, 200-349, and ≥350 cells/mm³ with values of 0.70, 0.82, and 0.94, respectively. Table 1 shows the base-case and sensitivity analysis values used.

Secondary Transmission of HIV

Differential transmission rates were modeled for the acute, subacute (2–9 months following infection), chronic, and AIDS phases. Because it was assumed that a test for HIV is 100% sensitive and specific, it was also assumed that any diagnosis of HIV occurs after the acute phase. Combined with the mortality estimates for untreated and undiagnosed HIV disease, the base-case transmission rates, shown in Table 1, result in an undiscounted lifetime average of 0.94 infections per HIV-infected person per lifetime. Rates of HIV transmission were assumed to decline by 20% in the base-case scenario (range, 0–50% in sensitivity analysis) if an individual is aware of his or her HIV-infected status, a conservative estimate based on several studies in sub-Saharan Africa and the United States.

Sensitivity Analysis

Comprehensive sensitivity analyses for each of the three incidence scenarios evaluated the effect of alternative assumptions for the model input parameters. For each variation of a single input parameter, the most cost-effective testing strategy was identified and compared with that of the base-case scenario. The sensitivity of the primary outcome of cost per QALY to a 1% change in each input parameter was also evaluated. The sensitivity of the results to downstream infections prevented due to testing and treatment in the primary cohort was studied by taking into account tertiary in addition to secondary infections averted.

### RESULTS

#### Base-Case Scenario

In low-risk environments, the most cost-effective testing frequency was testing every 7.5 years (Table 2). The total cost per QALY gained from this testing frequency was $998. When cost savings and QALYs gained from preventing secondary HIV infections were taken into account, the overall cost per QALY gained was $701. For testing every 7.5 years, the total cost per HIV-infected case identified was $2030. Of the total cost, 4.5%, 68.1%, and 27.3% were from HCT costs, HAART costs, and laboratory costs, respectively.

### TABLE 3. Effect of Changes in Input Parameters on Most Cost-effective Testing Interval for HIV Incidence of 0.8%

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable Low End</th>
<th>Most Cost-effective Testing Frequency</th>
<th>Variable High End</th>
<th>Elasticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years with HIV incidence</td>
<td>7.5 yrs</td>
<td>-</td>
<td>-</td>
<td>N/A</td>
</tr>
<tr>
<td>Average CD4 decline/year, untreated</td>
<td>22 cells/µL/yr</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Time to detect virologic failure</td>
<td>3 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reduction in transmission resulting from diagnosis</td>
<td>0%</td>
<td>15</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>HCT cost per tester</td>
<td>$2</td>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>First-line HAART cost, per patient-year</td>
<td>$30</td>
<td>30</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Second-line HAART cost, per patient-year</td>
<td>$200</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Linkage from diagnosis to care*</td>
<td>30%+</td>
<td>30</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Mortality rate, on effective HAART</td>
<td>Half base</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Failure rates on first-line HAART*</td>
<td>1%+</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Failure rates on second-line HAART*</td>
<td>1%+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HIV transmission rates, no treatment (reproductive number)</td>
<td>R0 = 0.5</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>HIV transmissions, on HAART (per person-year)</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quality-of-life values†</td>
<td>Small differential</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Maximal rise in CD4 count resulting from suppressive HAART</td>
<td>Half base</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Length of follow-up after Year 30 (base = 15)</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>40 years</td>
</tr>
</tbody>
</table>

*The center column represents the base case, represented with a dash. Outer columns represent the variable low and high values, whereas the columns between the base case and outer extremes represent an intermediate value. Dashes indicate that the most cost-effective frequency is the same as for the base case; otherwise, the frequency is indicated in years. Secondary infections averted were accounted for in the comparison of testing frequencies.

*Linkage to care starts at 50% linked to care in the first year after diagnosis and 3% each year thereafter. The failure rates from HAART range from 1% failure each year to 40% failure during the first year with 15% failing each year thereafter.

†Quality-of-life differentials refer to the magnitude of the difference between the values for the highest CD4 strata (greater than 350) and lowest (less than 200).

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### TABLE 4. Effect of Changes in Input Parameters on Most Cost-effective Testing Interval for HIV Incidence of 1.3%

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable Low End</th>
<th>Most Cost-Effective Testing Frequency</th>
<th>Base Case 5 Years</th>
<th>Variable High End</th>
<th>Elasticity $ \dagger $</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years with HIV incidence</td>
<td>5 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average CD4 decline/year, untreated</td>
<td>22 cells/µL/yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to detect virologic failure</td>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction in transmission resulting from diagnosis</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT cost, per tester</td>
<td>$2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-line HAART cost, per patient-year</td>
<td>$50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-line HAART cost, per patient-year</td>
<td>$200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linkage from diagnosis to care*</td>
<td>30%+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality rate, on effective HAART</td>
<td>Half base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure rates on first-line HAART*</td>
<td>1%+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure rates on second-line HAART*</td>
<td>1%+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV transmission rates, no treatment (reproductive number)</td>
<td>$R_0 = 0.5$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV transmissions, on HAART (per person-year)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quality-of-life value†</td>
<td>Small differential</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal rise in CD4 count resulting from suppressive HAART</td>
<td>Half base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of follow-up after Year 30 (base = 15 years)</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The center column represents the base case, represented with a dash. Outer columns represent the variable low and high values, whereas the columns between the base case and outer extremes represent an intermediate value. Dashes indicate that the most cost-effective frequency is the same as for the base case; otherwise, the frequency is indicated in years. Secondary infections averted were accounted for in the comparison of testing frequencies.

$ \dagger $ The elasticity refers to the ratio of a percent change in the cost-effectiveness ratio (with secondary infections accounted for) to a corresponding percent change in the parameter.

HCT, HIV counseling and testing; HAART, highly active antiretroviral treatment; N/A, not applicable.

### TABLE 5. Effect of Changes in Input Parameters on Most Cost-effective Testing Interval for HIV Incidence of 4.0%

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable Low End</th>
<th>Most Cost-Effective Testing Frequency</th>
<th>Base Case 2 Years</th>
<th>Variable High End</th>
<th>Elasticity $ \dagger $</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years with HIV incidence</td>
<td>2 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average CD4 decline/year, untreated</td>
<td>22 cells/µL/yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to detect virologic failure</td>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction in transmission resulting from diagnosis</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT cost, per tester</td>
<td>$2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-line HAART cost, per patient-year</td>
<td>$50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-line HAART cost, per patient-year</td>
<td>$200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linkage from diagnosis to care*</td>
<td>30%+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality rate, on effective HAART</td>
<td>Half base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure rates on first-line HAART*</td>
<td>1%+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure rates on second-line HAART*</td>
<td>1%+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV transmission rates, no treatment (reproductive number)</td>
<td>$R_0 = 0.5$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV transmissions, on HAART (per person-year)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quality-of-life value†</td>
<td>Small differential</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal rise in CD4 count resulting from suppressive HAART</td>
<td>Half base</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of follow-up after Year 30 (base = 15 years)</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The center column represents the base case, represented with a dash. Outer columns represent the variable low and high values, whereas the columns between the base case and outer extremes represent an intermediate value. Dashes indicate that the most cost-effective frequency is the same as for the base case; otherwise, the frequency is indicated in years. Secondary infections averted were accounted for in the comparison of testing frequencies.

$ \dagger $ The elasticity refers to the ratio of a percent change in the cost-effectiveness ratio (with secondary infections accounted for) to a corresponding percent change in the parameter.

HCT, HIV counseling and testing; HAART, highly active antiretroviral treatment; N/A, not applicable.
In a medium-risk environment, the most cost-effective testing frequency was every 9 years with a total cost per QALY gained of $977. Factoring in benefits derived from transmission reductions resulted in testing every 5 years being most cost-effective with a total cost per QALY gained of $681 (Table 2). The cost per HIV-infected case identified for this testing frequency was $2123. Of the intervention cost, 4.0%, 68.5%, and 27.4% were from HCT costs, HAART costs, and laboratory costs, respectively.

In a high-risk environment, testing every 5 years was most cost-effective with a cost per QALY of $942. Including secondary infections averted into the analysis resulted in testing every 2 years being the most cost-effective strategy with a total cost per QALY gained of $635. For this frequency, cost per HIV-infected case identified was $2325 with 3.2%, 69.2%, and 27.6% of the total cost from HCT costs, HAART costs, and laboratory costs, respectively. Annual testing and testing every 6 months resulted in incremental cost-effectiveness ratios of $833/QALY and $1899/QALY gained, respectively, when compared with the next least effective strategy and when benefits from secondary infections averted are accounted for.

Without testing, counseling, diagnosis, or treatment, the average number of undiscounted secondary infections per HIV-infected individual is 0.94 for the base-case scenarios. Values for reproductive numbers greater than 1.0 were assessed in the sensitivity analysis. Reductions in rates of HIV transmission resulting from testing, counseling, and treatment ranged from 5.4% (testing once after 30 years, 4.0% incidence) to 26.3% (testing every 3 months, 0.8% incidence). The percent reduction in transmission of HIV for each testing scenario is shown in Table 2.

**Sensitivity Analysis**

Tables 3 through 5 display the effects of varying the input parameters, at one a time, on the most cost-effective testing frequency taking into account benefits from secondary infections averted. For the low, medium, and high HIV-risk scenarios, the variations studied in the sensitivity analysis produced ranges of every 3 to 30 years, every 2 to 15 years, and every 6 months to 7.5 years, respectively, as the most cost-effective testing frequencies. For no scenario evaluated in the sensitivity analysis was testing every 3 months the most cost-effective frequency. The greatest variation was produced by varying assumptions about HCT cost, annual declines in CD4 counts for untreated HIV, and rates of HIV transmission; decreasing HCT costs, faster CD4 count decline, and greater reductions in HIV transmissions from diagnosis and treatment favored more frequent testing. Importantly, although some variations of parameters did not change which frequency was most cost-effective, all variations affected the cost per QALY gained from each testing strategy. When the cost savings and benefits due to tertiary infections averted in addition to secondary infections averted were factored into the analysis using base-case parameters, the most cost-effective testing intervals shortened further (data not shown).

**DISCUSSION**

Using a mathematical model, we compared alternative retesting strategies for HIV with best estimates for input parameters from sub-Saharan Africa. Expectedly, the most cost-effective testing frequency depended on the risk environment, with higher risk indicating more frequent testing.

Our sensitivity analysis shows that the most cost-effective strategy can vary substantially with changes in the input parameters. HCT cost, assumptions about the effect of a seropositive diagnosis on HIV transmission, and the cost of first-line HAART had the greatest effects across risk settings; rates of linkage to care, rates of CD4 count decline for untreated HIV, assumptions about quality-of-life values, rates of HIV transmission for untreated HIV, and cost of second-line HAART altered the optimal testing strategy for some risk scenarios.

The effect of the cost of HAART on Tables 3 through 5 merits further attention. Although HAART and laboratory costs are the primary drivers of overall cost in most scenarios considered, the percentage of total cost from HCT is what most influences the relative cost-effectiveness of the testing strategies. Lower treatment costs result in HCT costs comprising a greater percentage of the total intervention cost, approaching 67% for some testing strategies (data not shown), which creates a bias against frequent testing despite overall lower cost per QALY gained. For example, testing once after 30 years is the most cost-effective strategy for low-risk settings when first-line HAART is set at $50 per patient-year (Table 3), yet for this variation, even annual testing costs less per QALY gained than the most cost-effective strategy in the base case.

The results for the high-risk scenario approximate the recommendation for annual testing for high-risk individuals recently released from the World Health Organization, particularly if the cost of HCT per tester can be minimized and certainly in settings where the epidemic is rapidly growing, where including tertiary infections averted into the analysis is reasonable. The World Health Organization guidelines also discourage retesting for individuals who have no new exposure after a seronegative HIV test. However, knowing that no new exposure occurred may be difficult in the setting of a generalized epidemic, particularly for married women. In such circumstances, our results suggest that even populations of lower risk would benefit from continuing to retest for HIV.

Aside from uncertainties introduced by the input parameters, our model has several structural limitations and could be extended in several ways. Behavior change associated with HCT for seronegative testers, for which there is mixed evidence, would alter our cost-effectiveness estimates. Including a background of ongoing symptom-based case identification or exposure-related self-initiated testing at interim time points would affect the cost-effectiveness of the strategies as would allowing a mechanism for those who are lost to follow-up to later return to care. Treatment side effects and development of resistant strains that alter the effectiveness of available HAART options are not currently modeled. False-positive and false-negative test results are not taken into account, which would gain importance at more frequent testing intervals. Although studies have shown that CD4 counts at seroconversion vary with age, sex, and exposure group, and that rates of CD4 decline vary significantly with HIV-1 subtype, these factors were not included in the
REFERENCES


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Cost-Effectiveness of HIV Retesting


5.6. Changes in HIV risk behavior and seroincidence among clients presenting for repeat HIV counseling and testing in Moshi, Tanzania


CONTRIBUTION

My position as last author reflects my role as senior, supervising author on the paper. I conceived the research idea, assisted with seeking and obtaining funding, and provided on-site, day-to-day leadership of all aspects of the research including data collection, design of the analysis, and write up. I mentored Ms. Fiorillo as we conducted the analyses and wrote the paper together. I also mentored the medical students, Ms. Landman and Tribble from inception to completion of the project. They participated in data collection and data management. Mtalo and Itemba ensured that data collection was integrated with HIV counseling and testing service delivery, and contributed to the development of the research question. Ostermann and Thielman provided feedback on the analysis. All authors contributed to revisions of the manuscript.

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Changes in HIV risk behavior and seroincidence among clients presenting for repeat HIV counseling and testing in Moshi, Tanzania

Suzanne P. Fiorillo, Keren Z. Landman, Alison C. Tribble, Antipas Mtalo, Dafrosa K. Itemba, Jan Ostermann, Nathan M. Thielsman, and John A. Crump

Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, NC, United States; Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKUKI Women Against AIDS in Kilimanjaro), Moshi, Tanzania; Center for Health Policy, Duke University, Durham, NC, USA; Duke Global Health Institute, Duke University, Durham, NC, USA; Department of Pathology, Duke University Medical Center, Durham, NC, USA; Kilimanjaro Christian Medical Centre, Moshi, Tanzania; Kilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania

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Introduction

HIV Counseling and Testing (HCT) has been promoted as an HIV prevention strategy in sub-Saharan Africa. By focusing on individualized counseling using knowledge of HIV status, HCT aims to motivate people to change behaviors in order to prevent HIV transmission (Luo, 2000). With increased access to antiretroviral therapy (ART) in countries with generalized HIV epidemics, HCT also provides a means to identify HIV infection early (Voluntary HIV-1 Counseling and Testing Efficacy Study Group, 2000) and to facilitate entry into treatment programs (Van de Perre, 2000). However, for treatment programs to be sustainable there must be reduction in HIV incidence (Stover et al., 2006).

Although HCT has been considered a preventive measure against HIV infection in sub-Saharan Africa, behavior changes following HCT are not completely understood. With the scale-up of access to ART and testing services, more people are taking advantage of HCT with many returning for repeat testing. Studies in developed countries have found that returning HCT clients are often at the highest risk for HIV infection and have not reduced their sexual risk behavior in response to counseling and testing (Fernyak, Page-Shafer, Kellogg, McFarland, & Katz, 2002; MacKellar et al., 2002; Norton, Elford, Sherr, Miller, & Johnson, 1997; Phillips et al., 1995). Furthermore, there is some evidence that clients may actually be more likely to acquire HIV soon after receiving the results of a seronegative HIV test (MacKellar et al., 2002). Studies in sub-Saharan Africa have shown reduced risky behavior, such as reduction of the number of partners and increases in condom use, are most pronounced...
among testers who are HIV-seropositive or who are in serodiscordant partnerships. However, there has been little evidence to suggest that HCT outside these groups have successfully changed HIV risk behavior (Allen et al., 1992; Arthur et al., 2007; Cremin et al., 2010; Denison, O’Reilly, Schmid, Kennedy, & Sweat, 2008; Kamenga et al., 1991; Sherr et al., 2007, Turner et al., 2009; Weinhardt, Carey, Johnson, & Bickham, 1999). Several studies have examined behavioral trends among seronegative testers and some have found increased risk behavior over time in this population (Cremin et al., 2010; Matovu et al., 2007; Sherr et al., 2007).

The purpose of this study was to evaluate the sociodemographic characteristics, HIV risk behaviors and reasons for testing in a cohort of men and women presenting at a HCT center in Moshi, Tanzania. Since a large proportion of this cohort returned for recommended repeat testing (The United Republic of Tanzania Ministry of Health National AIDS Control Programme, 2005) we evaluated differences between these clients and clients who tested only once. We also studied returning HCT clients who previously tested HIV-seronegative and had reported history of sexual activity to evaluate changes in sexual risk behavior. HIV seroincidence rate was determined among repeat testers.

Methods

Study location

Participants were recruited at a freestanding HCT center operated by Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI; Women Against AIDS in Kilimanjaro). KIWAKKUKI, founded in 1990, is a women-led organization providing home-based care, counseling, education, and HCT services in the Kilimanjaro Region. Initially, KIWAKKUKI was the main provider of HCT services, although other providers began testing services before the study ended. Clients were initially charged 1000 Tanzanian shillings (US $0.95; 2003 exchange rate), but testing became free in May 2004 (Thielman et al., 2006). This was followed in September 2004 by expanding access to free ART provided through the Tanzanian government (Shorter et al., 2009). KIWAKKUKI saw an average of 13 clients each weekday (Thielman et al., 2006). Data collection was between November 2003 and January 2008.

HIV counseling and testing procedures

Consecutive clients 18 years and older presenting for HCT were invited to participate. Although the refusal rate was not consistently reported during the study period when measured it was always <5%. Presenting clients received confidential pretest counseling, including risk assessment and reduction planning with a trained, Tanzanian, Kiswahili-speaking counselor. Counseling and testing was done according to the Tanzanian Ministry of Health National AIDS Control Programme guidelines (The United Republic of Tanzania Ministry of Health National AIDS Control Programme, 2005). After informed consent was obtained, counselors administered a structured questionnaire. The questionnaire obtained data on sociodemographic characteristics, reasons for testing, HIV risk behavior, HIV testing history, and planned behavior changes after testing (Thielman et al., 2006). Client response was recorded on paper by the counselor.

After pretest counseling and questionnaire completion, a blood sample was obtained for HIV testing. HIV antibody testing was performed on whole blood using Capillus (Trinity Biotech, Bray, Wicklow, Ireland) and Determine (Abbott Laboratories, Abbott Park, IL, USA) rapid HIV antibody tests. If test results were discordant, a blood sample was tested with ELISA (Vironostika Uni-Form II plus O Ab, BioMerieux, Durham, NC, USA). If the ELISA was seronegative, no additional testing was done. If the ELISA was positive, a Western blot (Genetic Systems HIV-1 Western Blot kit, Bio-Rad, Hercules, CA, USA) was done to confirm the result (Mayhood et al., 2003). Clients received HIV results in approximately 30 minutes. Clients who tested seropositive were referred to care and treatment centers and encouraged to have sexual partners and children tested. If seronegative, clients received post-test counseling focusing on HIV prevention. Clients were encouraged to return for repeat testing three and six months after the initial test according to national guidelines.

Data collection

We identified “repeat testers” as those who were seen at KIWAKKUKI at least twice during the study period and tested HIV seronegative at their first test. If repeat testers tested more than two times, data from their first and second tests were used. “One-time testers” were identified as those who presented at KIWAKKUKI once during the study period and reported not having previously tested at KIWAKKUKI or any other testing center.

Estimation of HIV seroincidence was determined among repeat testers. An HIV seroconverter was defined as a repeat tester who had a seronegative initial HIV test and a seropositive HIV test at the second visit. The period of observation was the
interval between tests. Seroincidence is reported as number of infections per 100 person-years (PY).

Statistical analysis

Paper questionnaire data and HIV results were entered using Epi Info 2002 or Epi Info 3.3 (Center for Disease Control and Prevention, Atlanta, GA, USA) or Teleform 9.0 (Cardiff, Visa, CA, USA). Data were validated by randomly sampling 10% of the questionnaires, with an acceptable error rate of <1 error per 5 forms. During the study, the questionnaire was modified five times to improve data quality. Consequently, some behavior variables were not collected for all clients. Actual missing data due to client non-response was <0.5% for each variable.

For determining differences between repeat testers and one-time testers, data on sociodemographic information, reasons for HIV testing and HIV risk behaviors from the first test of repeat testers were compared to the same data from one-time testers. HIV risk behaviors changes and planned behavior changes were compared between first and second HCT encounters among repeat testers. Repeat testing clients' intended changes in risk behaviors reported at the first test were compared to risk behaviors reported at the second test. A stratified analysis using Mantel-Haenszel chi-square test (Mantel and Haenszel, 1959) was undertaken in order to adjust for potential confounding. The p-values were based on two-tailed test results and a p-value ≤ 0.05 was used to define statistical significance. All analyses were performed using SAS, version 9.2 (SAS Institute, Cary, NC, USA).

Research ethics

Ethical approval was granted by Kilimanjaro Christian Medical Centre Research Ethics Committee, Tanzania National Institutes for Medical Research Ethics Coordinating Committee, and an institutional review board of Duke University. All participants provided informed consent and were given the written consent document in Kiswahili.

Results

Characteristics

During the study period (4 years; 2 months), 6727 clients presented one or more times at KUWAKUKI for a total of 8682 HCT encounters. Among clients, 5345 (79.5%) had one HCT encounter and 1382 (20.5%) had two or more encounters. The median number of encounters was 1 (range 1–6). At the first HCT, 1235 (18.4%) clients were HIV seropositive. Women were significantly more likely to test HIV seropositive than men (OR 3.15; 95% CI 2.74, 3.63). The median age was 29.7 (range 18.0–87.3) years, 3712 (55.3%) were women and 3108 (47.2%) lived in urban Moshi (population 144,336) whereas the rest lived in rural villages (population 1,236,713) (United Republic of Tanzania National Bureau of Statistics, 2002) in the Kilimanjaro Region. Among clients, 1382 were identified as "repeat testers" and 4272 were identified as "one-time testers" (Table 1). Among repeat testers, 1296 (93.8%) reported at least one lifetime sexual partner.

Differences between repeat testers and one-time testers

Differences in sociodemographic characteristics, HIV risk behaviors and reasons for testing were evaluated between repeat testers and one-time testers. Repeat testers at their first test were more likely to be male, older, married, testing because of suspicion of an unfaithful partner, or having a new sexual partner (p < 0.01 for each variable). One-time testers were more likely to be students, widowed, testing due to illness, having a sexual partner who had died or having multiple partners (p < 0.02 for each variable). HIV risk behaviors and reasons for testing were adjusted for possible confounding by sex and age (Table 2).

Table 1. Baseline characteristics of all clients presenting at KIWAKKUKI Centre, Moshi, Tanzania, 2003–2008, n = 6727.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>3000 (44.7)</td>
</tr>
<tr>
<td>Women</td>
<td>3712 (55.3)</td>
</tr>
<tr>
<td>Age median (range) years</td>
<td>29.7 (18.0–87.3)</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>3108 (47.2)</td>
</tr>
<tr>
<td>Rural</td>
<td>3482 (52.8)</td>
</tr>
<tr>
<td>HIV seroprevalence</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1235 (18.4)</td>
</tr>
<tr>
<td>Men</td>
<td>292 (9.7)</td>
</tr>
<tr>
<td>Women</td>
<td>942 (25.4)</td>
</tr>
<tr>
<td>Number of HCT encounters</td>
<td></td>
</tr>
<tr>
<td>1 test only</td>
<td>5345 (79.5)</td>
</tr>
<tr>
<td>2 tests</td>
<td>833 (12.4)</td>
</tr>
<tr>
<td>3 tests</td>
<td>530 (7.9)</td>
</tr>
<tr>
<td>4 tests</td>
<td>16 (0.2)</td>
</tr>
<tr>
<td>5 tests</td>
<td>1 (&lt;0.1)</td>
</tr>
<tr>
<td>6 tests</td>
<td>2 (&lt;0.1)</td>
</tr>
<tr>
<td>One-time testers</td>
<td>4272 (63.5)</td>
</tr>
<tr>
<td>Repeat testers</td>
<td>1382 (20.5)</td>
</tr>
</tbody>
</table>

Missing data (n): 15 (gender); 18 (age); 137 (residence).

*One-time testers were HCT-naive and received HCT only once during the study period.
<table>
<thead>
<tr>
<th>Sociodemographic characteristics</th>
<th>Repeat testers (n = 1382) %</th>
<th>One time testers (n = 4272) %</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>645 (46.7)</td>
<td>1820 (42.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>Women</td>
<td>737 (53.3)</td>
<td>2441 (57.3)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 30 years</td>
<td>722 (52.2)</td>
<td>2031 (47.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>≤ 30 years</td>
<td>660 (47.8)</td>
<td>2230 (52.3)</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>620 (45.5)</td>
<td>1939 (46.9)</td>
<td>0.34</td>
</tr>
<tr>
<td>Rural</td>
<td>744 (54.5)</td>
<td>2215 (53.1)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Business</td>
<td>381 (27.6)</td>
<td>1208 (28.3)</td>
<td>0.61</td>
</tr>
<tr>
<td>Farming</td>
<td>337 (24.4)</td>
<td>896 (21.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Salaried worker</td>
<td>188 (13.6)</td>
<td>563 (13.2)</td>
<td>0.69</td>
</tr>
<tr>
<td>Skilled worker</td>
<td>136 (9.8)</td>
<td>391 (9.2)</td>
<td>0.44</td>
</tr>
<tr>
<td>Unskilled worker</td>
<td>111 (8.0)</td>
<td>283 (6.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>Student</td>
<td>92 (6.7)</td>
<td>380 (8.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Other</td>
<td>137 (9.9)</td>
<td>551 (12.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary or higher</td>
<td>420 (30.5)</td>
<td>1369 (32.2)</td>
<td>0.24</td>
</tr>
<tr>
<td>Primary</td>
<td>959 (69.5)</td>
<td>2888 (67.8)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>660 (47.8)</td>
<td>2118 (49.6)</td>
<td>0.24</td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>131 (9.5)</td>
<td>393 (9.2)</td>
<td>0.76</td>
</tr>
<tr>
<td>Cohabitating</td>
<td>99 (7.2)</td>
<td>382 (8.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Married</td>
<td>376 (27.2)</td>
<td>931 (21.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Widowed</td>
<td>116 (8.4)</td>
<td>448 (10.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Religion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catholic</td>
<td>578 (41.8)</td>
<td>1628 (38.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Muslim</td>
<td>237 (17.2)</td>
<td>902 (21.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Protestant</td>
<td>511 (37.0)</td>
<td>1555 (36.4)</td>
<td>0.70</td>
</tr>
<tr>
<td>Other</td>
<td>56 (4.0)</td>
<td>175 (4.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>Reasons for testing*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illness</td>
<td>149 (10.8)</td>
<td>937 (22.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Suspect unfaithful sexual partner</td>
<td>555 (40.2)</td>
<td>1514 (35.5)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Sexual partner died</td>
<td>114 (8.3)</td>
<td>466 (10.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>New sexual partner</td>
<td>85 (21.3)</td>
<td>245 (15.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>Multiple sexual partners</td>
<td>286 (20.7)</td>
<td>980 (23.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Premarriage</td>
<td>292 (21.1)</td>
<td>868 (20.4)</td>
<td>0.26</td>
</tr>
<tr>
<td>Preconception</td>
<td>55 (4.0)</td>
<td>167 (3.9)</td>
<td>0.79</td>
</tr>
<tr>
<td>HIV Risk Behaviors*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner died</td>
<td>160 (12.6)</td>
<td>571 (14.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>Partner(s) tested for HIV</td>
<td>227 (20.4)</td>
<td>607 (17.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Suspect partner(s) have HIV</td>
<td>109 (17.3)</td>
<td>349 (15.7)</td>
<td>0.49</td>
</tr>
<tr>
<td>Having concurrent sexual partners</td>
<td>166 (14.6)</td>
<td>471 (13.8)</td>
<td>0.70</td>
</tr>
<tr>
<td>Condom use past month</td>
<td>66 (14.5)</td>
<td>216 (13.8)</td>
<td>0.52</td>
</tr>
<tr>
<td>Had partner with other partners</td>
<td>367 (32.5)</td>
<td>1096 (32.1)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Missing data (n): 11 (gender); 11 (age); 116 (residence); 64 occupation; 18 (education); 18 (marital status); 12 (religion); 5 (illness); 12 (died); 13 (unfaithful partner); 18 (new partner); 14 (multiple partners); 13 (premarriage); 18 (preconception).

*All variables adjusted for sex and age.
**HIV risk behavior changes between first and second tests for repeat testers**

Compared to their first test, repeat testers at their second test were more likely to report having partners who tested for HIV, not have had concurrent sexual partners, not suspect partners are HIV-infected, not have had partners who are known to have other partners and to have used condoms in the past month \( (p < 0.04 \) for each variable). There was no difference in abstinence during the past year between tests \( (p = 0.16) \) (Table 3).

Before learning the results of their HIV test, all clients reported what behaviors they planned to change in the event of either a seropositive or a seronegative HIV test result. We compared intended behavior changes at the first HCT with behaviors reported by repeat clients at their second HCT. There were no differences in plans to change behavior between the first and second HCT for repeat testers (Table 3). However, planned intentions to change behavior at the first test were compared to the specific behavior at the second test. Clients who planned to remain abistent after the first test if receiving a seronegative test were more likely to have remained abistent by the second test compared to clients who did not plan to remain abistent (OR 2.58; \( p < 0.0001 \)). Clients who planned to use condoms after the first test if receiving a seronegative test were more likely to have used condoms by the second test compared to clients who did not plan to use condoms (OR 2.00; \( p = 0.003 \)) (Table 4).

**Table 3. Changes in HIV risk behavior from the first and second HIV tests among previously sexually active repeat HCT clients in Moshi, Tanzania, 2003–2008.**

<table>
<thead>
<tr>
<th>Behavior change/partner knowledge</th>
<th>First test ( n \ (%) )</th>
<th>Second test ( n \ (%) )</th>
<th>( p )-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partner(s) have tested for HIV</td>
<td>210 (20.7)</td>
<td>332 (32.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Do not have concurrent sexual partners</td>
<td>792 (83.3)</td>
<td>853 (89.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Have partner(s) who do not have other partners</td>
<td>510 (52.7)</td>
<td>556 (90.1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Have used condom in past month</td>
<td>622 (56.0)</td>
<td>686 (72.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Abstinent past year</td>
<td>181 (31.3)</td>
<td>203 (35.1)</td>
<td>0.16</td>
</tr>
<tr>
<td>Planned behavior change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Will remain abistent</td>
<td>826 (54.0)</td>
<td>868 (67.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>If test seropositive</td>
<td>574 (44.5)</td>
<td>583 (45.2)</td>
<td>0.66</td>
</tr>
<tr>
<td>Will use condoms</td>
<td>249 (19.3)</td>
<td>242 (18.8)</td>
<td>0.63</td>
</tr>
<tr>
<td>If test seropositive</td>
<td>263 (30.4)</td>
<td>262 (20.3)</td>
<td>0.88</td>
</tr>
<tr>
<td>Will reduce partners</td>
<td>298 (23.1)</td>
<td>325 (25.2)</td>
<td>0.21</td>
</tr>
<tr>
<td>If test seropositive</td>
<td>295 (22.9)</td>
<td>330 (25.6)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*Adjusted for sex and age.

**HIV seroincidence rate**

All repeat testers were HIV seronegative at their first test and seven repeat testers had seroconverted by their second test. The median time between the first and second tests was 94 (range 24–1920) days. Repeat testers contributed 468.43 PY of follow-up between their first and second tests. Thus, the HIV seroincidence rate among repeat testers was estimated at 1.49 infections per 100 PY (95% CI; 0.39, 2.60).

**Discussion**

This study provides evidence that initially HIV-seronegative clients presenting for repeat HCT demonstrated some reduction in risky behavior but were also more knowledgeable about their partner’s risk behaviors at the second test. Although partner’s risk behaviors are not in the control of the client, knowing the partner’s behaviors may influence the client’s perception of their risk and intention to change risky behavior. By the second HCT, repeat testers were more likely to report that partners were tested for HIV and less likely to have concurrent sexual partners. They were also more likely to not suspect a partner has HIV and know that their partner does not have other partners. There were no differences in planned behavior change between the first and second tests for abstinence, using condoms and reducing the number of partners. However, clients planning to be abstinent or use condoms at the first test were more likely to report that they...
Table 4. Intention to reduce risky behavior at first test and actual behavior change at the second test among previous sexually active repeat HCT clients, 2003–2008.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio*</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planned abstinence after 1st test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had not remained abstinent prior to 2nd test</td>
<td>1.00 (ref)</td>
<td>1.00, 1.00</td>
<td></td>
</tr>
<tr>
<td>Remained abstinent prior to 2nd test</td>
<td>2.58</td>
<td>1.89, 3.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Planned condom use after 1st test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had not used condoms prior to 2nd test</td>
<td>1.00 (ref)</td>
<td>1.00, 1.00</td>
<td></td>
</tr>
<tr>
<td>Used condoms prior to 2nd test</td>
<td>2.00</td>
<td>1.28, 3.14</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Note: OR: Odds ratio, 95% CI: 95% confidence interval.
*Adjusted for sex and age.

adhered to this behavior by the second test then those who did not plan to change behavior. Together, this provides some evidence that clients who returned for recommended follow-up HCT were more likely to have changed their own behavior and were more knowledgeable about their partners risk behaviors, both which could reduce risk for HIV infection.

Previously we have described characteristics of one-time testers (Shorter et al., 2009). However, significant differences in sociodemographic characteristics and reasons for testing were observed between one-time testers and repeat testing clients. Repeat testers were more likely to be older, male, married, testing because of new sexual partner, or suspected an unfaithful partner. One-time testers were more likely to be widowed or testing because of an illness. This suggests that repeat testers may test more often after any perceived possibility of HIV exposure whereas one-time testers are more likely to test only after a singular life event, such as being widowed, triggers concern they may be HIV infected. Consistent with our previous work (Chu et al., 2005; Landman et al., 2008; Shorter et al., 2009), we also found that women were less likely to test repeatedly and had higher HIV seroprevalence then men. It was suggested that women use HCT as a point of entry for seeking care and treatment and are more likely to present for HCT when they have an illness or are symptomatic (Shorter et al., 2009). Women may be more likely to seek HCT long after a specific exposure when they begin to experience an illness or symptoms whereas men are more likely to seek HCT sooner and more frequently after any potential exposure (Shorter et al., 2009).

We did not find differences in behavioral risk factors between repeat and one-time testers. Previous studies (Fernyak et al., 2002; MacKellar et al., 2002; Norton et al., 1997) have found that repeat testers reported more risky behavior than first time testers, although these studies were done in developed countries in different study populations. A study in Uganda (Matovu et al., 2007) also found that HIV-seronegative repeat testers were less likely to reduce their sexual risk behaviors following repeated HCT. However, HCT populations, regardless of repeat or one-time testing, have reported higher risk behaviors than in the general population (Chu et al., 2005).

Although we found differences between repeat and one-time testers, we are unable to ascertain whether the same behavior changes observed in repeat testers would have also been observed in one-time testers if we had been able to measure their behavior at a later time. These groups were similar in terms of HIV risks behaviors but it is unknown whether one-time testers also changed their behavior after receiving HCT. In this study, follow-up HCT was recommended to all clients. However, only 20% sought repeat testing. It may be suggested that clients who follow recommendations to return for testing are those who also follow counseling advice to change behavior.

Even with some reduction in risky behavior evident in repeat testers, HIV seroincidence in this cohort was 1.49 cases per 100 PY of follow-up, remaining relatively high compared to similar cohorts. HIV seroincidence among HCT cohorts, reported as cases per 100 PY of follow-up, was 1.21 in Harare, Zimbabwe (Corbett et al., 2007), 1.4 in Rakai, Uganda (Matovu et al., 2007), 1.3 in Nairobi, Kenya (Oyugi et al., 2009), and 0.69 and 1.04 among men and women, respectively, in Chiang Mai, Thailand (Kwichai et al., 2004). The high HIV seroincidence in this group suggests further efforts in prevention are still needed. However, HIV seroprevalence in this HCT cohort was 18.4%, more than twice as high as the overall HIV seroprevalence in Tanzania of 8.8% in 2005 (UNAIDS, 2004). Therefore it is likely that seroincidence rates in our study population reflect a group at higher than average risk for HIV infection that should not be extrapolated to the general population.

There are several limitations in this study. The cohort was comprised of people who self-selected for
testing likely due to their perception of higher personal risk and the results may not be generalized to the entire population. This study also relied on self-reporting of information to counselors, which could result in inaccurate estimation of behaviors due to social desirability bias. This may be more evident when clients returned for repeat testing as they were already familiar with counseling procedures and knew the socially acceptable response to questionnaires. It is also possible that some HCT clients were misclassified as one-time testers as they may have received further HCT at another testing site or after the study period ended. Finally, since one-time testers did not return for repeat testing, we do not know if they changed their behavior after receiving initial HCT.

In conclusion, we found that clients presenting for repeat HCT reported reducing risky behavior and improved knowledge of the sexual practices and HIV serostatus of their partners. It was promising to see that clients who planned to change their behavior after the first test were more likely to report that they adhered to this change at the second test. Unfortunately, clients who did not plan to change their behavior after the first test, did not report behavior change at the second test. Therefore, a goal of HCT should be to provide counseling to help all clients make their own decisions to successfully change their behavior, specifically targeting clients who may not feel behavior change is necessary. Women represent an important target group for HCT efforts and continued reinforcement is needed to engage them in HIV education before they are sexually active and their risk increases. The high HIV seroincidence rate in this and other cohorts of repeat HCT clients in sub-Saharan Africa, despite repeatedly receiving HIV education, suggests a need to aggressively tailor education and prevention interventions for this particular high risk group. Finally, the impact of HCT on HIV prevention among HIV-seronegative clients in sub-Saharan Africa requires further study and the effect of repeat testing beyond the initial repeat test needs to be explored as recommendations for regular retesting are rolled out (Waters et al., 2011). The continued assessment of HIV knowledge and promotion of behavior change is essential.

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References


5.7. Evaluation of the Abbott m2000rt RealTime HIV-1 assay with manual sample preparation compared with the ROCHE COBAS AmpliPrep/AMPLICOR HIV-1 MONITOR v1.5 using specimens from East Africa


CONTRIBUTION

I conceived the research idea, sought and obtained funding, designed the study, and supervised the research including overseeing participant enrollment and directing the Tanzania laboratory in which sample testing was conducted. Scott and Stevens conducted and oversaw testing at the comparison laboratory in South Africa. Msuya was the lead laboratory technologist in the Molecular Section of the Tanzania laboratory and Morrissey was laboratory supervisor. Kimaro coordinated sample collection from Tanzanian volunteers with and without HIV infection. Shao managed interactions with personnel at study sites and partner institutions. All authors contributed to revisions of the manuscript.

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Evaluation of the Abbott m2000rt RealTime™ HIV-1 assay with manual sample preparation compared with the ROCHE COBAS® AmpliPrep™/AMPLICOR™ HIV-1 MONITOR® v1.5 using specimens from East Africa

John A. Crump, John F. Shao, Wendy S. Stevens, Lesley E. Scott, Emma M. Msuya, Anne B. Morrissey, Ekyafyose E. Kimaro, Scott), Corresponding author at: Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Box 102359, Durham, NC 27710, USA.

1. Introduction

As access to antiretroviral therapy (ART) has expanded in the developing world the detection of treatment failure has become a priority (World Health Organisation, 2006a,b). While treatment failure may be defined using clinical and immunologic criteria, this approach lacks both sensitivity and specificity for detection of virologic treatment failure defined by increasing plasma HIV-1 RNA concentration (viral load). Consequently, the World Health Organization (WHO) has indicated that there is a strong argument for moving towards the wider availability of viral load testing in resource-constrained settings. In particular, the WHO suggests that simple point-of-care assays are needed (World Health Organisation, 2006a,b).

A variety of technologies have been developed and commercialized to measure HIV-1 RNA concentration, including reverse transcriptase polymerase chain reaction (RT-PCR) amplification, isothermal nucleic acid sequence-based amplification (NASBA), and branched-chain DNA signal amplification (bDNA) (Collins et al., 1997; Dyer et al., 1999; Johnson et al., 2001; Sun et al., 1998). The development of real-time (RT) PCR assays represents one step towards simpler assays for viral load monitoring. Several recent studies have shown that RT-PCR methods compare favorably with conventional assays for quantitation of HIV-1 RNA (de Mendoza et al., 2005; Rouet et al., 2005; Stevens et al., 2005; Yao et al., 2005). As in most of East Africa, HIV-1 from patients in Moshi, Tanzania, exhibit considerable subtype diversity with subtypes A, C, and...
D predominating and the frequent occurrence of HIV-1 recombinant forms (Kiwelu et al., 2003; Osmanov et al., 2002; Ramadhan et al., 2007). By contrast, most HIV-1 from patients in Europe, North and South America, and Australasia belongs to subtype B (Osmanov et al., 2002). A number of studies have shown that HIV-1 diversity can influence the reliability of RNA detection (Barlow et al., 1997; Swanson et al., 2005). Furthermore, inaccurate quantitation can have adverse consequences for patients (Geelen et al., 2003). Therefore, it is essential that as new, simpler assays for measurement of plasma HIV-1 RNA concentration are evaluated in settings where HIV-1 subtype and recombinant form diversity is substantial. The Abbott m2000rt RealTime HIV-1 assay (RealTime HIV-1) (Abbott Laboratories, Abbott Park, IL) offers the potential advantages of the reduced complexity of a real-time assay and, by targeting the pol integrase (IN) region of the HIV-1 genome, may be subject to less variability than assays targeting the gag gene (Geelen et al., 2003; Swanson et al., 2006a).

The RealTime HIV-1 Assay is an RT-PCR assay that has been evaluated against several LCX HIV RNA quantititative assay (Abbott Laboratories, Abbott Park, IL), ROCHE COBAS® System (Roche Molecular Systems, Branchburg, NJ) (Swanson et al., 2006a,b), the VERSANT HIV-1 RNA 3.0 (Bayer Diagnostics, Tarrytown, NY) (Swanson et al., 2006a, 2007), and the ROCHE AMPLICOR HIV-1 MONITOR 1.5 (Roche Molecular Systems, Branchburg, NJ) (Swanson et al., 2006a, 2007) using patient samples from Brazil (Swanson et al., 2006b), London (Garcia-Diaz et al., 2006; Swanson et al., 2006a), and assorted HIV-1 group M and O isolates (Swanson et al., 2007) and more recently against the NuclISENS HIV-1 (Xu et al., 2008). In these evaluations, the Abbott RealTime HIV-1 Assay performed well against other assays, reliably quantifying diverse HIV-1 strains, providing a wide dynamic range and good sensitivity. This study extends the evaluation of the RealTime HIV-1 assay using patient samples from East Africa where subtypes A, C and D, and HIV-1 recombinant forms predominate. Furthermore, the assay was evaluated in a resource poor setting using a simple, manual nucleic acid extraction method. In this study field data were compared directly to data generated in a national reference laboratory using the CAP/CA HIV-1 assay which is commonly used as a reference standard for HIV-1 RNA quantitation.

2. Materials and methods

2.1. Sample collection and laboratory testing sites

Participants were recruited among consecutive clients at the Kilimanjaro Christian Medical Centre (KCMM) Infectious Diseases Clinic, at a KCMM HIV voluntary counselling and testing clinic, and at the Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKUKUE: Women Against AIDS in Kilimanjaro) HIV voluntary counseling and testing clinic, all in Moshi, Tanzania. HIV antibody testing was done according to previously described methods (Mayhood et al., 2008). Forty milliliters of blood was collected from participants between November 2006 and January 2007 until the goal of 120 samples; 20 (17%) from HIV-infected persons and 100 (83%) from HIV-infected persons was reached. Whole blood was collected in tubes containing K3 EDTA. Within 4h of collection plasma was separated, divided into four aliquots each of 5 mL and frozen at −80°C. Frozen plasma was shipped to the University of Witwatersrand, Johannesburg, South Africa on dry ice. In Johannesburg, plasma was tested using the comparator assay to determine HIV-1 RNA concentration. When necessary to fulfill the requirements of the method validation plan, dilutions of nominal samples were prepared using plasma from donors not infected with HIV. The resulting samples were shipped back to the Kilimanjaro Christian Medical Centre, Moshi, Tanzania, on dry ice and were stored at −80°C before testing.

2.2. HIV-1 RNA concentration determination by RealTime HIV-1 Assay and the CAP/CA HIV-1 comparator assay

The RealTime HIV-1 Assay was performed in the Molecular Section of the Kilimanjaro Christian Medical Centre Biotechnology Laboratory, Moshi, Tanzania according to the manufacturer's specifications. RNA was manually extracted from 1 mL plasma samples using the Abbott Sample Preparation System (Abbott Laboratories, Abbott Park, IL). The Tanzania team was blinded to the results of the South Africa testing. The CAP/CA HIV-1 conventional and ultrasensitive assays were performed by the Department of Molecular Medicine and Haematology, University of Witwatersrand, Johannesburg, South Africa, according to the manufacturer's specifications. RNA was extracted from 0.7 mL plasma samples using the ROCHE COBAS® AmpliPrep™ System (Roche Molecular Systems, Branchburg, NJ). The South Africa laboratory complies with external quality assessment for viral load testing using the external quality assessment programs of both the College of American Pathologists and of the US National Institutes of Health Viral Quality Assessment program (VQA, Rush-Presbyterian-St. Luke's Medical Centre, Chicago, IL).

2.3. Method validation study plan

For within-run precision studies, three runs were performed, each containing replicates of 10 patient samples at high (>5.0 log copies/mL), medium (4.0–5.0 log copies/mL), or low (<4.0 log copies/mL) HIV-1 RNA concentration. Assay controls (low positive, high positive and negative) were included in every run in both Moshi and Johannesburg and results were only accepted if controls were in range according to the standard operating procedures for each assay. For between-run precision studies, runs each containing the same 15 patient samples and 3 control samples were repeated over 3 consecutive days. Accuracy studies used 100 samples from persons infected with HIV run in duplicate. Samples were compiled to represent the range of detection of the RealTime HIV-1 assay and the patient population in Moshi, Tanzania. For reportable range and linearity experiments, samples were selected from the collection of samples available; one with the closest value to the lower (40 copies/mL) and one with the closest value to the upper (1,000,000 copies/mL) ends of the reportable range for the Abbott m2000rt RealTime™ HIV-1 assay. In addition, four samples representing intermediate values were each run in duplicate. To verify the manufacturer's claims for the limit of blank and reference interval verification studies, plasma samples from 20 patients not infected with HIV were tested. To verify the limit of detection, 20 samples with HIV-1 RNA concentrations of <40 copies/mL were tested over 5 days. The CAP/CA HIV-1 ultrasensitive (reportable range 50–100,000 copies/mL) assay was used to measure these HIV-1 RNA concentrations. Four aliquots were tested per day for 5 days. Operational evaluations included measuring assay time for the Abbott m2000rt RealTime™ HIV-1 assay and for the CAP/CA HIV-1 assay.

2.4. Statistical analyses

For the purpose of this study, results from testing in South Africa were considered to be the reference value. Mean, median, minimum and maximum values were calculated for both assays. Correlation was used to determine the linear relationship between the assays and linear regression to quantitate this relationship. The coefficient of determination (R²) was calculated. Method comparison to determine agreement between the two assays was analyzed by Bland and Altman (1986) and percentage similarity (Scott et al., 2003). All statistical tests were two-sided and all statistical analyses were performed using SAS version 8.2 Enterprise Guide version 2.
software (SAS Institute, Inc., Cary, NC) and GraphPad Prism software version 4.02 (GraphPad Software, Inc., San Diego, CA).

2.5. Research ethics

Approval to collect patient samples for this study was granted by the Kilimanjaro Christian Medical Centre Research Ethics Committee.

3. Results

3.1. Instrument familiarization

The RealTime HIV-1 instrument was installed at the Moshis site over a period of 1 week using existing available bench space. Three laboratory technologists were trained over a subsequent period of 2 weeks and standard operating procedures were simultaneously developed. Two laboratory technologists each performed successfully three independent training runs before beginning the validation study. One laboratory technologist performed all validation study assays.

3.2. Within-run precision studies

The within-run precision experiment results compared well with the manufacturers’ claims. The mean, range, standard deviation and coefficient of variation (CV) of the CAP/CA HIV-1 and of the RealTime HIV-1 assay are shown in Table 1. Values for the CAP/CA HIV-1 assay were also within the limits of the manufacturer’s claims. The manufacturer’s claim for the RealTime HIV-1 assay is much tighter and showed some values, especially at log 5 copies/mL, with more variability or less precision relative to these claims (Table 1). Assay run control values were within limits for both assays. All negative samples gave negative values on all assay runs.

3.3. Between-run precision studies

All samples with continuous values yielded detectable values and no HIV-1 RNA was detected from any negative samples. After combining the results of between-run studies for each CAP/CA HIV-1 log HIV-1 RNA concentration interval, standard deviations were calculated for the RealTime HIV-1 method and compared with the manufacturer’s claims. For CAP/CA HIV-1 log HIV-1 RNA concentration interval 5.0 the RealTime HIV-1 standard deviation (and manufacturer’s SD claim) from repeat tests was 0.072 (0.08); for log 4.0 was 0.128 (0.06); for log 3.0 was 0.147 (0.10); and for log 2.0 was 0.07 (0.09). Total variability for CAP/CA HIV-1 log HIV-1 RNA concentration interval 5.0 was 0.192 (0.10); for 4.0 was 0.158 (0.14); and for 3.0 was 0.207 (0.13).

3.4. Accuracy studies

Of 100 patient samples, 39 (39%) were determined to have HIV-1 RNA concentrations <400 copies/mL on the Roche assay. Of the 39 samples in the first Moshis Abbott run, 24 (62%) were not detected, 12 (31%) were detected <40 copies/mL, and 3 (8%) were detected >40 copies/mL. On the second Moshis Abbott run, the only result that was discordant with the first was that one more sample was detected <40 copies/mL and one less at >40 copies/mL. All methods at all sites showed 100% concordance for discrete values irrespective of the range. Of the 100 patient samples, the remaining 61 (61%) yielded continuous values above 400 copies/mL. Of these 61 samples, the mean (minimum, maximum) log HIV-1 RNA concentration for the CAP/CA HIV-1 method was 4.63 (2.09, 6.83). The mean from the first Moshis RealTime HIV-1 run was 4.39 log copies/mL (1.67, 7.00) and 4.34 log copies/mL (1.60, 7.00) for the second run. The Bland-Altman difference plot for these data shows that the RealTime HIV-1 assay produced higher values than the CAP/CA in the low HIV-1 RNA concentration samples, but lower values on the higher HIV-1 RNA samples (Fig. 1). Assay controls passed for each run, with low and high positives within their acceptable ranges and all negative controls yielded negative results on both assays.

3.5. Reportable range and linearity experiments

Linearity was determined using known samples at intervals from 2.5 to 7.0 log HIV-1 RNA concentration. HIV-1 RNA levels were established using the CAP/CA HIV-1 assay. The equation of the line for the mean of the two RealTime HIV-1 assay runs was y = -1.09 + 1.21x (intercept p = 0.110); evaluation of correlation with nominal samples yielded an R² of 0.9684. HIV-1 copy number detected by the RealTime HIV-1 assay is displayed a linear relationship to the known viral concentration over the dynamic range of 2.5–7.0 log10 copies/mL (Fig. 2).

Table 1

<table>
<thead>
<tr>
<th>Sample run number</th>
<th>ROCHE COBAS® AMPLICOR™ HIV-1 MONITOR® v1.5</th>
<th>Abbott m2000rt RealTime™ HIV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=10)</td>
<td>5.51</td>
<td>5.07 (4.84, 5.22)</td>
</tr>
<tr>
<td>Mean</td>
<td>5.51</td>
<td>5.07</td>
</tr>
<tr>
<td>Range (low, high)</td>
<td>(5.43, 5.59)</td>
<td>(4.84, 5.22)</td>
</tr>
<tr>
<td>SD (manufacturer’s claim)</td>
<td>0.04 (0.1)</td>
<td>0.12 (0.04)</td>
</tr>
<tr>
<td>CV</td>
<td>0.71%</td>
<td>2.27%</td>
</tr>
<tr>
<td>2 (n=10)</td>
<td>4.32</td>
<td>4.20 (4.16, 4.24)</td>
</tr>
<tr>
<td>Mean</td>
<td>4.32</td>
<td>4.20</td>
</tr>
<tr>
<td>Range (low, high)</td>
<td>(4.19, 4.38)</td>
<td>(4.16, 4.24)</td>
</tr>
<tr>
<td>SD (manufacturer’s claim)</td>
<td>0.08 (0.14)</td>
<td>0.03 (0.02)</td>
</tr>
<tr>
<td>CV</td>
<td>1.76%</td>
<td>0.66%</td>
</tr>
<tr>
<td>3 (n=10)</td>
<td>3.07</td>
<td>3.29</td>
</tr>
<tr>
<td>Mean</td>
<td>3.07</td>
<td>3.29</td>
</tr>
<tr>
<td>Range (low, high)</td>
<td>(2.92, 3.31)</td>
<td>(3.15, 3.38)</td>
</tr>
<tr>
<td>SD (manufacturer’s claim)</td>
<td>0.13 (0.17)</td>
<td>0.06 (0.05)</td>
</tr>
<tr>
<td>CV</td>
<td>4.01%</td>
<td>1.85%</td>
</tr>
</tbody>
</table>

* Log transformed HIV-1 RNA values are reported.

* Standard deviation (manufacturer’s claims for total assay variability).
218-222

The Abbott HIV-1 assay showed excellent linearity between 2.5 and 7.0 log copies/mL with an $R^2$ value of 0.9684. Of negative samples, 100% showed negative results, and >95% of samples at 40 copies/mL were detected.

The manual extraction method and differences between real-time and traditional PCR technologies may contribute to increased variability, as may shipping of samples between Tanzania and South Africa for the validation study. A further contributor to variability may exist due to the differences in detection methodologies; the CAP/CA assay uses endpoint detection whereas the RealTime HIV-1 assay uses real-time detection. The RealTime HIV-1 assay used a larger input sample volume than the CAP/CA HIV-1 assay which may have contributed further to performance differences. Based on published comparisons of the RealTime HIV-1 with other assays, it is likely that use of the Abbott m2000sp automated sample preparation instrument would reduce the level of total variability observed in this study (Swanson et al., 2006a, 2007). This question will be addressed through a study currently ongoing in Tanzania.

It was possible to install the RealTime HIV-1 instrument at a site in East Africa in 1 week and to successfully train staff over 2 weeks with subsequent independent training runs. The small footprint of the Abbott m2000r allowed the use of a small amount of existing bench space and manual sample preparation used a similarly small area in another part of the laboratory. Despite the use of manual sample preparation, the RealTime HIV-1 assay with manual sample preparation performed favorably to the CAP/CA HIV-1 assay. The lower capital investment and lower complexity of manual sample preparation may make this an attractive option for other laboratories in resource-constrained settings with low to intermediate sample numbers of ≤21 per day (Fiscus et al., 2006).

The RealTime HIV-1 assay offers a number of potential advantages over the CAP/CA HIV-1 assay and other conventional assays. First, the overall turnaround time of real-time assays are shorter than for conventional PCR methods. This improves the likelihood that results will be available to clinicians when patients return to clinic, an important consideration in settings where patients travel long distances to receive care. The wider dynamic range of the RealTime HIV-1 assay covers the dynamic range of the CAP/CA and conventional assays, obviating the need to have more than one assay, and improving cost-effectiveness and work flow. The liquid plasma sample remains a short coming of both real-time and conventional PCR assays and this places constraints on access to the test for patients living in rural and remote areas. The evaluation of more durable dry blood spot samples for viral load monitoring provides a potential solution to this problem and has been the subject of past and ongoing research (Garrido et al., 2009; Leelawiwat et al., 2009).

5. Conclusions

These data suggest that the RealTime HIV-1 assay with manual sample preparation is an acceptable and feasible alternative to the conventional ROCHE COBAS AmpliPrep/AMPLICOR HIV-1 MONITOR v1.5 assay and that the RealTime HIV-1 assay performs well on samples from patients in East Africa. It is likely that manual sample preparation contributes to higher total assay variability, but this is offset by the lower cost and complexity of manual sample preparation. Further research is needed to compare the RealTime HIV-1 assay with automated sample preparation with non-subtype B samples and evaluating dry blood spot samples as an alternative to liquid plasma samples.

Conflict of interest

None declared.
Acknowledgements

The authors thank the staff and patients of the Kilimanjaro Christian Medical Centre Infectious Diseases Clinic and the staff and clients of Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI; Women Against AIDS in Kilimanjaro) for their participation. The authors thank Julitha Kimbi, Devotaha Lyimo, and Editha Mushii for assistance with sample collection. This research was supported by the Center for HIV/AIDS Vaccine Immunology, a United States National Institutes of Health (NIH) funded program (U01 AI067854). Authors received additional support from NIH awards International Studies of AIDS–associated Co-infections (ISAAc) (AI 062563 JAC, ABM), and the Duke Clinical Trials Unit and Clinical Research Sites (U01 AIO69484-01 JAC). The authors are grateful to Abbott Laboratories for donating reagents for sample testing in Moshi. This publication was made possible by support from the US Agency for International Development (USAID). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the US government. The authors acknowledge the National Health Laboratory Service PCR Laboratory staff in the Department of Molecular Medicine and Haematology for performing the routine analysis on the CAP/CA HIV-1.

References


5.8. Abbott RealTime HIV-1 m2000rt viral load testing: manual extraction versus the automated m2000sp extraction


CONTRIBUTION

I conceived the research idea, sought and obtained funding, designed the study, and supervised the research including overseeing participant enrollment and directing the Tanzania laboratory in which sample testing was conducted. Scott, Venter, and Stevens conducted and oversaw testing at the comparison laboratory in South Africa. Msuya was the lead laboratory technologist in the Molecular Section of the Tanzania laboratory and Morrissey was laboratory supervisor. All authors contributed to revisions of the manuscript.

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Short communication

Abbott RealTime HIV-1 m2000rt viral load testing: Manual extraction versus the automated m2000sp extraction

Lesley E. Scott, John A. Crump, Emma Msuya, Anne B. Morrissey, Willem F. Venter, Wendy S. Stevens

* University of the Witwatersrand, Department of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Science, 7 York Rd Parktown, Johannesburg 2000, South Africa
† National Health Laboratory Services, Johannesburg 2000, South Africa
‡ Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Box 102359, Durham, NC 27710, USA
§ Duke Global Health Institute, Duke University, Box 90519, Durham, NC 27708, USA
¶ Kilimanjaro Christian Medical Centre, PO Box 2240, Moshi, Tanzania
∥ Kilimanjaro Christian Medical College, Tumaini University, PO Box 2240, Moshi, Tanzania
** Reproductive Health and HIV Research Unit, University of Witwatersrand, Johannesburg, South Africa

A B S T R A C T

The Abbott RealTime HIV-1 assay is a real-time nucleic acid amplification assay available for HIV-1 viral load quantitation. The assay has a platform for automated extraction of viral RNA from plasma or dried blood spot samples, and an amplification platform with real time fluorescent detection. Overall, this study found no clinically relevant differences in viral load, if samples were extracted manually.

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HIV viral load testing is used for monitoring treatment, determining prognosis and risk of disease progression, and for ascertaining treatment failure (Yilmaz, 2001). The newer laboratory viral load assays are based on real time detection and can detect viral load values as low as 20 copies/ml (Scott et al., 2009a). The FDA approved Abbott RealTime HIV-1 assay uses the automated extraction and detection m2000 system platform (Abbott Molecular Inc., Des Plaines, IL, USA). This assay targets the pol/JIN region of HIV-1 using partially double-stranded probes (Huang et al., 2007) with a dynamic range of 40–1.0E+7 copies/ml. This fully automated system has a reporting capacity of 93 patient results per an 8-h day and has been fully validated against other real time assays (Scott et al., 2009b). Laboratories also have the option of performing manually the front end extraction for reasons such as lower sample throughput (21 reported patient results per 8-h day), the m2000sp purchase cost, lack of space for the m2000sp or a desire to minimise the use of complex equipment requiring service and maintenance (Crump et al., 2009).

This study therefore investigates any differences in viral load values between samples processed manually and using the m2000sp automated platform. K3 EDTA blood samples were recruited from 147 participants with written informed consent in Moshi, Tanzania (approval obtained from the Kilimanjaro Christian Medical Centre (KCMC) Research Ethics Committee approval number 156). Forty milliliters of blood was collected from each participant and the plasma separated within 4 h. This was divided into four aliquots of 5 ml each, and frozen at −80 °C. Frozen plasma was shipped to the University of the Witwatersrand, Johannesburg, South Africa, on dry ice, where replicates and dilutions for another method validation plan were prepared (Crump et al., 2009) and then shipped back to the KCMC in Moshi, Tanzania, on dry ice. Storage at both sites was at −80 °C before testing. In Johannesburg, the 1 ml plasma extraction protocol (including additional for the ‘dead’ volume) was performed on neat samples using the automated m2000sp platform according to the manufacturer’s instructions. In Moshi, samples were extracted manually from 1 ml plasma samples using the CE marked Abbott Sample Preparation System (Abbott Laboratories, Abbott Park, IL), as per the manufacturer’s instructions. Extracted samples from both methods were amplified and detected on the m2000rt platforms at each site according to the manufacturer’s instructions. Agreement in viral load values between the different extraction methods was measured using the Bland–Altman and percentage similarity (Bland and Altman, 1995; Scott et al., 2003).
The total 147 samples (neat plasma tested at both sites) yielded 62 samples that were quantified by both extraction methods. The remaining 85 were either <40 copies/ml (n = 12 (14.1%)) or 'target not detected' by both methods of extraction. Manual extraction yielded two additional quantifiable samples (46 copies/ml and 83 copies/ml) that were <40 copies/ml after automated extraction, and therefore there was 97.7% concordance between the two extraction methods. The mean viral load, for the automated extraction samples yielding quantifiable results, was log 4.44 copies/ml (25,118 copies/ml) with a range of log 2.0 (100 copies/ml) to log 7.0 copies/ml (10 million copies/ml). The mean difference between the automated and the manual extraction values was log 0.0015 copies/ml (confidence interval of the mean difference -0.076; 0.079), with a standard deviation of this difference being log 0.306 copies/ml. The limits of agreement (0.536; -0.688 copies/ml) and the mean percentage similarity (100.4% with 4.0% standard deviation and 4.0% percentage similarity CV) showed good overall agreement between the methods of extraction. The Bland–Altman plot (Fig. 1) shows no values >log 1.0 difference and therefore no clinically relevant outliers. Seven (11.3%) values had differences in viral load values >log 0.5 copies/ml and generate higher values after automated extraction, and similarly viral load values <log 4.0 copies/ml generate higher values after manual extraction. This observation may also explain why manual extraction could quantify an additional two samples in the lower range than automated extraction, but this would have to be confirmed on a larger sample size.

In summary, samples extracted manually or by the automated protocol on the m2000sp platform for further quantification on the Abbott RealTime HIV-1, show no clinically relevant differences in viral load values. Although the manual extraction was performed here by a single skilled technical laboratory operator, inter-operator variability may occur with manual sample preparation, but this variability should be minimised if operators adhere to strict laboratory standard operating procedures. The 11.3% of samples that had viral load values >log 0.5 copies/ml indicate that samples with low viral load values are more readily quantified after manual extraction and that samples with high viral load values are more readily quantified after automated extraction. The automated extraction protocol offers the advantages of requiring fewer operational staff for a higher throughput of specimens and less chance for contamination (although contamination was not found to be an issue in this study). Manual extraction has the advantage of requiring less laboratory space, requiring less complex and expensive instrumentation, yet still generates reliable real time viral load results. Although potentially any RNA extraction method with sufficient yield and clean RNA product could be used for sample processing, the authors chose the Abbott Sample Preparation System since its CE marking ensures consistent, site-independent assay performance. Both automated and manual extraction methods provide accurate and reliable viral load results for patient management.

Acknowledgments

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and the Duke Clinical Trials Unit and Clinical Research Sites (U01 AI069484-01, JAC). The authors are grateful to Abbott Laboratories for donating reagents for sample testing in Moshi and to Dr. Lawrence Phillips from Abbott Molecular for assistance in addressing queries around manual extraction methods.

References


5.9. Evaluation of a dried blood spot HIV-1 RNA program for early infant diagnosis and viral load monitoring at rural and remote health care facilities


CONTRIBUTION

My position as last author reflects my role as senior, supervising author in the paper. I conceived the research idea, sought and obtained funding, designed the study, and supervised all aspects of the research including mentorship of my student doctoral student Ms. Sarah Lofgren. Ms. Lofgren oversaw day-to-day operations of the study, did the preliminary statistical analysis, and wrote the first draft of the manuscript. Morrissey, Chevallier, Amos, Sifuna, Stevens, and Crump led laboratory aspects of the research. Malabeja and von Seidlein coordinated research and administrative activities at the Korogwe site and Edmonds at the Muheza site. Schimana and Bartlett participated in study design and assisted with integration of the research with clinical and programmatic activities locally and nationally. All co-authors contributed to revision of the manuscript.

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Evaluation of a dried blood spot HIV-1 RNA program for early infant diagnosis and viral load monitoring at rural and remote healthcare facilities

Sarah M. Lofgren, Anne B. Morrissey, Caroline C. Chevallier, Anangisye I. Malabeja, Sally Edmonds, Ben Amos, David J. Sifuna, Lorenz von Seidleing, Werner Schimanac, Wendy S. Stevens, John A. Bartlett, and John A. Crump

Objective: To assess technical and operational performance of a dried blood spot (DBS)-based HIV-1 RNA service for remote healthcare facilities in a low-income country.

Design: A method comparison and operational evaluation of DBS RNA against conventional tests for early infant diagnosis of HIV and HIV RNA quantitation under field conditions in Tanzania.

Methods: DBSs were prepared and plasma was frozen at −80°C. DBSs were mailed and plasma couriered to a central laboratory for testing using the Abbott m2000 system. Infant diagnosis DBSs were also tested for HIV-1 DNA by ROCHE COBAS AmpliPrep/COBAS TaqMan System. Results of DBS RNA were compared with conventional tests; program performance was described.

Results: Among 176 infant diagnosis participants, using a threshold of at least 1000 copies/ml, sensitivity and specificity of DBS versus plasma RNA were 1.00 and 0.99, and of DBS RNA versus DBS DNA were 0.97 and 1.00. Among 137 viral load monitoring participants, when plasma and DBS RNA were compared, r value was 0.9709; r value was 0.9675 for at least 5000 copies/ml but was 0.7301 for less than 5000 copies/ml. The highest plasma RNA value at which DBS RNA was not detected was 2084 copies/ml. Median (range) turnaround time from sample collection to result receipt at sites was 23 (4–69) days. The Tanzania mail service successfully transmitted all DBS and results between sites and the central laboratory.

Conclusion: Under program conditions in Tanzania, DBS provided HIV-1 RNA results comparable to conventional methods to remote healthcare facilities. DBS RNA testing is an alternative to liquid plasma for HIV-1 RNA services in remote areas.

AIDS 2009, 23:2459-2466

Keywords: blood specimen collection, diagnosis, HIV, laboratory techniques and procedures, reverse transcriptase PCR, Tanzania
**Introduction**

In 2007, an estimated 33 million people were living with HIV globally [1]. Access to antiretroviral therapy (ART) has increased markedly since 2004, particularly in low-income and middle-income countries. In order to initiate life-saving ART early, HIV care and treatment programs in resource-constrained settings are increasing efforts to diagnose HIV infection among infants using nucleic acid amplification testing (NAT) [2,3]. Furthermore, the recognition that virologic failure among patients receiving ART is poorly predicted by clinical and immunologic monitoring [4,5] has led to growing interest in expansion of NAT services for monitoring plasma HIV-1 RNA levels to patients receiving ART in low-income and middle-income countries [3,4].

Conventional HIV NAT has relied on liquid plasma samples. In many resource-constrained settings, NAT services are scarce and are highly centralized. Because of centralization of testing and that liquid plasma samples must be assayed within 6 h or frozen to −80°C to avoid deterioration, HIV NAT has been available only to persons able to travel to reference centers or to clinics with a robust cold chain. Dried blood spots (DBS) represent an alternative sample type to liquid plasma that are easy to prepare, robust, and that do not require a cold chain [6,7]. HIV-1 DNA PCR of DBS (DBS DNA) compares favorably with HIV-1 DNA PCR of liquid plasma (plasma DNA) for early infant diagnosis of HIV [8]. Furthermore, HIV-1 RNA PCR of plasma samples (plasma RNA) has been shown to be a valid test for early infant diagnosis of HIV infection [9–11]. Similarly, several studies have evaluated DBS HIV-1 RNA (DBS RNA) for measurement of HIV-1 RNA concentration [12] and for early infant diagnosis [13,14] under laboratory conditions. Taken together, these findings suggest that it might be possible to establish a DBS RNA service suited to the needs of remote health facilities that uses a single platform in centralized laboratories that provide simultaneous early infant diagnosis of HIV with baseline HIV-1 RNA measurement as well as quantitation of HIV-1 RNA levels for monitoring patients on ART.

In order to investigate the feasibility of a DBS RNA service for early infant diagnosis and for HIV-1 RNA concentration monitoring under field conditions in a resource-constrained setting, we studied the technical and operational performance of a DBS RNA program in Tanzania in partnership with two rural and remote healthcare facilities. The assessment included a method comparison of DBS RNA against conventional assays following transportation of DBS by mail, an evaluation of DBS sample stability over time, and operations research that monitored the reliability of the mail service for delivering DBS and that measured turnaround times.

**Methods**

**Study sites**

Participants were recruited among HIV care and treatment clinic attendees and pediatric inpatients at two rural healthcare facilities, Magunga Hospital in Korogwe District and Teule Hospital in Muheza District. These hospitals are located in northeastern Tanzania approximately 300 and 350 km, respectively, from the Kilimanjaro Christian Medical Centre (KCMC) Biotechnology Laboratory in Moshi, Tanzania, which served as the central laboratory. The climatic conditions in this area have been described elsewhere [15]. ART has been available free through the national care and treatment program since 2004, starting at consultant referral hospitals, and subsequently decentralized to regional and then district hospital level. DBS RNA and plasma RNA testing were performed at the central laboratory, and DBS DNA testing was done at the University of Witwatersrand, Parktown, South Africa.

**Participant selection**

Infants aged less than 18 months old who were HIV-1 exposed or were clinically suspected to have HIV infection were eligible for enrollment in part A. HIV-infected persons of at least 18 months were eligible for enrollment in part B. After obtaining informed consent, patients were administered a standardized questionnaire that assessed demographic, epidemiologic, clinical, and treatment information.

**Sample collection, preparation, and transport**

Five to 10 ml of blood was drawn by venipuncture and collected in EDTA tubes. To prepare DBS, 50 μl whole blood aliquots were spotted onto Guthrie 903 filter paper cards and (Munktell GmbH, Bärenstein, Germany) air-dried for at least 4 h on a drying rack on the laboratory bench. Up to 10 DBS were prepared per individual for testing and to provide surplus spots in case of losses during mailing or testing. After drying, the cards were placed in a gas-impermeable zip-locked bag with desiccant and stored in a safe location at an ambient temperature. The DBS were mailed weekly from the two rural hospital sites to the central laboratory using a mail service of the Tanzania Posts Corporation. The remaining EDTA blood was separated at 6 h, or less after collection by centrifugation, the plasma frozen at −80°C, and transported weekly on dry ice to the central laboratory via courier.

**Sample testing and result reporting**

Upon receipt at the KCMC Biotechnology Laboratory, DBS were cut from the 903 cards using a 16 mm diameter card cutter. Two DBS were transferred to a 50ml conical tube with 1.7 ml of lysis buffer (Abbott m Sample Preparation System buffer; Abbott Laboratories, Abbott Park, Illinois, USA). These tubes were incubated at room temperature for 2 h with inter-
mittent mixing; 1.0 ml of the resulting solution was assayed for HIV-1 RNA by the Abbott m2000 system (Abbott Laboratories), using the HIV-1 RNA DBS quantitative protocol. A 0.6 ml aliquot of liquid plasma from each patient was tested using the Abbott m2000 system and results were compared.

DBS from part A were also sent to the University of Witwatersrand, South Africa, under ambient conditions for HIV-1 DNA testing. Each spot was transferred to a 1.8 ml S-tube, and 1100 µl of COBAS AmpliPrep/COBAS TaqMan specimen preextraction reagent (Roche Diagnostics, Indianapolis, Indiana, USA) was added. The tubes were incubated in an Eppendorf Thermomixer comfort (Eppendorf AG, Hamburg, Germany) at 56°C and 1000 rpm continuous shaking for 10 min; at least 1 ml of the resulting solution was assayed for HIV-1 DNA using the ROCHE COBAS AmpliPrep/COBAS TaqMan System (Roche Diagnostics) [13]. The quality of results of the Tanzania Abbott m2000 assay and the South Africa ROCHE COBAS AmpliPrep/COBAS TaqMan was assured by successful participation in the AIDS Clinical Trials Group Viral Quality Assurance program.

To assess the stability of DBS samples under field conditions, DBS from 32 patients enrolled in part B were stored for approximately 10 weeks before retesting. The baseline HIV-1 RNA level was defined as the first DBS RNA measurement at 40 days, or less from sample collection. The follow-up level was defined as the second DBS RNA measurement taken 41–80 days following collection. DBS were sealed in gas-impermeable shipment bags with desiccant, placed in plastic shipping envelopes, and were grouped by received date. DBS were kept in the central laboratory, which is temperature monitored and maintained between 18 and 25°C.

Assessment of program performance
To assess operational performance of the DBS program under field conditions in Tanzania, we monitored samples and results transported by mail, measuring the duration of transit times and the proportion of samples damaged or lost. Turnaround times were calculated using time from sample collection to shipping, time in transit, time in the central laboratory prior to testing, and time from sample testing to result shipment. Assay performance was tracked by documenting the number and underlying causes of run failures. The cost of sample transport was recorded.

Statistical analysis
The technical performance of the DBS RNA method was compared against conventional plasma RNA and against DBS DNA for early infant diagnosis. Methods were compared with linearity plot and Bland–Altman difference plot. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were estimated along with 95% confidence intervals (CIs). Plasma RNA thresholds examined for early infant diagnosis were at least 1000 copies/ml [16] and the American Academy of Pediatrics (AAP) threshold of at least 10 000 copies/ml [11]. Plasma RNA thresholds examined for HIV-1 RNA quantitation were at least 400 copies/ml and the 2004 National Antiretroviral Treatment Guidelines of South Africa threshold of at least 5000 copies/ml [17]. Stability was assessed by comparing the differences in log HIV-1 RNA levels between baseline and follow-up DBS. Results were entered into Microsoft Access (Microsoft Corporation, Redmond, Washington, USA) database using Teleform (Verity, Inc., Sunnyvale, California, USA). Analysis was done using Microsoft Excel (Microsoft Corporation), EP Editor (David G. Rhoads Associates, Kennett Square, Pennsylvania, USA), and JMP software (SAS worldwide, Cary North Carolina, USA).

Research ethics
This study was approved by the KCMC Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an Institutional Review Board of Duke University Medical Center.

Results
Of 375 participants enrolled from 29 October 2008 to 6 March 2009, 313 (83.5%) had samples available for analysis. Of potential participants approached, there were no refusals. The remaining samples were unavailable owing to hemolysis, insufficient volume, collection outside the study period, failure to meet enrollment criteria, or were lost owing to assay errors (Fig. 1). Of the 313 participants, 176 (56%) were enrolled into part A. The Magunga Hospital site enrolled 133 (42%) of all participants.

Part A: early infant diagnosis
Among part A participants, the median (range) age was 6 months (1 day–17 months) and 82 (46%) were female; 101 (38%) were enrolled because they were HIV exposed, five (3%) were enrolled because of clinical suspicion for HIV, and 70 (40%) met both criteria.

In the early infant diagnosis group, 39 (22%) participants had detectable plasma RNA compared with 35 (20%) by DBS RNA. At the threshold of at least 1000 copies/ml, 34 (19%) infants were classified as infected by plasma RNA compared with 35 (20%) by DBS RNA. At the threshold of at least 10 000 copies/ml, 31 (18%) infants were classified as infected by plasma RNA compared with 31 (18%) for DBS RNA. In one participant aged 17 months with a plasma RNA level of 53 copies/ml, no HIV RNA was detected from DBS, whereas DBS HIV
DNA was detected. Excluding this sample, there was a complete concordance between DBS RNA and DBS DNA at the DBS RNA threshold of at least 1000 copies/ml. Of 36 samples positive by DBS DNA, four (11.1%) had DBS RNA levels of 1000–10,000 copies/ml from patients aged 0, 1, 7, and 15 months. There were also three patients aged 10, 13, and 14 months, negative by DBS DNA, with DBS RNA not detected and plasma RNA levels of 70, less than 40, and 237 copies/ml, respectively. None was on ART.

Comparing plasma RNA with DBS RNA at the threshold of at least 1000 copies/ml, estimated sensitivity and specificity (95% CI) were 1.00 (0.90–1.00) and 0.99 (0.96–1.00), and were 1.00 (0.89–1.00) and 1.00 (0.97–1.00) at the threshold of at least 10,000 copies/ml. Comparing DBS RNA with DBS DNA at the threshold of at least 1000 copies/ml, estimated sensitivity and specificity (95% CI) were 0.97 (0.86–1.00) and 1.00 (0.97–1.00), respectively, and were 0.86 (0.71–0.94) and 1.00 (0.97–1.00), respectively, at the threshold of at least 10,000 copies/ml (Table 1).

Part B: viral load monitoring

Among part B participants, the median (range) age was 34 years (21 months–77 years) and 108 (79%) were female. Of the 137 patients, 110 (80%) had CD4-positive T-lymphocyte counts (CD4 cell count) performed and the median (range) CD4 cell count was 253 (6–2586) cells/μL. All 137 patients provided ART information and 73 (53%) reported receiving it. All patients were taking fixed-dose combination stavudine, lamivudine, and nevirapine.

At the threshold of at least 400 copies/ml, 82 (60%) participants were classified as having virologic failure by plasma RNA compared with 88 (64%) by DBS RNA. At the threshold of at least 5000 copies/ml, 74 (54%) participants were classified as having virologic failure by plasma RNA compared with 76 (55%) by DBS RNA. Compared with plasma RNA, estimated sensitivity and specificity (95% CI) for classifying patients with virologic failure of DBS RNA at the threshold of at least 40 copies/ml was 0.99 (0.93–1.00) and 0.87 (0.76–0.94), respectively, and at the threshold of at least 5000 copies/ml was 1.00 (0.95–1.00) and 0.97 (0.89–0.99), respectively (Table 2).

The relationship between DBS RNA and plasma RNA levels is shown in Fig. 2. Using data from both parts A and B of the study, the r value produced was 0.9709; r value was 0.9675 for at least 5000 copies/ml but was 0.7301 for less than 5000 copies/ml. Twenty-five (8.0%) samples had HIV-1 RNA detected in plasma but not on DBS; 24 of these had plasma HIV-1 RNA concentrations below 400 copies/ml. The highest plasma HIV-1 RNA level at which DBS RNA was not detected was 2084 copies/ml. A Bland–Altman difference plot comparing Abbott m2000 system plasma and DBS RNA showed a range of difference between DBS RNA and plasma RNA levels for detectable results of 1.60–0.62 log copies/ml and 97% of difference values were within 2 logs of zero (Fig. 3).

Stability testing

Thirty-two samples were re-tested to assess stability of D3S RNA results during approximately 10 weeks of storage. Baseline DBS RNA testing was done at a median (range) of 21 (9–37) days after sample collection, whereas follow-up testing was done at 68 (42–76) days following sample collection. There was no statistically significant change in log RNA levels between the baseline and follow-up time points.

Program performance

All 28 packages containing DBS, 14 (50%) from each study site, were received at the central laboratory. During the course of the study, 29 packages were sent to the sites, seven with supplies, two with case report forms and consent forms needing clarification, and 20 with plasma RNA results. All documents and samples reached their destination, and none was damaged.

The interval from sample collection to shipping from the sites to the KCMC Biotechnology was 0–7 days. Of 20 packages sampled, the median (range) transit time from sites to the KCMC Biotechnology Laboratory was 1.5 (1–2) days. The interval from receipt of a patient care sample to completion of HIV RNA testing at the KCMC Biotechnology Laboratory was median (range) 13 (1–51) days. When this was calculated excluding a vacation period during which plasma collection was suspended and the laboratory was operating with reduced staffing levels, the median (range) interval from sample receipt to
Table 1. Classification of infant HIV infection comparing dried blood spot HIV-1 RNA results with HIV-1 plasma RNA results at the threshold of at least 1000 copies/ml (a) and at least 10 000 copies/ml (b) and comparing dried blood spot HIV-1 RNA results with dried blood spot HIV-1 DNA results at the threshold of at least 10 000 copies/ml (c) and at least 10 000 copies/ml (d), Tanzania, 2008–2009.

<table>
<thead>
<tr>
<th>(a)</th>
<th>Plasma RNA</th>
<th>(b)</th>
<th>Plasma RNA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>≥ 1000</td>
<td>&lt; 1000</td>
<td>Total</td>
</tr>
<tr>
<td>DBS RNA &lt; 1000</td>
<td>0</td>
<td>143</td>
<td>143</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>142</td>
<td>176</td>
</tr>
</tbody>
</table>

SN 1.00 (0.90, 1.00), SP 0.99 (0.96, 1.00)  
PPV 0.97, NPV 1.00  

(c) | DBS DNA | (d) | DBS DNA |
<table>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>DBS RNA &lt; 1000</td>
<td>35</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>140</td>
<td>176</td>
</tr>
</tbody>
</table>

SN 0.97 (0.66, 1.00), SP 1.00 (0.97, 1.00)  
PPV 1.00, NPV 0.99  

SN 0.96 (0.71, 0.94), SP 1.00 (0.97, 1.00)  
PPV 1.00, NPV 0.97  

DBS, dried blood spot; NPV, negative predictive value; PPV, positive predictive value; SN, sensitivity; SP, specificity. 95% confidence intervals are shown in brackets.

The median (range) transit time from sample receipt to the Biotechnology Laboratory was estimated to be 1.5 (1−2) days. The interval from sample receipt to completion of testing was 7 (1−21) days. The interval from testing to shipping of results to sites was 0−7 days, and the median (range) transit time from the KCMC Biotechnology Laboratory to the site was 23 days overall and was 17 days excluding the vacation period, ranging from 4 to 69 days or from 4 to 39 days excluding the vacation period. The interval from sample receipt to completion of testing in the Biotechnology Laboratory contributed the most to total turnaround time. Factors contributing to delays in the KCMC Biotechnology Laboratory or loss of samples included three plasma run failures; two runs failed during the sample preparation, one because of a problem with the automated liquid handler, and one owing to a power outage. The third run failed at the amplification and detection stage. DBS RNA samples were lost owing to six runs with errors: three with problems during sample preparation resulting in loss of the whole run, two with out-of-range controls, and one with an inadequate sample volume detected in 50% of samples because of technician error.

At the time of the study, the weekly cost of mailing DBS from healthcare facilities to the central laboratory was US $6, whereas the weekly ground transport of frozen plasma samples on dry ice costs US $515.

Table 2. Classification of virologic failure comparing plasma HIV-1 RNA results with dried blood spot HIV-1 RNA results at the threshold of at least 400 copies/ml (a) and at least 5000 copies/ml (b), Tanzania 2008–2009.

<table>
<thead>
<tr>
<th>(a)</th>
<th>Plasma</th>
<th>(b)</th>
<th>Plasma</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>≥ 400</td>
<td>&lt; 400</td>
<td>Total</td>
</tr>
<tr>
<td>DBS RNA &lt; 400</td>
<td>1</td>
<td>48</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>55</td>
<td>137</td>
</tr>
</tbody>
</table>

SN 0.98 (0.93, 1.00), SP 0.87 (0.76, 0.94)  
PPV 0.92, NPV 0.98  

SN 1.00 (0.95, 1.00), SP 0.97 (0.89, 0.99)  
PPV 1.00, NPV 0.97  

DBS, dried blood spot; NPV, negative predictive value; PPV, positive predictive value; SN, sensitivity; SP, specificity.

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**Discussion**

We demonstrate under field conditions in Tanzania that an HIV-1 RNA DBS program performs well against liquid plasma HIV-1 RNA for viral load monitoring and against quantitative liquid plasma HIV-1 RNA and qualitative DBS DNA for early infant diagnosis. Performance was best for samples from patients with plasma RNA levels above 5000 copies/ml. DBS RNA levels appear to be stable after approximately 10 weeks of storage compared with a baseline sample tested at a median of 21 days following collection. Furthermore, we show that a DBS program performs well using existing staff at remote healthcare facilities, a robust central laboratory, and that using the local mail service for DBS transport is reliable and less expensive than ground transportation of frozen plasma on dry ice.

For early infant diagnosis, our study showed an agreement between DBS RNA and both plasma RNA and DBS DNA using an RNA threshold of at least 1000 copies/ml. Although we were able to test only one sample rather than the two recommended by the AAP, several participating samples positive by DBS DNA would have been classified as HIV uninfected using the AAP threshold of at least 10,000 copies/ml [11]. As untreated HIV-infected infants usually have high HIV RNA levels, it is unclear why four patients positive by DBS DNA had low HIV RNA levels, that is, between 1000 and 10,000 copies/ml. Low plasma HIV RNA levels have been seen in other studies [16]. Possible explanations include that these patients are able to control HIV RNA levels [18,19], they had early acute HIV infection with low HIV RNA levels prior to viral load ramp-up [20] either following breastfeeding [21] or peripartum transmission, or that HIV-1 subtype variation resulted in an underquantitation. Additionally, cross-contamination of DBS samples at sites could cause false-positive results. The training provided to staff for this study would make cross-contamination unlikely but difficult to rule out. Other groups have shown that most infants with initial low levels of plasma RNA followed over time subsequently are confirmed to be HIV infected [14,22], but a proportion are found to be HIV uninfected [16,22–24].

Our findings on the use of DBS RNA for early infant diagnosis are consistent with those of others. A study [7] in Thailand yielded a sensitivity of 97–100% and a specificity of 100%, using DBS RNA with the ROCHE AmpliCor (Roche Diagnostics), NucliSens (Organon Teknika, Molenaarstraat, The Netherlands), and an in-house assay. A South African study [13] found sensitivity and specificity of 99.7 and 100%, respectively, using the Cobas AmpliPrep/Cobas TaqMan HIV-1 Qual test.

Compared with plasma RNA, DBS RNA was highly sensitive and specific for diagnosis of virologic failure at a threshold of at least 5000 copies/ml and retained high sensitivity but lower specificity for virologic failure at a threshold of at least 400 copies/ml. Across a range of HIV-1 RNA levels, there was an excellent agreement of at least 5000 copies/ml and a fair agreement of less than 5000 copies/ml between plasma and DBS RNA (Figs. 2 and 3). HIV-1 RNA levels from DBS tended to be higher than from plasma of less than 5000 copies/ml, and we identified 25 samples with HIV RNA not detected on DBS but with low levels of plasma RNA detected. Entrapment of RNA in filter paper and amplification of proviral DNA may contribute to these findings. Our study was consistent with others showing a disagreement and the frequency of lack of detection of DBS RNA increasing below 4000–6000 copies/ml [6,25].

Our data suggest that DBS are stable over an 80-day period under laboratory conditions. DBS RNA samples for early infant diagnosis using a qualitative assay have been shown to remain 99.2% sensitive and 100% specific on re-testing 4 years later [26]. Using a quantitative HIV-1 RNA assay, DBS RNA concentration was shown stable at 9 months when stored at a range of temperatures [7], and in another study stable over 1 year if stored at room
temperature or −70°C [6]. Thus, DBS RNA samples appear to be stable over a duration that exceeds the period of clinical value of the result.

We demonstrate that a DBS RNA program performs well under field conditions in Tanzania. The local mail service rapidly and reliably transported DBS samples to the central laboratory and plasma RNA results to the clinical sites. The program achieved a median turnaround time from sample collection to receipt of the result at the remote site of 23 days. As many follow-up visits in HIV Care and Treatment programs in Tanzania are scheduled monthly, most results were available to the clinician at the next follow-up visit. Some results took longer, leading to an inconvenience for clinicians and for patients. The main contributor to total turnaround time was the interval from sample receipt to testing at the central laboratory. Laboratory turnaround time could be shortened by an improved maintenance of back-up electricity infrastructure, increased technical support, and greater experience of laboratory staff with the instrument and with DBS. Total turnaround time could be further reduced by using electronic or telephone transmission of results. Despite the total turnaround time, all RNA results were deemed to be clinically useful for patient management.

A DBS RNA service with centralized testing could reach a very large population requiring or receiving ART. In 2007, it was estimated that 136,000 people were receiving ART in Tanzania [27]. The WHO recommends that, where available, HIV-1 RNA testing should be offered to patients receiving ART. With annual testing, given that the assay evaluated in this study can process 93 patient samples per run, and assuming that an instrument at a central laboratory completes one run per day 365 days a year, then 26,242 DBS samples could be processed per year. If two runs were done per day then 52,484 DBS RNA tests could be done per year. These test volumes would cover 39% of Tanzanians receiving ART in 2007.

Our study had a number of limitations. The remote healthcare facilities in this study had active research programs and capacities, which may not be found in all rural and remote areas. The sites were served by a reliable and rapid mail service, which may not be available everywhere. Our stability testing data are limited by a small sample size and would need to be verified with large numbers of samples. A number of samples were lost with assay errors and could not be included in the final analysis, possibly resulting in bias. Finally, because the study used patient samples, the range of HIV-1 RNA levels may not have thoroughly evaluated the entire dynamic range of the assay.

We demonstrate that a DBS HIV RNA program serving rural and remote healthcare settings is feasible in Tanzania both in terms of the technical quality of the results and the operation of the program. We suggest that DBS are a viable alternative to a plasma sample type within RNA programs, and DBS RNA services could be scaled up to a national level. Having established the feasibility of such a program technically and operationally, we suggest that detailed cost-effectiveness analyses should be conducted to allow Ministries of Health to determine costs and benefits of such a program. Larger studies that evaluate DBS program performance at nonresearch sites and that incorporate the impact of HIV-1 RNA results on patient management are needed.

Acknowledgements

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J.A.C., W.S., and J.A.B. conceived the study, and J.A.C. and J.A.B. obtained funding. All authors contributed to the study design, implementation, and manuscript writing; S.M.L., A.I.M., and S.E. contributed to the daily follow-up of study participants. A.B.M., C.C.C., B.A., and D.J.S. coordinated laboratory aspects of the study. S.M.L. and J.A.C. did the statistical analyses and prepared the first draft of the manuscript.

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11. Read JS. Diagnosis of HIV-1 infection in children younger than 18 months in the United States. Pediatrics 2007; 120; e1547-e1562.


CONTRIBUTION

My position as last author reflects my role as senior, supervising author on the paper. Thielman and I conceived the research idea in discussion with Kiwera, Kaale, and Mtweve. We sought and obtained funding, designed the study, and supervised the research including the study team members responsible for participant enrollment, data collection, data analysis, and write up. We mentored the medical students, Ms. Tillekeratne and Ms. Chu. Kiwera, Kaale, and Mtweve managed interactions between the research team and the HIV community home based care program. Morpeth and Ostermann assisted with data analysis. Shao and Bartlett managed interactions with personnel at study sites and partner institutions as assisted with obtaining funding. All authors contributed to revisions of the manuscript.

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Morbidity and mortality among a cohort of HIV-infected adults in a programme for community home-based care, in the Kilimanjaro Region of Tanzania (2003–2005)


* Duke University Medical Center, Box 3867, Durham, NC 27710, U.S.A.
† Duke Global Health Institute, Box 90519, Durham, NC 27708, U.S.A.
‡ Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI; Women Against AIDS in Kilimanjaro), P.O. Box 567, Moshi, Tanzania
§ Terry Sanford Institute of Public Policy, Duke University, Box 90239, Durham, NC 27708, U.S.A.
¶ Kilimanjaro Christian Medical College, Tumaini University, P.O. Box 3010, Moshi, Tanzania
** Kilimanjaro Christian Medical Centre, P.O. Box 3010, Moshi, Tanzania

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Community home-based care (CHBC) plays an integral role in the care of HIV-infected patients living in resource-limited regions. A longitudinal cohort study has recently been conducted, in the Kilimanjaro Region of northern Tanzania, in order to identify the components of an effective CHBC programme. Structured questionnaires were administered to clients over two census rounds, one in October 2003–February 2004 and the other in January 2005–October 2005. In the second round, follow-up interviews were completed for 226 (87.9%) of the 257 clients included in the first round. The clients included in the first round had a median (range) age of 38 (20–66) years and 182 (75.2%) of them were female. Although only 27 (12.9%) of them were using antiretroviral therapy (ART) when first interviewed, 108 (44.6%) were taking trimethoprim-sulfamethoxazole (SXT) prophylaxis. By the time of the follow-up interviews, 102 (45.1%) of the clients included in the first round had died, giving a mortality of 51/100 person-years of observation. The primary cause of death for 87 (85.3%) of the clients who had died was respiratory and/or gastrointestinal infection, and the most common contributory causes of death were malnutrition (81.4%) and anaemia (42.2%). On bivariable analysis, the following first-round conditions were found to be significantly associated with death by the second census round: weakness for >1 month (odds ratio (OR)=2.64; P=0.008); oral thrush (OR=2.31; P=0.013); painful swallowing (OR=2.02; P=0.036); staying in bed for part of the day over most of the previous month (OR=1.94; P=0.017); fever for >1 month (OR=1.95; P=0.016); and severe bacteraemia (OR=1.80; P=0.036). The high mortality was associated with advanced, symptomatic HIV disease for which antiretroviral therapy was indicated. Clients who were in the advanced stages of HIV disease (as defined by the World Health Organization's criteria) in the first census round were significantly more likely to have died by the time of the second round than the other clients investigated (log-rank χ²=8.115; P=0.044).

The high level of morbidity observed in this study, and the causes of mortality that were identified, emphasise the need for CHBC programmes to provide HIV-infected patients with improved access to basic resources such as SXT and isoniazid prophylaxis, clean water, oral rehydration therapy, and micronutrient supplementation, in addition to increased access to ART.

The current burden posed by HIV/AIDS is estimated at 33 million infected people, with two-thirds of those infected living in sub-Saharan Africa. In Tanzania, the prevalence of HIV/AIDS among adults aged 15–49

Reprint requests to: J. A. Crump, Division of Infectious Diseases and International Health, Duke University Medical Center, Box 3867, Durham, NC 27710, U.S.A. E-mail: crump017@mc.duke.edu; fax: +1 919 684 8902.

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years was estimated at 6.2% in 2007, indicating that about 1.3 million adults in the country were HIV-infected (Anon., 2008).

The current capacity of Tanzania’s health services to care for the country’s HIV-infected individuals is limited. A survey of healthcare facilities in the Northern Zone of Tanzania in 2004 (Landman et al., 2006) revealed a need for additional trained personnel, medications and laboratory capacity to care for the region’s HIV-infected patients—a challenge also illustrated by the results of a nation-wide survey in 2006 (Anon., 2007a). In government hospitals in the Kilimanjaro and Kagera Regions, 21.6% and 32.8% of inpatient beds, respectively, may already be occupied by patients living with HIV/AIDS (Kwesigabo et al., 1999; Ole-Nguyaine et al., 2004). It has been estimated that delivering antiretroviral therapy (ART) to all those in need of such treatment in Tanzania would require the full-time services of almost half of the country’s current healthcare workforce (Beckmann and Rai, 2004). With formal healthcare services overwhelmed, alternative models—such as community home-based care (CHBC), a service in which basic care is delivered to a patient’s home by trained members of the community—will become increasingly important in delivering healthcare to Tanzania’s HIV-infected population.

CHBC has already been recognised as a vital component of the care of HIV-infected populations by both the World Health Organization (WHO) and the Tanzanian government (Anon., 2001; WHO, 2002). It has the potential to provide care for large numbers of HIV-infected patients, especially in rural areas, where the majority of Tanzanians live and where prevention and care services are often lacking (Somi et al., 2006). Unfortunately, although such data are needed to determine the essential components of successful CHBC programmes, and to help structure such programmes to deliver effective care, few data exist on the current needs of the HIV-infected clients whose main care is community home-based (Bowie et al., 2006). To address this issue, a longitudinal cohort study among HIV-infected adults receiving CHBC in the Kilimanjaro Region of northern Tanzania was recently conducted. The methodology, results and conclusions of this study are detailed below.

SUBJECTS AND METHODS

Location
The Kilimanjaro Region of northern Tanzania has an estimated population of 1.4 million (Anon., 2003). At the time of the present study (2003–2005), the region was divided into the six districts of Hai, Moshi Rural, Moshi Urban, Mwanga, Rombo and Same. The subjects of the present study, who were all HIV-infected individuals (‘clients’) receiving CHBC, were surveyed through an existing AIDS-service organization in Moshi, known as Women Against AIDS in Kilimanjaro or, in the local Swahili, Kikundi cha Wanawake Kilimanjaro Kapambana na UKIMWI (KIWAKKUKI). This organization is a community-based women’s organization that provides HIV voluntary counselling and testing (VCT), CHBC for HIV-infected individuals, and education and support for people living with HIV/AIDS.

Home-based Care
Clients presenting to KIWAKKUKI are each checked for anti-HIV antibodies (Chu et al., 2005) using two commercial rapid tests: the Capillus™ HIV-1/HIV-2 (Trinity Biotech, Bray, Ireland) and the Determine™ HIV-1/2 (Abbott Laboratories, Abbott Park, IL). All clients found seropositive are offered enrollment in KIWAKKUKI’s CHBC programme. At the start of the present study, this programme provided care for approximately 800 of the estimated 98,000 HIV-infected individuals in the Kilimanjaro Region. It was maintained by
180 'providers' who each visited a median of five clients/month. A typical home visit included basic nursing care, health education, nutritional and spiritual counselling, and the provision of food and basic medications. The providers were community members who volunteered their services, and who had received training according to a Tanzania Ministry of Health curriculum. The training included an intensive, 5-day course, covering topics such as social and emotional support, basic medical care and essential medications, networking with community members, and assessing the need for referrals to formal healthcare services.

**Questionnaire Administration**

Those HIV-infected clients enrolled in the KIWAKUKUKI's CHBC programme who received home visits during the period of the organization's first census of its clients, which ran from October 2003 to February 2004, were recruited for participation in the present study. The aim of the first census round was to improve the quality of the CHBC programme, by improving understanding of the number and health status of the clients and by providing the information needed to advocate for increased resources for rural clients who could benefit from CHBC. The programme's providers administered structured questionnaires during home visits in both this first census and, as follow-up for the present study, in a second census round that ran from the January to the October of 2005. All those clients for whom identifying information was available and who were aged ≥18 years at the time of the first census round were sought for follow-up in the second round.

The questionnaires captured information on socio-demographic characteristics, self-reported medical history and symptoms, measured height and weight, and medication usage. No laboratory data were recorded as a part of this study. If, in the second census round, a client was found to have died since the first census round, a verbal-autopsy form was administered, by the CHBC provider, to a member of the client's family (Anon., 1997). The autopsy forms captured information on symptoms experienced prior to death, medical history, and the documented causes of death. Each autopsy form was reviewed by two physicians, who independently coded the primary and contributory causes of death, using a condensed list from the tenth revision of the International Classification of Diseases (Anon., 1997).

**Analysis**

Data were entered into electronic databases using the Epi Info 2004 software package (Centers for Disease Control and Prevention, Atlanta, GA). They were validated by sampling 10% of the questionnaires, with less than one error/five pages being considered acceptable. Analysis was carried out using Epi Info 2004 and the JMP IN 2005 software package (SAS Institute, Cary, NC). Limited clinical staging was performed using the following criteria (WHO, 2005): weight loss and herpes zoster (stage 2); diarrhoea for >1 month, fever for >1 month, oral thrush, pulmonary tuberculosis within the previous year, and severe bacterial infections (stage 3); and oral or genital ulcers for >1 month, presumed to result from infection with *Herpes simplex* virus (stage 4). These clinical criteria were recorded by the CHBC providers on the basis of the clients' self-reported clinical histories (during both census rounds), with the corresponding staging assigned during the data analysis. The level of mortality recorded between the census rounds was expressed as deaths/100 person-years of observation. Survival curves were constructed using the Kaplan–Meier method and compared using the log-rank test. Bivariable analysis was performed to determine which of the first-round characteristics of the clients were significantly associated with mortality by the second census round.
A *P*-value of <0.05 was considered indicative of a statistically significant difference.

**Research Ethics**

Ethical approval for the study was obtained from the Kilimanjaro Christian Medical Centre's Research Ethics Committee, an Institutional Review Board of Duke University Medical Center, and the Tanzania National Institute of Medical Research's National Medical Research Coordinating Committee. All clients included in the second census round gave their written informed consent for use of the data collected on them in both census rounds.

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**RESULTS**

Overall, 378 CHBC clients were surveyed during the initial census round, which was carried out as an internal quality-improvement activity. Although age eligibility (≥18 years at the time of the first round) and the availability of identifying information allowed follow-up to be attempted, in the second census round, on 257 clients, only 226 (88%) of these were successfully followed up. The 31 clients lost to follow-up had all reportedly moved out of the study area between the two census rounds. The median time elapsed between the interviews in the two census rounds was 16.6 months (range=12.1–24.6 months).

**Demographic Characteristics**

The socio-demographic characteristics recorded, in the first census round, for the 257 clients for whom follow-up was attempted are summarized in Table 1. All districts of the Kilimanjaro Region were represented. Most (75.2%) of the clients for

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<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NO. AND (%) OF CLIENTS:</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>182 (75.2)</td>
</tr>
<tr>
<td>District of residence</td>
<td></td>
</tr>
<tr>
<td>Hai</td>
<td>50 (19.5)</td>
</tr>
<tr>
<td>Moshi Rural</td>
<td>41 (16.0)</td>
</tr>
<tr>
<td>Moshi Urban</td>
<td>46 (17.9)</td>
</tr>
<tr>
<td>Mwanga</td>
<td>17 (6.6)</td>
</tr>
<tr>
<td>Rombo</td>
<td>42 (16.3)</td>
</tr>
<tr>
<td>Same</td>
<td>61 (23.7)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
</tr>
<tr>
<td>Farming</td>
<td>146 (62.4)</td>
</tr>
<tr>
<td>Business</td>
<td>55 (23.5)</td>
</tr>
<tr>
<td>Skilled work</td>
<td>12 (5.1)</td>
</tr>
<tr>
<td>Other</td>
<td>14 (9.0)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>Primary or less</td>
<td>220 (92.1)</td>
</tr>
<tr>
<td>Secondary</td>
<td>19 (7.9)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>73 (31.5)</td>
</tr>
<tr>
<td>Married</td>
<td>80 (34.5)</td>
</tr>
<tr>
<td>Widowed</td>
<td>49 (21.1)</td>
</tr>
<tr>
<td>Other</td>
<td>30 (12.9)</td>
</tr>
<tr>
<td>Weekly household expenditure (Tanzanian shillings)</td>
<td></td>
</tr>
<tr>
<td>0-7000 (U.S.$0–6.68)</td>
<td>154 (62.3)</td>
</tr>
<tr>
<td>&gt;7000 (&gt;U.S.$6.68)</td>
<td>93 (37.7)</td>
</tr>
<tr>
<td>Median age and (range) (years)</td>
<td>38 (20–66)</td>
</tr>
</tbody>
</table>
whom follow-up was attempted were female, most (62.4%) were farmers, and most (92.1%) had completed no more than primary education. Only 34% were married. Most (62.3%) spent $6.68/week on household expenses (Anon., 2007b). The median age of these clients was 38 years (range=20–66 years).

**Morbidity**

Table 2 depicts the morbidity reported by clients during the first census round. Most (66.9%) of the clients were categorized as being in WHO stage 3, with a history of severe bacterial infections being the most common stage-3-defining condition (52.5% of all clients). The median body-mass index (BMI) was 20.3 kg/m$^2$ (range=12.5–39.3 kg/m$^2$), and having to stay in bed for part of the day over most of the previous month was the predominant functional capacity among the clients (50.4%). The predominant symptoms reported included any weight loss in the previous 3 months (81.7%), weakness for >1 month (78.5%), chronic headache (52.7%), and fever for >1 month (51.4%).

<table>
<thead>
<tr>
<th>Symptom/condition</th>
<th>No. and (%) of clients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WORLD HEALTH ORGANIZATION DISEASE STAGE</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>26 (10.1)</td>
</tr>
<tr>
<td>2</td>
<td>31 (12.1)</td>
</tr>
<tr>
<td>3</td>
<td>172 (66.9)</td>
</tr>
<tr>
<td>4</td>
<td>28 (10.9)</td>
</tr>
<tr>
<td><strong>ACTIVITY LEVEL IN PREVIOUS MONTH</strong></td>
<td></td>
</tr>
<tr>
<td>No symptoms</td>
<td>71 (28.9)</td>
</tr>
<tr>
<td>Had symptoms, but normal activities possible</td>
<td>51 (20.7)</td>
</tr>
<tr>
<td>Bedridden for less than half of each day</td>
<td>22 (8.9)</td>
</tr>
<tr>
<td>Bedridden for most of each day</td>
<td>102 (41.5)</td>
</tr>
<tr>
<td><strong>BODY-MASS INDEX (kg/m$^2$)</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5 (underweight)</td>
<td>60 (30.2)</td>
</tr>
<tr>
<td>18.5–24.9 (healthy weight)</td>
<td>121 (60.1)</td>
</tr>
<tr>
<td>25.0–29.9 (overweight)</td>
<td>12 (6.0)</td>
</tr>
<tr>
<td>≥30.0 (obese)</td>
<td>6 (3.0)</td>
</tr>
<tr>
<td><strong>WEIGHT CHANGE OVER PREVIOUS 3 MONTHS</strong></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>15 (6.3)</td>
</tr>
<tr>
<td>Decreased</td>
<td>196 (81.7)</td>
</tr>
<tr>
<td>No change</td>
<td>29 (12.1)</td>
</tr>
<tr>
<td>Weakness for &gt;1 month</td>
<td>193 (78.5)</td>
</tr>
<tr>
<td>Chronic headache</td>
<td>129 (52.7)</td>
</tr>
<tr>
<td>Previous bacterial infection</td>
<td>125 (52.5)</td>
</tr>
<tr>
<td>Fever for &gt;1 month</td>
<td>125 (51.4)</td>
</tr>
<tr>
<td>Skin rash</td>
<td>96 (39.3)</td>
</tr>
<tr>
<td>Oral or genital ulcers</td>
<td>81 (34.6)</td>
</tr>
<tr>
<td>Herpes zoster in previous 5 years</td>
<td>70 (29.4)</td>
</tr>
<tr>
<td>Diarrhoea for &gt;1 month</td>
<td>69 (27.6)</td>
</tr>
<tr>
<td>Pulmonary tuberculosis in past year</td>
<td>66 (27.4)</td>
</tr>
<tr>
<td>Vaginal candidiasis &gt;1 month</td>
<td>46 (25.3 of women)</td>
</tr>
<tr>
<td>Swollen lymph nodes</td>
<td>57 (23.4)</td>
</tr>
<tr>
<td>Painful swallowing</td>
<td>51 (20.9)</td>
</tr>
<tr>
<td>Oral thrush</td>
<td>46 (18.6)</td>
</tr>
<tr>
<td>Urethral discharge in men</td>
<td>10 (16.7 of men)</td>
</tr>
</tbody>
</table>

*Category of underweight, healthy weight, overweight and obese according to Anon. (2006).
FIG. Kaplan–Meier survival curves for 189 of the 226 clients surveyed in both the first and second census rounds for whom follow-up or death dates were available. The curves show the overall results (a) and the results stratified according to whether, in the first census round, the clients were in (World Health Organization) stages 1 (——), 2 (–––), 3 (——–) or 4 (———) of their HIV disease (b). Of the 189 living clients considered from the first census round (time '0'), 149, 125, 115 and 110 remained alive 6, 12, 18 and 24 months later, respectively.

Mortality
Of the 226 clients who were successfully followed up in the second census round, 102 (45%) had died since the first round [Fig. (a)]. There were 51 deaths/100 person-years of observation. The median time to death, since interview in the first census round, was 5.6 months (range=0.2–25.1 months).

Causes of Death
Coding of the verbal-autopsy forms (Table 3) revealed that the primary cause of death was infectious for 94 (92.2%) of the deceased clients and non-infectious or unknown for the other eight (7.8%). Among the infectious causes, respiratory and/or gastro-intestinal infections accounted for the majority of the deaths (85.3% of all
deaths). Of the deaths attributed to respiratory and/or gastro-intestinal infections, 25 (24.5% of all deaths) were attributed only to acute or chronic diarrhoea, 17 (16.7%) to pulmonary tuberculosis, 11 (10.8%) only to chronic cough, and 31 (30.4%) to a combination of chronic cough and chronic diarrhoea. Among the eight deaths with non-infectious or unknown causes, two (2.0% of all deaths) were attributed to complications of pregnancy/childbirth, and two (2.0%) to suicide. The most common contributory causes of death included malnutrition (81.4%), anaemia (42.2%), infectious skin diseases (38.2%), and liver diseases (26.5%).

TABLE 3. The primary and contributory causes of death for the 102 clients who died between the first and second census rounds

<table>
<thead>
<tr>
<th>Cause of death*</th>
<th>No. and (%) of dead clients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary cause</td>
</tr>
<tr>
<td>INFECTIOUS</td>
<td></td>
</tr>
<tr>
<td>Acute diarrhoea</td>
<td>94 (92.2)</td>
</tr>
<tr>
<td>Chronic diarrhoea</td>
<td>10 (9.8)</td>
</tr>
<tr>
<td>Chronic cough</td>
<td>15 (14.7)</td>
</tr>
<tr>
<td>Chronic diarrhoea and chronic cough</td>
<td>11 (10.8)</td>
</tr>
<tr>
<td>Pulmonary tuberculosis, probable or confirmed</td>
<td>31 (30.4)</td>
</tr>
<tr>
<td>Acute respiratory-tract infection</td>
<td>17 (16.7)</td>
</tr>
<tr>
<td>Respiratory disease, unknown cause</td>
<td>3 (2.9)</td>
</tr>
<tr>
<td>Febrile illness, acute or chronic</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Infectious skin disease</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>NON-INFECTIOUS/UNKNOWN</td>
<td>8 (7.8)</td>
</tr>
<tr>
<td>Maternal death</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Intentional self-harm/suicide</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Alcohol-related</td>
<td>–</td>
</tr>
<tr>
<td>Anaemia</td>
<td>–</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>–</td>
</tr>
<tr>
<td>Renal failure</td>
<td>–</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>–</td>
</tr>
<tr>
<td>Hypertensive diseases</td>
<td>–</td>
</tr>
<tr>
<td>Blood loss</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Gastric/duodenal ulcer</td>
<td>–</td>
</tr>
<tr>
<td>Excessive vaginal bleeding</td>
<td>–</td>
</tr>
<tr>
<td>Liver disease</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Skin disease</td>
<td>–</td>
</tr>
<tr>
<td>Skin cancer</td>
<td>–</td>
</tr>
<tr>
<td>Lymphoma/leukaemia</td>
<td>–</td>
</tr>
<tr>
<td>Cancer, unspecified</td>
<td>–</td>
</tr>
<tr>
<td>Nervous system problem</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Epilepsy/status epilepticus</td>
<td>–</td>
</tr>
<tr>
<td>Paraplegia/quadruplegia</td>
<td>–</td>
</tr>
<tr>
<td>Headache</td>
<td>–</td>
</tr>
<tr>
<td>Blindness</td>
<td>–</td>
</tr>
<tr>
<td>Ear disease</td>
<td>–</td>
</tr>
<tr>
<td>Other family member died of HIV</td>
<td>–</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1.0)</td>
</tr>
</tbody>
</table>

*Results were obtained by coding verbal autopsy forms using a condensed version of the tenth revision of the International Classification of Diseases (Anon., 1997).
Predictors of Mortality

Advanced WHO stage at the first census round was found to be significantly positively associated with between-census mortality [log-rank test \(\chi^2=8.115; P=0.044\); Fig. (b)]. Certain first-round symptoms were also strongly associated with mortality, the clients who died between censuses being more than twice as likely to have reported weakness for >1 month [odds ratio (OR)=2.64; \(P=0.008\)], oral thrush (OR=2.32; \(P=0.015\)), and painful swallowing (OR=2.02; \(P=0.036\)) during the first round than the clients who survived to the second round. Other first-round conditions found to be significantly associated with mortality by the second round included having to stay in bed for part of the day over most of the previous month (OR=1.94; \(P=0.017\)), fever for >1 month (OR=1.95; \(P=0.016\)), and a history of severe bacterial infections (OR=1.80; \(P=0.036\)).

Medication Use

At the start of the present study, ART in Tanzania was only available to those who could afford to pay for it themselves, at a cost of approximately U.S.$35/month. The Tanzanian government began providing free ART to patients with disease of WHO stages 3 or 4 in September 2004 (i.e. shortly before the second census round), starting at referral hospitals and expanding to regional and district hospitals.

At the time of the first census round, ART use — reported by 27 (12.9%) of the clients investigated at that time — was uncommon. It remained just as uncommon by the time of the second census round, when only 15 (12.7%) of the clients were recorded as using ART (\(P=0.970\)). The use of SXT prophylaxis was much more wide-spread and increased, among the clients investigated, during the present study. Overall, 108 (44.6%) of the clients investigated in the first census round and 77 (63.6%) of the clients surviving at the time of the second round were recorded as receiving such prophylaxis (at the time of the first and second rounds, respectively; \(P<0.001\)). Neither the use of ART at the time of the first round nor the use of SXT at that time was, however, significantly associated with survival to the second round.

DISCUSSION

In the present study, baseline morbidity and mortality in a cohort of HIV-infected adults receiving CHBC in northern Tanzania were found to be high. The majority of the clients had advanced, symptomatic HIV disease for which treatment with ART was indicated. The level of morbidity and causes of mortality indicate that CHBC programmes need to improve access to basic medications and resources, in addition to expanding ART, in order for effective care to be delivered to HIV-infected patients.

The socio-demographic characteristics of the present CHBC cohort were generally representative of the wider Tanzanian population (Anon., 2002), with the majority of clients being farmers and having only a primary or lower level of education. The proportion of women in the cohort was, however, high, which may reflect KIWAKKUKI's original status as a women's organization (although it now serves both men and women). There is some gender bias in HIV infection in Tanzania, with females accounting for 58.5% of the HIV burden in individuals aged \(\geq 15\) years (Anon., 2008).

A high level of morbidity was seen at the time of the first census round, with the majority of clients having WHO stage-3 disease and almost half having had to stay in bed for part of the day over most of the previous month. The symptoms reported are similar to those found in a cohort in Malawi, in which 95% of patients were clinically diagnosed with AIDS-related disease and were receiving home-based care (HEC) services from teams consisting of community volunteers and healthcare
workers (Bowie et al., 2006). The majority of the patients in the Malawian cohort had disease of WHO stages 3 or 4 (95%), and experienced symptoms including weight loss (94%), headache (74%), and fever (70%). The level of debility in both the Malawian and the present, Tanzanian cohort can be attributed to the progression of HIV disease in the absence of wide-spread ART. In addition, the prevalence and range of symptoms indicate that basic medications and resources, such as antimicrobials, antipyretics and adequate nutrition, were also scarce in the Tanzanian cohort, with many clients probably lacking the financial resources and physical strength to visit centralized medical centres.

In the present study, as described in several other investigations, mortality was found to be associated with advanced WHO clinical stage (Jerene and Lindtjorn, 2005). Weakness, oral thrush, painful swallowing, fever, severe bacterial infections, and being bedridden were all independently associated with mortality. These are same or similar to the predictors of mortality detected in other studies. Lindan et al. (1992), for example, described low BMI, chronic diarrhoea, a history of herpes zoster disease and oral thrush as being associated with mortality in a cohort of HIV-infected women in Kigali, Rwanda.

The mortality rate in the Tanzanian cohort was high but similar, as far as can be seen from the limited data available, to that of other HIV-infected cohorts receiving HBC but without widespread access to ART. Among a cohort of HIV-infected Ugandan children receiving HBC services from a state hospital, for example, O’Hare et al. (2005) observed mortality of 26% over a period of 10 months. Similarly, of the HIV-infected patients receiving HBC in Malawi investigated by Bowie et al. (2006), 56% died within the first 18 months. In contrast, only 5% of a cohort of HIV-infected Ugandan adults receiving SXT and ART as part of their HBC died over a period of 16 weeks (Mermin et al., 2008).

The use of ART by the clients investigated in the present study remained low, even though most of the clients had advanced HIV disease. The Tanzanian government began distributing free ART to government healthcare centres in September 2004, shortly before the second census round. It is therefore likely that, during the period of the present study, patients in rural communities would not have access to such medications. The use of SXT increased over the study period, a trend that may indicate that CHBC services are becoming more informed and effective at providing clients with some essential medications and services. The lack of a statistically significant association between ART or SXT use and survival, although disappointing, is probably the result of factors such as the targeting of medications towards the sicker clients and the sporadic access to such medications.

The primary and contributory causes of death, as well as the conditions associated with mortality, indicate that improved access to ART, as well as the provision of other medications, nutrition and care, is essential. A basic prevention and care package, tailored to the region and resources available, has previously been advocated to reduce morbidity and mortality in HIV-infected patients living in Africa (Mermin et al., 2005). In the present cohort, of HIV-infected patients in northern Tanzania, much of the mortality was attributable to chronic illness and infections of the respiratory and gastro-intestinal systems, with malnutrition and anaemia among the most common contributory causes of death. Besides ART, SXT and isoniazid prophylaxis, micronutrient supplementation and the provision of safe drinking water have been shown to prevent respiratory and gastro-intestinal disease in people living with HIV in Africa, and many such interventions have been shown to be cost-effective (Rose, 1998; Anglaret et al., 1999; Fawzi et al., 2004; Lule et al., 2005). The present results indicate that a treatment package targeted at
respiratory and gastro-intestinal disease may be of benefit to HIV-infected patients receiving CHBC.

Following the dissemination of the data collected in the present study to KIWAKKUKI, a CHBC liaison officer was placed fulltime at the Kilimanjaro Christian Medical Centre (KCMC) — the government referral hospital serving northern Tanzania. This liaison officer now helps link CHBC clients with the government services offering free ART. Given the need for a two-pronged approach, with provision of both ART and basic resources, the liaison officer also provides KCMC patients with information on KIWAKKUKI’s CHBC programme, through which patients could receive home visits and basic treatment needs. It is recommended that four basic and cost-effective components be incorporated into all CHBC programmes in order to improve care for HIV-infected patients: SXT and isoniazid prophylaxis; micronutrient provision; oral rehydration therapy; and supplies to generate clean water. The trends in morbidity and mortality following the incorporation of such improvements (which would be useful topics for future research) need to be carefully monitored, so that CHBC programmes can be optimised.

Some limitations in the present study must be noted. As mentioned above, most of the clients investigated were female. The main findings are not, however, gender-specific. Approximately 12% of the initial cohort was lost to follow-up, apparently because they had moved out of the region. It is unknown whether the clients lost to follow-up had travelled home to die, or whether they were travelling because they were well. The WHO staging was based on an abbreviated list of criteria. More detailed staging would, however, only be expected to show increased severity of a client’s HIV-associated disease.

Along with efforts to scale-up access to ART and prevent the further spread of the HIV epidemic in sub-Saharan Africa, there is great need for basic care and treatment to be delivered to the large numbers of patients living with HIV. CHBC and other alternative methods of delivering healthcare are becoming increasingly important in managing some of this healthcare burden. The present results indicate that countries need to address both basic interventions and ART access when scaling-up CHBC programmes for HIV-infected patients living in resource-limited settings.

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REFERENCES


5.11. HIV-associated morbidity, mortality, and diagnostic testing opportunities among inpatients at a referral hospital in northern Tanzania


CONTRIBUTION

My position as second author reflects my role providing on-site, day-to-day guidance and collegial mentorship to Dr. Ole-Nguyaine. With Dr. Thielman, I co-developed the database that formed the basis of the analysis and co-designed and implemented that data analysis. I worked with Dr. Ole-Nguyaine to write the first draft of the manuscript. Ole-Nguyaine, Kibiki, Kiang, Taylor, and Schimana coordinated and undertook data collection. Bartlett, Shao, and Hamilton assisted with institutional relationships and ensured that research activities with integrated with patient care delivery. All authors contributed to revisions of the manuscript.

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HIV-associated morbidity, mortality and diagnostic testing opportunities among inpatients at a referral hospital in northern Tanzania

S. OLE-NGUYAINE*, J. A. CRUMP†, G. S. KIEIKI*, K. KIANG‡, J. TAYLOR‡, W. SCHIMANAN§, J. A. BARTLETT†, J. F. SHAO§, J. D. HAMILTON§ and N. M. THIELMAN‡

*Department of Medicine, Kilimanjaro Christian Medical Centre, Moshi, Tanzania
†Division of Infectious Diseases and International Health, Department of Medicine, Box 3152, Duke University Medical Center, Durham, NC 27710, U.S.A.
‡Department of Paediatrics, Kilimanjaro Christian Medical Centre, Moshi, Tanzania
§Department of Microbiology, Kilimanjaro Christian Medical Centre, Moshi, Tanzania

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Hospitalized patients with HIV infection are among the most likely to benefit from the expanding availability of anti-retroviral therapy in sub-Saharan Africa. Between 1990 and 2000, 3667 people known to be HIV-infected were admitted to Kilimanjaro Christian Medical Centre (KCMC) in Moshi, northern Tanzania. The level of inpatient mortality among these patients varied from 15%-21%, and the proportion of the HIV-infected patients admitted who were female increased significantly, from 45% at the start of the study period to 52% at the end (P<0.001). When the medical records for 1683 of the HIV-infected patients who had been admitted between 1996 and 2001 were reviewed, the most prevalent diagnoses on admission were found to be pulmonary tuberculosis (21%), malaria (14%) and gastro-enteritis/diarrhoea (12%) among the adults, and non-tubercular pulmonary infection (21%), pulmonary tuberculosis (19%) and gastro-enteritis/diarrhoea (12%) among the children. The crude odds ratios (OR) for inpatient death were greatest for adults presenting with meningitis [OR=3.7; 95% confidence interval (CI)=2.1-6.7], septicaemia (OR=2.9; CI=1.2-7.3) or renal disease (OR=2.6; CI=1.2-5.7), and mortality was higher for men than for women (OR=1.4; CI=1.1-1.8). A single-day, point-prevalence survey in September 2001, among the KCMC's inpatients, identified HIV infection in 21% of those surveyed, many (44%) of the patients found positive being previously unaware of their infection. HIV infection remains a major cause of hospitalization and mortality in Moshi. A policy of routine testing would increase the number of HIV infections detected, allowing improvements in case management and in the prevention of infection.

Of the 42 million people living with HIV/AIDS at the end of 2002, over 70% resided in sub-Saharan Africa, making it easily the most affected region of the world (Anon., 2002). In Tanzania, by the end of 2001, approximately 2.2 million individuals aged ≥15 years were living with HIV/AIDS — a 3% increase from the previous year. In the Kilimanjaro region, the prevalence of HIV infection among women attending some antenatal clinics has nearly tripled since 1992, with estimates for 1997–2000 ranging from 17% to 20% (Anon., 2003). Between 1992 and 1998, across 51 villages surveyed in the Hai district of this region, HIV/AIDS accounted for 57% of all deaths, reflecting the dramatic impact HIV has made among young, sexually active adults (Setel et al., 2000). Unfortunately, in Tanzania, as in other countries in sub-Saharan Africa, many of those infected with HIV are unaware of their serostatus. This not only hampers
efforts to prevent transmission of the virus but also limits the efficacy of the treatment and care services. The problem of the late recognition of many cases of HIV infection is beginning to be addressed by the development of a growing number of sites for voluntary counselling and testing (VCT). Many opportunities to detect seropositive individuals are still missed, however, because many hospitalized individuals are not routinely offered HIV tests.

As increased international attention and resources are focused on the AIDS crisis in sub-Saharan Africa and plans for more intensive and effective therapeutic interventions are developed, it has become increasingly important to describe the clinical manifestations of HIV infection among hospitalized patients. Such patients are particularly likely to have advanced HIV-attributable disease and most of them could derive immediate benefit from anti-retroviral medications. Although there have been some hospital-based surveys of HIV-associated morbidity and mortality in sub-Saharan Africa (Tembo et al., 1994; Arthur et al., 2000; Lewis et al., 2003), including other regions of Tanzania (Kwesigabo et al., 1999), there has been none in northern Tanzania. Recently, a policy of offering VCT to everyone admitted to hospitals in sub-Saharan Africa has been advocated (De Cock et al., 2002). However, the number of additional HIV infections that might be detected if this strategy were implemented has not been estimated.

In the present study, the clinical characteristics of HIV infection and HIV seroprevalence, among patients admitted to the major referral hospital in northern Tanzania, were determined. For this, the medical records of patients found to be HIV-positive over an 11-year period were reviewed, and a cross-sectional seroprevalence survey was conducted among the hospital's inpatients. To estimate the proportion of HIV infections missed by a testing policy based on clinical and behavioral criteria, the data obtained routinely, following this policy, were compared with those of the cross-sectional survey.

PATIENTS AND METHODS

Study Site
Kilimanjaro Christian Medical Centre (KCMC) is located in the Moshi municipality in the Kilimanjaro region of northern Tanzania. As one of four national referral centres, the 450-bed hospital serves those (>10 million) living in the Northern and Central zones of Tanzania. In addition, the KCMC hosts a medical school and 15 other schools of allied health sciences. In 2001, 17,812 admissions and 1121 inpatient deaths were recorded in the KCMC, and bed occupancy was nearly 100%.

Hospital-inpatient Series
Between 1990 and 2001, all patients suspected by clinicians at the KCMC to be at relatively high risk of HIV infection were offered HIV testing; such testing was not offered routinely to all inpatients. In following the relevant guidelines of the World Health Organization (WHO, 1997), each patient who consented to HIV testing was checked for anti-HIV antibodies using two rapid tests, commonly the Capillus® HIV-1/HIV-2 rapid test (Trinity Biotech, Bray, Ireland) and the Vironostika® HIV Uni-Form II Ag/Ab microwell enzyme immunoassay (bioMérieux, Marcy l'Etoile, France). Demographic information and the status of the patient at discharge were extracted from the KCMC's discharge and HIV-testing logs. An additional set of data, based on a retrospective review of medical records, was generated for patients admitted between 1997 and 2001, using a standardized form. These data included patient age, gender, status at discharge, and admission diagnoses. The recorded admission diagnoses reflected the opinions of the senior consultant physicians who reviewed each case on the day of his or her admission; if further investigation resulted in an alternate, definitive diagnosis, this was recorded as the admitting diagnosis. For analysis, composite diagnostic categories were formulated, by combining aetologically
or syndromically related diagnoses, to shorten the long list of diagnoses observed in the medical records. Any patient aged >13 years was considered an adult.

Cross-sectional Seroprevalence Survey
To assess the seroprevalence of HIV among some of the KCMC’s inpatients and, specifically, to help determine what percentage of HIV infections was being routinely detected, a point-prevalence survey was performed on 18 September 2001. The protocol for this survey was approved by the Research Ethics Committee at KCMC, and patients were enrolled only after their informed consent (or that of a parent/guardian, if the patient was a child) had been obtained. All patients on the general medical and paediatric wards at the KCMC were invited to participate in the study. Patients who were younger than 6 months of age, those in the intensive care unit and those receiving private medical care were excluded from the study.

Prior to the survey, clinical information, including recorded HIV serostatus (if known), was abstracted from the routine medical records for the subjects. The subjects were asked about their medical histories and given physical examinations to see if, according to the World Health Organization’s case definition (WHO, 1994), they had AIDS.

Four drops of blood from each subject, collected from a fingerprick, were transferred to filter paper, allowed to air dry, and sealed in plastic for transfer to Duke University’s Medical Center (in Durham, NC). A waiver for the anonymised testing of the dry blood spots, for anti-HIV antibody, was granted by Duke University’s Institutional Review Board. The dried blood was eluted from the filter paper so that it could be tested in the Vironostika® HIV-1 micro-ELISA (Organon Teknika, Durham, NC) according to the manufacturer’s instructions. Every sample found positive was tested again, using the same kit, and only samples found positive twice were considered to have come from seropositive patients.

Statistics
All of the statistical computations and comparisons were made using version 4.04 of the JMP software package (SAS Institute, Cary, NC) and Epi Info 2002 (Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS
Hospital-inpatient Series
The number of patients known to be seropositive for HIV increased approximately 2-fold from the first half of the study period to the second (see Figure), and the male:female ratio for the known seropositives gradually decreased so that, from 1998, there was a preponderance of females ($\chi^2$ test for trend; $P < 0.001$). The proportion of identified seropositives who were female was consistently <50% between 1990 and 1996 (with a low of 38% in 1994) and consistently >50% from 1998. The inpatient mortality for the known seropositives generally increased over the study period, varying from 14.6% to 21.2% for each calendar year.

Between 1997 and 2001, 1553 adults — 814 (52%) women and 739 (48%) men — known to be seropositive for HIV were inpatients at the KCMC. The median age and (range) of these 1553 adults was 35 (13–92) years. Among the known seropositive patients, the females were significantly younger (median = 33 years; range = 13–92 years) than the males (median age = 38 years; range = 14–80 years; $P < 0.001$). Data on in-hospital mortality were available for 1549 of the patients; the median age and (range) of the 386 patients known to have died while hospitalized was 36 (13–77) years, the known seropositives who were male being more likely to die in hospital than their female counterparts [28% v. 22%; odds ratio (OR) = 1.4; 95% confidence interval (CI) = 1.1–1.8; $P = 0.004$].
Table 1 lists the diagnostic categories assigned to 1242 HIV-infected adult patients between 1997 and 2001 and the level of inpatient mortality among those with each of these diagnoses. The most common diagnosis, for the adults known to be HIV-positive, was pulmonary tuberculosis (21%), followed by malaria (13.6%), gastro-enteritis/diarrhoea (12.2%) and non-tubercular pulmonary infection (10.1%). Taken together, pulmonary infections accounted for nearly one-third of all admissions. Central-nervous-system disease was more frequently recorded in the women than in the men (47 cases v. 26; OR = 1.7; \( P = 0.034 \)) but the women were less likely to have Kaposi’s sarcoma (15 v. 30; OR = 0.4; \( P = 0.009 \)) and renal disease (nine v. 18; OR = 0.5; \( P = 0.04 \)) than the men. No significant gender differences were seen among the other diagnoses. With the exception of intra-abdominal infections, which tended to occur in the older patients (median age = 42 years; range = 24–57 years), there was little variation in the median patient age across the diagnostic categories. The frequencies of in-hospital death were relatively high for those presenting with meningitis (OR = 3.7; CI = 2.1–6.7), septicaemia (OR = 2.9; CI = 1.2–7.3), renal disease (OR = 2.6; CI = 1.2–5.7) or non-tubercular pulmonary infection (OR = 1.9; CI = 1.3–2.8).

Table 2 shows the diagnoses assigned to 130 paediatric patients (aged 1.5–12 years) who were known to be positive for anti-HIV-1 antibody. High prevalences of chest disease, particularly of non-tubercular pulmonary infection, were noted in this population.

FIG. The annual numbers of male (■) and female (□) inpatients at the Kilimanjaro Christian Medical Centre who were known to be infected with HIV. The percentages shown next to the bars indicate the corresponding levels of inpatient mortality.
<table>
<thead>
<tr>
<th>Diagnostic category*</th>
<th>N</th>
<th>Prevalence (%)</th>
<th>Female:male ratio</th>
<th>Median age (years)</th>
<th>No. of inpatient deaths</th>
<th>Mortality (%)</th>
<th>Crude odds ratio and (95% confidence interval) for inpatient mortalityf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary tuberculosis</td>
<td>262</td>
<td>21</td>
<td>1.0</td>
<td>37</td>
<td>62</td>
<td>24</td>
<td>1.0 (0.7–1.4)</td>
</tr>
<tr>
<td>Malaria</td>
<td>169</td>
<td>14</td>
<td>1.1</td>
<td>34</td>
<td>52</td>
<td>31</td>
<td>1.5 (1.1–2.2)</td>
</tr>
<tr>
<td>Gastro-enteritis/diarrhoea</td>
<td>152</td>
<td>12</td>
<td>1.1</td>
<td>34</td>
<td>31</td>
<td>20</td>
<td>0.8 (0.5–1.2)</td>
</tr>
<tr>
<td>Non-tubercular pulmonary infection</td>
<td>126</td>
<td>10</td>
<td>0.9</td>
<td>36</td>
<td>45</td>
<td>36</td>
<td>1.9 (1.3–2.8)</td>
</tr>
<tr>
<td>Oral candidiasis</td>
<td>114</td>
<td>9</td>
<td>1.4</td>
<td>35</td>
<td>22</td>
<td>19</td>
<td>0.7 (0.5–1.2)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>73</td>
<td>6</td>
<td>1.1</td>
<td>36</td>
<td>25</td>
<td>34</td>
<td>1.7 (1.0–2.9)</td>
</tr>
<tr>
<td>Central-nervous-system disease</td>
<td>73</td>
<td>6</td>
<td>1.8</td>
<td>37</td>
<td>20</td>
<td>27</td>
<td>1.2 (0.7–2.1)</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>48</td>
<td>4</td>
<td>1.1</td>
<td>37</td>
<td>1</td>
<td>2</td>
<td>0.1 (0.01–0.4)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>46</td>
<td>4</td>
<td>1.0</td>
<td>34</td>
<td>24</td>
<td>52</td>
<td>3.7 (2.1–6.7)</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
<td>45</td>
<td>4</td>
<td>0.5</td>
<td>33</td>
<td>6</td>
<td>13</td>
<td>0.5 (0.2–1.1)</td>
</tr>
<tr>
<td>Extrapulmonary tuberculosis</td>
<td>43</td>
<td>4</td>
<td>1.2</td>
<td>35</td>
<td>9</td>
<td>21</td>
<td>0.8 (0.4–1.8)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>34</td>
<td>3</td>
<td>1.1</td>
<td>35</td>
<td>10</td>
<td>29</td>
<td>1.3 (0.6–2.8)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>27</td>
<td>2</td>
<td>0.5</td>
<td>35</td>
<td>12</td>
<td>44</td>
<td>2.6 (1.2–5.7)</td>
</tr>
<tr>
<td>Malignancy†</td>
<td>27</td>
<td>2</td>
<td>2.0</td>
<td>37</td>
<td>5</td>
<td>19</td>
<td>0.7 (0.3–1.9)</td>
</tr>
<tr>
<td>Urinary-tract infection</td>
<td>24</td>
<td>2</td>
<td>1.7</td>
<td>34</td>
<td>4</td>
<td>17</td>
<td>0.6 (0.2–1.9)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>23</td>
<td>2</td>
<td>0.6</td>
<td>38</td>
<td>5</td>
<td>22</td>
<td>0.9 (0.3–2.4)</td>
</tr>
<tr>
<td>Septicaemia</td>
<td>19</td>
<td>2</td>
<td>1.4</td>
<td>33</td>
<td>9</td>
<td>47</td>
<td>2.9 (1.2–7.3)</td>
</tr>
<tr>
<td>Skin/soft-tissue infection</td>
<td>18</td>
<td>1</td>
<td>0.6</td>
<td>35</td>
<td>1</td>
<td>6</td>
<td>0.2 (0.02–1.4)</td>
</tr>
<tr>
<td>Sexually transmitted disease</td>
<td>18</td>
<td>1</td>
<td>2.6</td>
<td>36</td>
<td>5</td>
<td>28</td>
<td>1.2 (0.4–3.5)</td>
</tr>
<tr>
<td>Intra-abdominal infection</td>
<td>16</td>
<td>1</td>
<td>1.3</td>
<td>42</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>13</td>
<td>1</td>
<td>1.2</td>
<td>35</td>
<td>3</td>
<td>23</td>
<td>1.0 (0.3–3.5)</td>
</tr>
<tr>
<td>Otitis media</td>
<td>8</td>
<td>1</td>
<td>1.7</td>
<td>36</td>
<td>3</td>
<td>38</td>
<td>1.9 (0.5–8.1)</td>
</tr>
<tr>
<td>Other, unclassified diagnoses</td>
<td>142</td>
<td>11</td>
<td>1.2</td>
<td>35</td>
<td>18</td>
<td>13</td>
<td>0.6 (0.4–1.0)</td>
</tr>
</tbody>
</table>

*Multiple diagnoses per patient were possible; the mean number of diagnoses/patient was 1.2 (with a range of one to three).
†Calculated with death as the dependent variable and diagnostic category as the independent variable.
‡Other than Kaposi's sarcoma or lymphoma.
TABLE 2. Diagnoses assigned to 130 HIV-infected paediatric inpatients (aged 1.5–12 years) at the Kilimanjaro Christian Medical Centre between 1997 and 2001

<table>
<thead>
<tr>
<th>Diagnostic category*</th>
<th>N</th>
<th>Prevalence (%)</th>
<th>Median age and (range) years</th>
<th>No. of inpatient deaths</th>
<th>Crude odds ratio and (95% confidence interval) for inpatient mortality†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-tubercular pulmonary infection</td>
<td>27</td>
<td>21</td>
<td>4 (1.5–10)</td>
<td>8</td>
<td>2.3 (0.8–6.1)</td>
</tr>
<tr>
<td>Pulmonary tuberculosis</td>
<td>25</td>
<td>19</td>
<td>4 (2–12)</td>
<td>3</td>
<td>0.5 (0.1–2.0)</td>
</tr>
<tr>
<td>Gastro-enteritis/diarrhoea</td>
<td>16</td>
<td>12</td>
<td>3 (1.5–10)</td>
<td>6</td>
<td>3.2 (1.0–9.9)</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>15</td>
<td>11</td>
<td>3 (2–9)</td>
<td>6</td>
<td>3.6 (1.1–11.3)</td>
</tr>
<tr>
<td>Malaria</td>
<td>14</td>
<td>11</td>
<td>3.5 (2–11)</td>
<td>3</td>
<td>1.2 (0.3–4.8)</td>
</tr>
<tr>
<td>Otitis media</td>
<td>6</td>
<td>5</td>
<td>4 (2–12)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Anaemia</td>
<td>7</td>
<td>5</td>
<td>4 (2–9)</td>
<td>2</td>
<td>1.8 (0.3–10.1)</td>
</tr>
<tr>
<td>Extrapulmonary tuberculosis</td>
<td>10</td>
<td>8</td>
<td>9 (2–10)</td>
<td>0</td>
<td>2.0 (0.5–8.5)</td>
</tr>
<tr>
<td>Septicaemia</td>
<td>7</td>
<td>5</td>
<td>3 (2–9)</td>
<td>1</td>
<td>3.6 (0.8–17.4)</td>
</tr>
<tr>
<td>Kapost's sarcoma</td>
<td>6</td>
<td>5</td>
<td>3.5 (2–8)</td>
<td>1</td>
<td>0.9 (0.1–7.8)</td>
</tr>
<tr>
<td>Other</td>
<td>16</td>
<td>19</td>
<td>4 (1.5–12)</td>
<td>1</td>
<td>0.3 (0.03–2.1)</td>
</tr>
</tbody>
</table>

*Multiple diagnoses per patient were possible.
†Calculated with death as the dependent variable and diagnostic category as the independent variable.

Whereas the median age for most of the diagnostic categories was quite young, that for extrapulmonary tuberculosis was relatively high, at 9 years (range = 2–10 years).

Cross-sectional Seroprevalence Survey

Of the 61 adults on the general wards and 29 children on the paediatric wards offered testing on 18 September 2001, consent was provided by 58 of the adults (median age = 45 years; range = 20–94 years) and by the guardians of 25 children (median age = 1.9 years; range = 0.7–14 years). Only the 16 children who were aged >18 months and for whom consent had been obtained were tested. Twelve (21%) of the 58 adults tested and four (25%) of the 16 children were seropositive for HIV. Four (33%) of the seropositive adults were not known to be HIV seropositive when tested. One of the four seropositive children was previously thought to be seronegative and two others had unknown serostatus when tested in the survey. No significant differences in sex or age were found between those testing positive for anti-HIV antibodies and those who tested negative. Of those tested, 15% of the 26 women and 25% of the 32 men were found seropositive (P = 0.3686). The adult seropositives were generally younger than the adult seronegatives, with mean ages of 44 and 52 years, respectively (P = 0.2415).

For the adult inpatients, the sensitivity of the World Health Organization’s case definition for AIDS surveillance was 0.58 (CI = 0.31–0.74). In terms of specific symptoms and signs, the sensitivity was 0.75 (CI = 0.47–0.91) for weight loss, 0.33 (CI = 0.47–0.91) for chronic diarrhoea, 0.50 (CI = 0.25–0.75) for prolonged fever, 0.58 (CI = 0.32–0.81) for cough, and 0.25 (CI = 0.09–0.53) for thrush.

DISCUSSION

This article describes the diagnoses and mortality associated with HIV infection in a large referral hospital for the Northern zone of Tanzania, based on retrospective but systematic review of medical records. The present findings are consistent with those of other researchers in sub-Saharan Africa, in highlighting the considerable morbidity and mortality of HIV/AIDS seen in a relatively young and potentially economically-productive population and the large number of missed cases. It is clear that a more aggressive testing strategy would markedly increase the numbers of HIV infections being identified.
Significant gender differences in case age and mortality were noted. The longitudinal data reveal a decreasing male:female ratio for those hospitalized with known HIV infection, and the women known to be seropositive were, in general, approximately 5 years younger than the men. Similar age differences have been noted in hospital-based studies in Kenya, Uganda and Malawi (Tembo et al., 1994; Arthur et al., 2000; Lewis et al., 2003). It remains unclear why the hospitalized seropositive women are generally younger than the men but this may reflect gender differences in age at acquisition of HIV (Fylkesnes et al., 1998), health-seeking behaviour, and/or in the rate of disease progression. In the present study, the women with (known) HIV infections were less likely to die in hospital than the men (22% v. 28%; \( P = 0.004 \)). Others have noticed more striking differences in gender-associated mortality in sub-Saharan Africa, speculating that cultural reasons may account for such variation. Compared with very sick men, for example, very sick women may be less likely to be taken to hospital (Arthur et al., 2000).

The most prevalent diagnoses on admission recorded in the adults known to be seropositive were pulmonary tuberculosis, malaria, gastro-enteritis/diarrhoea and non-tubercular pulmonary infection. With the exception of malaria, these diagnoses are similar to those reported among seropositives from other regions of sub-Saharan Africa (Tembo et al., 1994; Floyd et al., 1999; Arthur et al., 2000; Colvin et al., 2001). Among the patients at the KCMC who were known to be HIV-positive, those considered to have meningitis, septicaemia, renal disease or non-tubercular pulmonary infection on admission were quite likely to die while hospitalized, with in-hospital mortality levels of 52%, 47%, 44% and 36%, respectively. Although relatively high mortality levels are expected with meningitis, septicaemia and renal disease, even among seronegative patients in resource-poor settings, the relatively high mortality seen, in the present study, among the seropositive patients with non-tubercular pneumonia is surprising. It highlights the need for more intensive investigations of chest disease in seropositive patients at the KCMC. The true case fatality ‘rates’ for several of the diagnoses recorded on admission (e.g. cryptococcal meningitis) are probably higher than the inpatient levels reported here, as many of the seropositive patients, though critically ill, were probably discharged so that they could die at home.

The present, single-day, point-prevalence survey documented HIV seroprevalence of 21% on the general adult wards and 25% on the paediatric. Even within the small sample investigated, almost half (44%) of those found seropositive had not previously been so identified. Although a prevalence study conducted over a period of a few weeks and with many more subjects may give a more precise picture of the prevalence of HIV seropositivity and clinical symptomatology at the KCMC, it seems clear that the use of a testing policy directed by clinical suspicion leads to a considerable number of HIV infections going undiagnosed. De Cock et al. (2002) argued for the routine testing of all those admitted to general medical wards. This policy, of offering HIV testing to all patients admitted, irrespective of perceived risk, would allow many more patients living with HIV/AIDS to be detected and therefore provide additional prevention and treatment opportunities.

Although the point-prevalence survey revealed several HIV infections that had not been recognized previously, the seroprevalence of HIV infection at the KCMC appeared to be relatively low compared with that seen in similar studies in sub-Saharan Africa (Miller et al., 1995; Kwesigabo et al., 1999; Arthur et al., 2000; Colvin et al., 2001; Lewis et al., 2003). There may therefore be marked regional differences in overall seroprevalence. An alternative explanation is that, in northern Tanzania, a relatively high
proportion of those infected with HIV either never become hospital inpatients or are only admitted relatively late, when in the advanced stages of AIDS. In this region, much of the burden of chronic HIV/AIDS care is carried by the communities in which the cases live, often with the support of a programme of home-based care run by Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (Women Against AIDS in Kilimanjaro). This organization currently cares for between 700 and 1000 patients in Kilimanjaro region (L. Kaale, unpubl. obs.). At Kenyatta National Hospital in Nairobi, curiously, despite predictions of overwhelming numbers of AIDS cases, a decrease in clinical AIDS presentations was noted between 1992 and 1997 (Arthur et al., 2000).

Although HIV seroprevalence is not as high in the Kilimanjaro region as in many other parts of sub-Saharan Africa, it remains a significant contributor to admissions and mortality at the KCMC. It appears that HIV infection is increasingly affecting local women, and disproportionately affects an otherwise economically productive segment of the population. The results of the present, relatively small, point-prevalence survey indicate that >40% of patients in the KCMC who were HIV-infected had not been identified as HIV-positives by the centre’s routine procedures. A more liberal testing strategy would probably lead to the identification of many more HIV-positives, who could then be offered access to the expanding HIV-care options (including anti-retroviral therapy) and be targeted for health education to help prevent further transmission.

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REFERENCES


5.12. Effect of point-of-use disinfection, flocculation, and combined flocculation-disinfection on drinking water quality in western Kenya


CONTRIBUTION

I conceived the research idea, designed the study, and did all aspects of the research under the mentorship of Luby. Luby and Keswick sought and obtained funding. Okoth and Ogaja assisted me with sample collection and transportation. I conducted all laboratory analyses and data analyses, and wrote the first draft of the paper in partnership with Okoth. Slutsker directed the research site that hosted the study team; he assisted with adaption of the study design to the site. All authors contributed to revisions of the manuscript.

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Effect of point-of-use disinfection, flocculation and combined flocculation–disinfection on drinking water quality in western Kenya*

J.A. Crump¹, G.O. Okoth², L. Slutsker², D.O. Ogaja², B.H. Keswick³ and S.P. Luby¹

¹Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA, ²Centers for Disease Control and Prevention/Kenya Medical Research Institute Kisian Research Station, Kisumu, Kenya, and ³Procter & Gamble Company, Mason, OH, USA

ABSTRACT


Aims: Point-of-use drinking water disinfection with sodium hypochlorite has been shown to improve water quality and reduce diarrhoeal disease. However, the chlorine demand of highly turbid water may render sodium hypochlorite less effective.

Methods and Results: We evaluated a novel combined flocculant-disinfectant point-of-use water treatment product and compared its effect on drinking water quality with existing technologies in western Kenya. In water from 30 sources, combined flocculant-disinfectant reduced Escherichia coli concentrations to <1 CFU/100 ml for 29 (97%) and reduced turbidity to <5 nephelometric turbidity units (NTU) for 26 (87%). By contrast, water from 30 sources treated with sodium hypochlorite reduced E. coli concentrations to <1 CFU/100 ml for 25 (83%) and turbidity to <5 NTU for 5 (17%).

Conclusions: For source waters over a range of turbidities in western Kenya, combined flocculant-disinfectant product effectively reduces turbidity to <5 NTU and reduces E. coli concentrations to <1 CFU/100 ml⁻¹.

Significance and Impact of the Study: The novel flocculant-disinfectant product may be acceptable to consumers and may be effective in reducing diarrhoeal disease in settings where source water is highly turbid.

Keywords: alum compounds, disinfection, flocculation, nephelometry and turbidimetry, sodium hypochlorite, water purification.

INTRODUCTION

The World Health Organisation estimates that 1·1 billion persons do not have access to improved water supplies (United Nations Development Program 1996). Drinking contaminated water contributes substantially to the estimated 2·2 million annual deaths from diarrhoea (World Health Organization 1999). The least expensive methods for home treatment of drinking water that have been proved to reduce waterborne diarrhoeal disease include the addition of dilute sodium hypochlorite solution (Semenza et al. 1998; Quick et al. 1999, 2002) and solar disinfection (Conroy et al. 1996, 1999) combined with safe storage of water in a narrow-mouth container (Mintz et al. 1995). However, surface waters heavily contaminated with organic matter and microorganisms are commonly used as drinking water sources in resource-poor settings. When sodium hypochlorite solution is added to highly turbid water the chlorine rapidly binds to the organic matter, and so is unavailable to kill pathogens. Likewise, highly turbid water prevents adequate penetration of ultraviolet light and may protect organisms from the
thermal activity of solar disinfection. If water is not too heavily contaminated with organic matter, higher dosages of sodium hypochlorite can render it microbiologically safe, but such high levels of chlorine adversely affect the taste of the water and so may decrease the willingness of people to treat the water (Reller et al. 2003; World Health Organisation 2003). Although the benefits of water disinfection with sodium hypochlorite are likely to far outweigh the risks of not doing so, the treatment of highly turbid water with large doses of sodium hypochlorite may lead to higher concentrations of potentially toxic chlorinated aromatic compounds (Karol 1995).

In response to these limitations and the persistent unmet need for water treatment, a new flocculant-disinfectant technology for treating water in the home that incorporates techniques used in municipal water purification was developed. The product is a powder that is added to water; uses precipitation, coagulation, and flocculation to remove heavy metals, organic matter, and micro-organisms; and leaves a free chlorine residual. After decanting, the treated water is microbiologically and chemically cleaner, and looks clearer (Rangel et al. 2003; Souter et al. 2003). The immediate improvement in clarity may encourage more people to treat their water. Traditional in-home water clarification practices such as filtration, settling and decanting, and the use of inorganic (Oo et al. 1993) and organic coagulants and flocculants (Dhekane et al. 1970; Tripathi et al. 1976; Jahn 1988; Ndabigengesere and Narasiah 1998) are common in many resource-poor settings. Alum is used frequently for point-of-use flocculation of turbid water in western Kenya and is also used worldwide as a flocculant in municipal water treatment plants. Elsewhere, alum has been demonstrated to improve water quality (Ahmad et al. 1984; Oo et al. 1993; Chowdhury et al. 1997; Karanis and Kimura 2002) and to prevent cholera during an epidemic (Khan et al. 1984). In addition, a dilute sodium hypochlorite solution is available for water disinfection in many areas. Information can be found at the United States Centers for Disease Control and Prevention Safe Water website (http://www.cdc.gov/safewater).

We evaluated the impact of point-of-use water treatment with alum, with sodium hypochlorite, and with combined flocculation–disinfection on drinking water quality in western Kenya.

MATERIALS AND METHODS

Study site

The study was conducted in areas known locally as Asembo and Gem. These areas are located in Rained, Wagai, and part of Yala Division, Siaya and Bondo Districts, western Kenya, near Lake Victoria. The demographic characteristics of persons living in this area has been described elsewhere (Phillips-Howard et al. 2003) and a longstanding surveillance system monitors the aetiology of diarrhoea among this rural population (Shapiro et al. 1999, 2001; Brooks et al. 2000). Persons live in family compounds and rely predominantly on subsistence agriculture and migrant employment. The study was conducted from 15 to 25 October 2002. October falls prior to the commencement of the 'short rains' and is characterized by low rainfall (approx. 60 mm mean monthly rainfall) and warm temperatures (daily mean low and high temperatures of approx. 18 and 29°C) (Bloland et al. 1999).

Water sources

Thirty drinking water sources were sought to represent a range of water source turbidities. Available water sources include Lake Victoria, rivers, streams, excavated ponds, springs, and boreholes. At each water source, six locally purchased, cleaned plastic buckets (buckets A–F) were filled with 10 l of untreated source water.

Interventions

The flocculant–disinfectant product (Procter & Gamble Co., Mason, OH, USA) includes ferric sulphate and calcium hypochlorite (Reller et al. 2003). Each of the ingredients is commonly used in municipal water treatment plants. The ingredients have been especially formulated in single use sachets to work quickly on small volumes of water. The water treatment process combines precipitation, coagulation, and flocculation with disinfection. It aggregates and facilitates the removal of suspended organic matter, bacteria, viruses, parasites, and heavy metals in treated water (Rangel et al. 2003; Souter et al. 2003). A high- and a low-dose formulation of the product were evaluated in this study. The single-use sachet contained sufficient calcium hypochlorite to leave a residual chlorine concentration for the high-dose product of 3.5 mg l⁻¹ and of 2.0 mg l⁻¹ for the low-dose product when added to 10 l of demineralized water. One sachet of the flocculant–disinfectant is added to 10 l of water, stirred vigorously intermittently for 5 min, and allowed to stand until the water is clear and a floc has formed. The clear water is then poured into a second bucket through a 100% cotton cloth to remove any remaining floc particles and large objects such as twigs. The floc remains in the first bucket.

The water disinfectant product solution (Klorin or WaterGuard, Jet Chemicals, Nairobi, Kenya) consisted of 1% sodium hypochlorite manufactured specifically for home water disinfection. As a point-of-use disinfectant, sodium hypochlorite solution has been proved to improve water quality (Sobel et al. 1998; Luby et al. 2001) and to reduce diarrhoeal disease (Semenza et al. 1998; Quick et al. 1999, © 2004 The Society for Applied Microbiology, Journal of Applied Microbiology, 97, 225–231, doi:10.1111/j.1365-2672.2004.02309.x
A recommended dose of 5 ml yielded a residual chlorine concentration of 5 mg L\(^{-1}\) when added to 10 l of demineralized water.

Locally available alum (aluminium sulphate) flocculant is used commonly for the clarification of turbid drinking water in western Kenya. Traditionally, a block of alum (ca 100 g) is mixed gently for approx. 60 s in 10 l of water to initiate flocculation and is then removed. Clear water is decanted after a floc has formed.

**Water treatment and sampling techniques**

Water in bucket A was used as the control and was not treated. Water in bucket B was mixed with the high-dose formulation of the combined flocculant-disinfectant was then mixed vigorously using a spoon intermittently for 5 min and let stand until the water was clear and the floc had grown in size. Treated water was then poured through a new, thick 100% cotton cloth into a second bucket. Water in bucket C was mixed with the low-dose formulation of the combined flocculant-disinfectant and was mixed vigorously intermittently for 5 min and let stand until the water was clear and the floc had grown in size. Treated water was then poured through a new, thick 100% cotton cloth into a second bucket. For both formulations of the combined flocculant-disinfectant product, all measurements were made after treatment with a single sachet. However, when floc failed to form with a single sachet, a second sachet was added to determine if floc formation would occur at a higher dose although the resulting water was not tested further. Water in bucket D was treated with 5 ml of locally available 1% sodium hypochlorite solution for water disinfection (Klorin, Jet Chemical Industries Ltd) and was poured into a second bucket after 30 min. Water in bucket E was treated manually using a 100 g (fist-sized) alum block mixed gently for 60 s using a gloved hand. Treated water was poured into a second bucket after a 30-min settling period, taking care to leave the floc in the original bucket, consistent with local practices. Water in bucket F was treated manually using a 100-g alum block mixed gently for 60 s using a gloved hand and with 5 ml of locally available 1% sodium hypochlorite solution for water disinfection (Klorin, Jet Chemical Industries Ltd). Treated water was poured into a second bucket after a 30-min settling period, taking care to leave the floc in the original bucket, consistent with local practices. Large stainless steel spoons were used for mixing all buckets. All waters were exposed to intervention for 30 min before testing. Buckets were designated ‘dirty’ (untreated water) and ‘clean’ (treated water), and were dedicated to only one type of water treatment. Buckets were cleaned with gloved hands using copious distilled water between water sources.

**Water chemistry**

Free residual and total chlorine levels were measured in the field using a Hach Portable DR/890 Colorimeter (Perma-Chem, Hach Company, Loveland, CO, USA). The system uses the \(n,n\)-diethyl-p-phenylenediamine colorimetric method and the reagent for 10 ml drinking water samples has an upper limit of detection of 2-20 mg L\(^{-1}\) free and total chlorine. The turbidity of the samples was determined in the field using a Hach 2100P Portable Turbidimeter (Hach Co.). This instrument provides a direct readout in nephelometric turbidity units (NTU). pH was measured in the field using a IQ150 Handheld pH Meter (IQ Scientific Instruments Inc., San Diego, CA, USA).

**Water bacteriology**

Water samples were collected using sterile technique in 150 ml sterile plastic containers with thiosulphate (IDEXX Laboratories Inc., Westbrook, ME, USA) and were transported on ice to the Centers for Disease Control and Prevention/Kenya Medical Research Institute (CDC/KEMRI) water bacteriology laboratory for processing within 8 h of collection. The samples were processed with the Colilert Quantitray 2000 system (IDEXX Laboratories Inc.). The media reagent was added to the water sample and shaken until dissolved. The sample mixture was then poured into the Colilert Quantitray 2000 culture tray, sealed, and incubated at 35°C for 24 h. The tray wells were then analysed for colour change and fluorescence. A most probable number table was used for quantification of *Escherichia coli* and total coliform concentrations based on the number of positive wells. All water samples were tested undiluted and at 1 : 10, 1 : 100, and 1 : 1000 dilutions to provide broad range of possible values.

**Statistical methods**

Data were analysed using SAS System for Windows, Release 8.02 (SAS Institute, Cary, NC, USA). Bacterial concentrations are reported as colony forming units per 100 ml derived from most probable number tables. The arithmetic mean or median were used as the measures of central tendency. Median was selected for data series that included values greater than the limit of detection of the instrument. Minimum and maximum values are presented as a measure of spread and to indicate instances where the upper limit of detection was exceeded for the test. Continuous data were compared using the Wilcoxon rank sum test. Proportions were compared using the chi-square test. Comparisons with \(P\)-values <0.05 were considered to be statistically significant.
RESULTS

Water source characteristics

A total of 30 water sources were tested. Eleven samples were collected from ponds or earth pans, eight from streams, two from rivers, three from Lake Victoria, three from springs, and three from boreholes (Table 1). Overall, mean (minimum, maximum) turbidity was 331.9 (0.3, 1724) NTU, pH was 7.19 (5.66, 8.72). E. coli concentration was 3938 (0, 43,900) CFU 100 ml⁻¹ and total coliform concentration was 25,553 (8,4, 122,300) CFU 100 ml⁻¹.

Treated water characteristics

The effect of water treatments on free and total chlorine, turbidity, and pH are summarized in Table 2. Combined flocculant-disinfectant and sodium hypochlorite-based treatments left detectable free chlorine residuals after 30 min. The high-dose formulation of the flocculant-disinfectant product was the only treatment to produce a median free chlorine residual >1 mg l⁻¹. Compared with untreated water, both high- and low-dose formulations of combined flocculant-disinfectant reduced turbidity significantly (331.9 vs 243 and 33.9 NTU, P < 0.001) whereas sodium hypochlorite alone did not mitigate turbidity (331.9 vs 273.4 NTU, P = 0.953). Alum-based treatments lowered pH substantially. In high-turbidity (>100 NTU) water compared with no treatment, high- (0% vs 70%, P = 0.005) and low-dose (0% vs 50%, P = 0.039) product reduced turbidity to the WHO turbidity water quality standard of <5 NTU in the majority of waters (World Health Organization 2003). Although reducing turbidity substantially (Table 2), combined with no treatment alum (0% vs 10%, P = 0.305) or alum plus sodium hypochlorite (0% vs 10%, P = 0.305) did not consistently reduce turbidity to <5 NTU (Table 3). A single sachet of both the low-dose and the high-dose formulation of the flocculant-disinfectant product failed to form a floc in two source waters. In both instances when a floc did not form with a single sachet of flocculation-chlorination product, the addition of a second sachet led to floc formation. The results are reported after treatment with one sachet only.

Disinfectant-containing water treatments performed well in low- and medium-turbidity water for achieving the WHO bacteriologic water quality standard of <1 E. coli CFU 100 ml⁻¹ (World Health Organization 2003) on the majority of occasions. In high-turbidity water compared with no treatment, only the high-dose formulation of combined flocculant-disinfectant product (0% vs 100%, P < 0.001) reduced E. coli concentrations to <1 CFU 100 ml⁻¹ in all 10 samples (Figure 1). Compared with no treatment, low-dose product (0% vs 60%, P = 0.015), sodium hypochlorite (0% vs 60%, P = 0.015), and alum plus sodium hypochlorite (0% vs 90%, P < 0.001) also reduced E. coli concentrations to <1 CFU 100 ml⁻¹ in a significant proportion of samples. However, all high-turbidity samples treated with alum alone failed to reach the WHO bacteriologic potability standard (Fig. 1).

DISCUSSION

These data demonstrate that for source waters over a range of turbidities in western Kenya, high-dose formulation of combined flocculant-disinfectant product effectively reduces turbidity to <5 NTU and reduces E. coli concentrations to <1 CFU 100 ml⁻¹. The combined flocculant-disinfectant product has been demonstrated to remove poliovirus, rotavirus, Cryptosporidium parvum oocysts, and Giardia lamblia cysts from seeded waters from a range of developing countries (Souter et al. 2003) and to improve the microbiologic quality of drinking water in rural Guatemala (Rangel et al. 2003). More recently in Guatemala, the combined flocculant-disinfectant product has

<table>
<thead>
<tr>
<th>Source type</th>
<th>Turbidity*</th>
<th>pH</th>
<th>Escherichia coli concentration†</th>
<th>Total coliform concentration†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond</td>
<td>11 (37)</td>
<td>331.9</td>
<td>7.23 (6.35, 8.72)</td>
<td>6024 (625, 43,900)</td>
</tr>
<tr>
<td>Stream</td>
<td>8 (27)</td>
<td>1007</td>
<td>7.20 (6.69, 8.45)</td>
<td>5924 (135, 42,600)</td>
</tr>
<tr>
<td>River</td>
<td>2 (7)</td>
<td>728</td>
<td>7.29 (7.13, 7.44)</td>
<td>1320 (700, 1860)</td>
</tr>
<tr>
<td>Lake</td>
<td>3 (10)</td>
<td>7.3</td>
<td>8.25 (7.68, 8.49)</td>
<td>420 (5.12, 111)</td>
</tr>
<tr>
<td>Spring</td>
<td>3 (10)</td>
<td>6.4</td>
<td>5.76 (5.66, 5.47)</td>
<td>90 (7, 199)</td>
</tr>
<tr>
<td>Borehole</td>
<td>3 (10)</td>
<td>0.5</td>
<td>7.07 (7.00, 7.5)</td>
<td>76 (0, 228)</td>
</tr>
<tr>
<td>Total</td>
<td>30 (100)</td>
<td>0.3</td>
<td>7.19 (5.66, 8.72)</td>
<td>3938 (0, 43,900)</td>
</tr>
</tbody>
</table>

*Nephelometric turbidity units (NTU).
†Colony-forming units per 100 ml (CFU 100 ml⁻¹).

Table 2 Effect of water treatments on water characteristics

<table>
<thead>
<tr>
<th>Treatment type</th>
<th>Free chlorine*</th>
<th>Total chlorine*</th>
<th>Turbidity†</th>
<th>pH</th>
<th>Escherichia coli concentration‡</th>
<th>Total coliform concentration‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median, Min, Max</td>
<td>Median, Min, Max</td>
<td>Mean, Min, Max</td>
<td>Mean, Min, Max</td>
<td>Mean, Min, Max</td>
<td>Mean, Min, Max</td>
</tr>
<tr>
<td>High-dose product</td>
<td>1.44, 0.09, &gt;2.20</td>
<td>1.64, 0.31, &gt;2.20</td>
<td>24.3, 0.6, 3.410</td>
<td>6.64, 4.56, 7.46</td>
<td>0.0, 0.0, 10.0</td>
<td>0.5, 0.0, 9.7</td>
</tr>
<tr>
<td>Low-dose product</td>
<td>0.42, 0.01, 2.17</td>
<td>0.60, 0.17, &gt;2.20</td>
<td>3.39, 0.5, 5.040</td>
<td>6.67, 5.61, 7.32</td>
<td>4.8, 0.0, 12.46</td>
<td>7.9, 0.0, 12.46</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>0.92, 0.0, &gt;2.20</td>
<td>1.26, 0.34, &gt;2.20</td>
<td>2.734, 0.5, 15.760</td>
<td>7.41, 5.83, 8.85</td>
<td>0.5, 0.0, 10.9</td>
<td>11.6, 0.0, 10.9</td>
</tr>
<tr>
<td>Alum</td>
<td>0.02, 0.00, 0.25</td>
<td>0.05, 0.00, 0.25</td>
<td>3.07, 1.5, 3.570</td>
<td>4.04, 3.71, 4.93</td>
<td>159.4, 0.0, 2490.0</td>
<td>517.5, 0.0, 5860.0</td>
</tr>
<tr>
<td>Alum + sodium hypochlorite</td>
<td>0.78, 0.00, &gt;2.20</td>
<td>1.34, 0.06, &gt;2.20</td>
<td>25.8, 1.4, 27.30</td>
<td>4.14, 3.71, 6.83</td>
<td>0.0, 0.0, 10.0</td>
<td>1.0, 0.0, 11.0</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.03, 0.00, 0.44</td>
<td>0.06, 0.00, 0.55</td>
<td>33.19, 0.3, 1724.0</td>
<td>7.19, 5.66, 8.72</td>
<td>2397.6, 0.0, 43900.0</td>
<td>25535.5, 8.4, 122300.0</td>
</tr>
</tbody>
</table>

*mg/l⁻¹. †Nephelometric turbidity units (NTU). ‡Colony-forming units per 100 ml (CFU 100 ml⁻¹).

Table 3 Proportion of waters with turbidity <5 NTU after treatment by starting turbidity category

<table>
<thead>
<tr>
<th>Treatment type</th>
<th>Waters reaching WHO turbidity standard* n (%) by starting turbidity category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10 NTU (n = 10)</td>
</tr>
<tr>
<td>High-dose product</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Low-dose product</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Alum</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Alum + sodium hypochlorite</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Untreated</td>
<td>5 (50)</td>
</tr>
</tbody>
</table>

*World Health Organization turbidity standard <5 NTU.

Fig. 1 Percentage of water samples rendered potable (World Health Organisation potability standard <1 Escherichia coli CFU 100 ml⁻¹) by starting turbidity category, western Kenya, 2002

been showed to reduce diarrhoea prevalence among persons using it to treat water in the home (Reller et al. 2003). Our study is the first to evaluate the effect of the combined flocculant-disinfectant product on the physical and bacteriologic quality of drinking water over a range of turbidities while simultaneously comparing performance with traditional technologies. The results suggest that the combined flocculant-disinfectant product will be particularly useful in settings where source waters are both highly contaminated and turbid.
Alum combined with sodium hypochlorite also reduces *E. coli* concentrations to <1 CFU 100 ml⁻¹ over a range of source water turbidities. However, locally available alum appears to be inferior to the flocculant-disinfectant for mitigating turbidity. Alum usually failed to reduce turbidity levels to the WHO turbidity standard of <5 NTU.

Traditional alum flocculation without disinfectant mitigates turbidity but does not reliably reduce *E. coli* concentrations to <1 CFU 100 ml⁻¹. Nonetheless, traditional alum flocculation does substantially reduce both *E. coli* and total coliform concentrations. Previous evaluation of alum for decontaminating shallow well water also showed substantial reductions in faecal coliform concentrations (Oo et al. 1993). Shallow well water is both less turbid and less heavily contaminated with faecal coliforms than the majority of source waters evaluated in our study and alum would more readily render such waters potable. Experiments using well and pond water treated with a range of doses of alum and inoculated with *E. coli* or *Vibrio cholerae* at fixed concentrations show that alum doses of 500 µg ml⁻¹ reduced 8-h survival of both organisms (Ahmad et al. 1984). This indicates that alum may also have some residual bactericidal effect for stored water. Furthermore, alum treatment of household water has been demonstrated to reduce secondary transmission within households during a cholera epidemic in Bangladesh (Khan et al. 1984). We show that traditional alum treatment of water substantially reduces the pH of drinking water. Although this pH reduction may contribute to the bacteriologic and clinical impact of alum on *V. cholerae* (Ahmad et al. 1984; Khan et al. 1984) which is exquisitely susceptible to low pH, it also leads to a sour taste (Ahmad et al. 1984) that could result in lower acceptability and use in field studies. Indeed, several villagers reported that stirring the alum in the water for too long was associated with abdominal discomfort. Studies are needed to determine if alum doses required to confer health benefit are associated with reduced use due to unpleasant taste.

We confirm that sodium hypochlorite alone does not mitigate turbidity and achieves *E. coli* concentrations <1 CFU 100 ml⁻¹ in low and medium turbidity water, but not highly turbid water. Sodium hypochlorite solution is one of a very limited range of options available to households with unimproved water supplies to make their water safer. Sodium hypochlorite solution at standard doses performs poorly in the highly turbid waters of western Kenya. As highly turbid source waters are relied on for drinking water by numerous of persons in the developing world, our findings highlight the importance of identifying and evaluating point-of-use water treatments suitable for these conditions.

Potential solutions for the provision of effective water treatments for persons with access to highly turbid source water might include the combination of traditional alum flocculation with sodium hypochlorite solution. However, this requires a two-step treatment process and results in water with residual turbidity and a sour taste. The combined flocculant-disinfectant product shows promise as an alternative point-of-use treatment that requires a single treatment step, maintains a neutral pH, and renders highly turbid source water clear and safe for drinking.

Worldwide, the overwhelming majority of people who are drinking contaminated water are not treating their water before consuming it. New ways to encourage communities whose children are at high risk of death from diarrhoeal disease to treat their water could save millions of lives. Further research is needed to characterize the performance of various point-of-use water treatments in different geographic settings. Our data indicate that combined flocculant-disinfectant products are particularly effective where source waters are highly turbid. A health outcome study comparing the novel flocculant-disinfectant, sodium hypochlorite, and traditional water-handling practices is planned at the western Kenya site to evaluate the effect on diarrhoeal disease of these water treatments among families using highly turbid source water for drinking.

ACKNOWLEDGEMENTS

We thank the staff of the CDC/KEMRI Kisian Research Station for their assistance and advice in conducting this study. We thank Dr Davey K. Koech (Director of the Kenya Medical Research Institute) for his cooperation and permission to publish this paper. This study was supported by a cooperative research and development agreement between the Centers for Disease Control and Prevention and the Procter & Gamble Company.

REFERENCES


5.13. **Household based treatment of drinking water with floculant-disinfectant for preventing diarrhoea in areas with turbid source water in rural western Kenya: cluster randomized controlled trial**


**CONTRIBUTION**

I conceived the research idea, designed the study, and lead all aspects of the research under the mentorship of Luby. Luby and Keswick sought and obtained funding. Otieno served as study coordinator, managing day-to-day operations of the study team under my guidance. Rosen and Hoekstra oversaw statistical analysis. I hired and trained all staff, developed all standard operating procedures, and implemented all aspects of the research in the field and the laboratory. I conducted initial analysis and wrote the first draft of the paper. Slutsker directed and Vulule oversaw the research site that hosted the study team; they assisted with adaption of the study design to the site and with project administration. All authors contributed to revisions of the manuscript.

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Household based treatment of drinking water with flocculant-disinfectant for preventing diarrhoea in areas with turbid source water in rural western Kenya: cluster randomised controlled trial

John A Crump, Peter O Otieno, Laurence Slutsker, Bruce H Keswick, Daniel H Rosen, R Michael Hoekstra, John M Vulule, Stephen P Luby

Abstract

Objective To compare the effect on prevalence of diarrhoea and mortality of household based treatment of drinking water with flocculant-disinfectant, sodium hypochlorite, and standard practices in areas with turbid water source in Africa.

Design Cluster randomised controlled trial over 20 weeks.

Setting Family compounds, each containing several houses, in rural western Kenya.

Participants 6650 people in 605 family compounds.

Intervention Water treatment: flocculant-disinfectant, sodium hypochlorite, and usual practice (control).

Main outcome measures Prevalence of diarrhoea and all cause mortality. Escherichia coli concentration, free residual chlorine concentration, and turbidity in household drinking water as surrogates for effectiveness of water treatment.

Results In children < 2 years old, compared with those in the control compounds, the absolute difference in prevalence of diarrhoea was –25% in the flocculant-disinfectant arm (95% confidence interval –40 to –5) and –17% in the sodium hypochlorite arm (–34 to 4). In all age groups compared with control, the absolute difference in prevalence was –19% in the flocculant-disinfectant arm (–34 to –2) and –26% in the sodium hypochlorite arm (–39 to –9). There were significantly fewer deaths in the intervention compounds than in the control compounds (relative risk of death 0.58, P = 0.036). Fourteen per cent of water samples from control compounds had E coli concentrations < 1 CFU/100 ml compared with 82% in flocculant-disinfectant and 76% in sodium hypochlorite compounds. The mean turbidity of drinking water was 8 nephelometric turbidity units (NTU) in flocculant-disinfectant households, compared with 55 NTU in the two other compounds (P < 0.001).

Conclusions In areas of turbid water, flocculant-disinfectant was associated with a significant reduction in diarrhoea among children < 2 years. This health benefit, combined with a significant reduction in turbidity, suggests that flocculant-disinfectant is well suited to areas with highly contaminated and turbid water.

Introduction

Studies in developing countries have shown that household based disinfection of drinking water reduces the incidence of diarrhoea by 20-48%.1-4 Disinfectants, however, may adversely affect the taste of drinking water and may not improve its appearance. Sodium hypochlorite—a widely used household based disinfectant—is less effective in highly turbid water6 and for pathogens resistant to chlorine.7

A new flocculant-disinfectant technology has been developed for treating water in the home.7,8 This treatment could be useful in areas with turbid source water as the improvement in water clarity would encourage use, reduce chlorine demand, and remove some chlorine-resistant organisms.

We conducted a 20 week study to evaluate the efficacy of the flocculant-disinfectant in preventing diarrhoea in rural western Kenya, an area with heavily faecally contaminated and highly turbid source water. The primary hypothesis was that children < 2 years living in family compounds that received flocculant-disinfectant would have fewer episodes of diarrhoea than children in compounds using sodium hypochlorite. We also compared the effect on prevalence of diarrhoea in all ages compared with usual water handling practices (see bmj.com) and assessed the relative acceptability of the two interventions.

Methods

Setting

The study was conducted in 49 villages near Lake Victoria in Siaya and Bondo Districts, western Kenya. Infant mortality is about 150 per 1000 inhabitants.9 Drinking water is usually obtained from ponds, rivers, and springs; it is regularly contaminated with both human and animal faeces. Water is typically carried in 20 l plastic drums and stored in wide mouthed clay vessels holding 20-30 l.10
Interventions
The flocculant-disinfectant comes in single use sachets for use with small volumes of water. It aggregates and facilitates the removal of suspended organic matter, bacteria, viruses, parasites, and heavy metals in treated water. One packet contains enough calcium hypochlorite to leave a residual chlorine concentration of 3.5 mg/l in 10 l of demineralised water. The sodium hypochlorite treatment used 1% sodium hypochlorite solution. In the control group, participants continued their usual water collection, treatment, and storage practices (see bmj.com).

We identified 600 family compounds with at least one child aged <2 years; 300 used pond water and 300 used river water. Family compounds were randomly assigned to one of the three study arms at each of the two sites.

Data collection
Field workers visited participating compounds weekly and recorded the presence or absence of diarrhoea and any deaths during the seven days since the last visit for each person. They also assessed the mothers’ knowledge of and attitudes towards the interventions during the fifth and 15th week of the study. During the baseline survey and during unannounced visits every four weeks field workers collected samples of stored drinking water to measure free chlorine concentration and turbidity and samples of source water to measure turbidity. During the baseline survey and the 10th week of the study, the concentration of *Escherichia coli* was measured in samples of stored drinking water.

To assess compliance each week field workers collected and counted empty sachets of flocculant-disinfectant and empty sodium hypochlorite bottles and replaced them. At the end of the study, partially used bottles were collected and weighed to determine the total use of sodium hypochlorite.

Residual free chlorine concentration was measured in samples of stored household water collected in sterile plastic bags. During routine weekly visits, residual free total chlorine concentration and turbidity was measured in the field. Household water samples were also tested for bacteriology. See bmj.com for further details of methods.

Statistical analysis
We calculated that we would need 200 family compounds, each containing at least one child <2 years, per intervention group to detect relevant difference in the prevalence of diarrhoea between the children <2 years in the two intervention groups. To evaluate the effects of interventions on prevalence of diarrhoea, we aggregated and compared results at the level of randomisation (the compound level) and over time to account for clustering and repeated measures. We fitted a generalised linear model to the data, with log link binomial distribution and adjustment for overdispersion, to compare the proportional reduction in overall prevalence of diarrhoea.

Results
Of 1860 family compounds, 605 had a child aged <2 years, used turbid drinking water, and agreed to participate. Of these, 201 were assigned to flocculant-disinfectant, 203 to sodium hypochlorite, and 201 to standard water handling. Of 133 000 potential person weeks of observation for diarrhoea, 24 525 (18.4%) were missing because of short or long term outward migration or death. We did not exclude any family compound from the analysis. The study team completed 108 475 person weeks of observation for 6650 people, including 9999 person weeks of observation for 715 children <2 years. At baseline, all groups had similar in terms of family compound sizes, education level of the household head, sanitation, and water handling practices.

**Weekly prevalence of diarrhoea**
The crude weekly prevalence of diarrhoea among control compounds varied during the study period from 1.69 weeks with diarrhoea per 100 person weeks in July 2003 to 4.61 weeks with diarrhoea per 100 person weeks in May 2003 at the onset of the long seasonal rains (figure).

The adjusted absolute difference in prevalence of diarrhoea among children <2 years was –25% in the flocculant-disinfectant compounds and –17% in the sodium hypochlorite compounds. Children <2 years in control compounds had 9.64 weeks with diarrhoea per 100 person weeks. Children in the two intervention compounds had similar prevalence of diarrhoea (table). The adjusted absolute difference in prevalence of diarrhoea was –19% in the flocculant-disinfectant compounds and –26% in the sodium hypochlorite compounds (table).

There were 282277 deaths in the control group, 142124 in the flocculant-disinfectant group (relative risk of death 0.83, P=0.052 compared with control), and 172249 in the sodium hypochlorite group (0.61, P=0.108). The pooled data showed that there were significantly fewer deaths in the intervention compounds than the control compounds (0.58, P=0.036). Fifteen (54%) of the 28 who died in the control compounds were children <5 years compared with five (36%) in the flocculant-disinfectant compounds and four (26%) in the sodium hypochlorite compounds.
Prevalence of diarrhoea by water treatment intervention group among various age groups, western Kenya

<table>
<thead>
<tr>
<th>Age group (intervention)</th>
<th>Person level observations</th>
<th>Compound level observations*</th>
<th>Absolute difference in diarrhoea prevalence v control (%)</th>
<th>Absolute difference in diarrhoea prevalence v sodium hypochlorite (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Person weeks of observation</td>
<td>Weeks with diarrhoea</td>
<td>Compounds under observation</td>
<td>Weeks with diarrhoea</td>
</tr>
<tr>
<td>Age &lt;2 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flocculant-disinfectant</td>
<td>3381</td>
<td>251</td>
<td>177</td>
<td>7.60</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>3151</td>
<td>258</td>
<td>169</td>
<td>7.81</td>
</tr>
<tr>
<td>Control</td>
<td>3467</td>
<td>342</td>
<td>176</td>
<td>9.64</td>
</tr>
<tr>
<td>All ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flocculant-disinfectant</td>
<td>34 775</td>
<td>689</td>
<td>201</td>
<td>2.22</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>36 438</td>
<td>675</td>
<td>203</td>
<td>2.06</td>
</tr>
<tr>
<td>Control</td>
<td>37 062</td>
<td>929</td>
<td>201</td>
<td>2.67</td>
</tr>
</tbody>
</table>

Ref-reference category.
*Data aggregated and compared at the level of randomisation (compound level).
†Compared by generalised linear model with log link binomial distribution and adjustment for overdispersion.

Acceptability of intervention
By the fifth week, all 191 respondents from the flocculant-disinfectant compounds reported that their water looked better after treatment compared with 149 (77%) of 193 in the sodium hypochlorite compounds (relative risk 1.3, 95% confidence interval 1.2 to 1.4). Ratings of taste and smell did not differ significantly between the two groups. All respondents from flocculant-disinfectant compounds and 99% from sodium hypochlorite compounds preferred treated water to untreated water.

Use of intervention
During scheduled visits 86% of drinking water samples from flocculant-disinfectant compounds and 85% from sodium hypochlorite compounds had free chlorine concentrations >0.1 mg/l. In samples collected during unannounced visits, 44% of flocculant-disinfectant households and 61% of sodium hypochlorite households had free chlorine concentrations >0.1 mg/l. The median free residual chlorine concentrations in treated waters, however, was only 0.4 mg/l in both intervention arms, which may indicate substantial binding of free chlorine to residual organic and inorganic material in drinking water or prolonged storage of treated water in open containers.

Samples of drinking water from intervention households were more likely to meet WHO guidelines for bacteriological quality than samples from control households. Furthermore, drinking water from flocculant-disinfectant households had much lower turbidity than samples from control or sodium hypochlorite households (8 Å ≈ 555 nephelometric turbidity units, P < 0.001, by Student's t test).

Discussion
In this setting where diarrhoea is a leading cause of childhood death and drinking water is highly turbid and contaminated with faeces, we found that children <2 years from family compounds that treated their drinking water with flocculant-disinfectant had significantly less diarrhoea than compounds that used standard practices (control). Among people of all ages, those in compounds where water was treated with flocculant-disinfectant or sodium hypochlorite had significantly less diarrhoea than control compounds. There was no significant difference in prevalence of diarrhoea between the two interventions in either age group.

This is the first study of household based water treatment to show a significant reduction in mortality, despite a modest reduction in prevalence of diarrhoea. The trend towards younger age at death in the control arm suggests an effect on mortality among infants and children in the intervention arms.

The lack of observed difference in prevalence of diarrhoea between the two intervention arms may have been due to a lack of statistical power. Weekly prevalence of diarrhoea in the sodium hypochlorite arm reached only one third of that modelled in our estimation of sample size. The lack of observed differences between study arms may also have been due to limited intervention effects. Initially, water may not have been turbid enough for us to show the differential effects of the flocculant-disinfectant on water quality compared with sodium hypochlorite. The effect on health may have been greater if use had been higher or if it had been possible to minimise the drinking of untreated water outside the home.

The flocculant-disinfectant was highly acceptable to consumers, and this was closely linked to its ability to reduce turbidity. If the flocculant-disinfectant was available in the market place, the visible effect on turbidity may lead more families to use household based water treatment. Low use has been a key challenge, so properties that encourage purchase and use could improve effectiveness. Sodium hypochlorite makes water safer to drink but also alters the taste and does not reduce turbidity. The flocculant-disinfectant offers improvements in the aesthetic qualities of water while also providing a health benefit.
Skin biopsy rates and incidence of melanoma: population based ecological study

H Gilbert Welch, Steven Woloshin, Lisa M Schwartz

Abstract

Objectives To describe changes in skin biopsy rates and to determine their relation to changes in the incidence of melanoma.

Design Population based ecological study.

Setting Nine geographical areas of the United States.

Participants Participants of the Surveillance Epidemiology and End Results (SEER) programme aged 65 and older.

Main outcome measures For the period 1986 to 2001, annual skin biopsy rates for each surveillance area from Medicare claims and incidence rates for melanoma for the same population.

Results Between 1986 and 2001 the average biopsy rate across the nine participating areas increased 2.5-fold among people aged 65 and older (2847 to 7222 per 100 000 population). Over the same period the incidence rate for melanoma increased 2.4-fold (45 to 108 per 100 000 population). Assuming that the occurrence of true disease was constant, the extra number of melanoma cases that were diagnosed after carrying out 1000 additional biopsies was 12.6 (95% confidence interval 11.2 to 14.0). After controlling for a potential increase in the true occurrence of disease, 1990 additional biopsies were still associated with 6.9 (3.1 to 10.8) extra melanoma cases diagnosed. Stage specific analyses suggested that 1000 biopsies were associated with 4.4 (2.1 to 6.8) extra cases of in situ melanoma diagnosed and 2.5 (0.0 to 4.6) extra cases of local melanoma, but not with the incidence of advanced melanoma. Mortality from melanoma changed little during the period.

Conclusion The incidence of melanoma is associated with biopsy rates. That the extra cases diagnosed were confined to early stage cancer while mortality remained stable suggests overdiagnosis—the increased incidence being largely the result of increased diagnostic scrutiny and not an increase in the incidence of disease.

Detailed model outputs are on bmj.com
5.14. Effect of trimethoprim-sulfamethoxazole prophylaxis on antimicrobial resistance of fecal Escherichia coli in HIV-infected patients in Tanzania


CONTRIBUTION

My position as last author reflects my role as senior, supervising author on the paper. I conceived the research idea, sought and obtained funding, designed the study, and supervised the research including the study team members responsible for participant enrollment, data collection, laboratory assessments, data analysis, and write up. I mentored the post-doctoral fellow, Dr. Morpeth. Morpeth and Ramadhani managed day-to-day operations of the study and wrote the first draft of the manuscript. Ostermann led statistical analysis. Kisenge and H.J. Shao assisted in the study clinic. Reller and Sam led laboratory assessments. Itemba oversaw patient referral from the HIV counseling and testing site. Hamilton, Bartlett, J.F. Shao, and Itemba managed interactions with personnel at study sites and partner institutions. All authors contributed to revisions of the manuscript.

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Effect of Trimethoprim-Sulfamethoxazole Prophylaxis on Antimicrobial Resistance of Fecal Escherichia coli in HIV-Infected Patients in Tanzania

Susan C. Morpeth, MB, ChB, DTM&H,*† Nathan M. Thielman, MD, MPH,* Habib O. Ramadhani, MD,‡ John D. Hamilton, MD,* Jan Ostermann, PhD,§ Peter R. Kisenge, MD, MMed,‡ Humphrey J. Shao, MD,‡ L. Barth Reller, MD, DTM&H,† Dafrosa K. Itemba, BA,|| Noel E. Sam, MD, MMed,†¶ John A. Bartlett, MD,**¶ John F. Shao, MD, MSc, PhD,‡¶ and John A. Crump, MB, ChB, DTM&H*†¶

Background: Trimethoprim-sulfamethoxazole (SXT) reduces morbidity and mortality among HIV-infected persons in Africa, but its impact on antimicrobial resistance is of concern.

Methods: HIV-uninfected (group A), HIV-infected but not requiring SXT (group B), and HIV-infected and eligible for SXT (group C) adults were recruited into a prospective observational cohort study in Moshi, Tanzania. Stool was examined for Escherichia coli nonsusceptible to SXT at baseline and at weeks 1, 2, 4, and 24. General estimating equation models were used to assess differences in susceptibility over time and cross-resistance to other antimicrobials.

Results: Of 181 subjects, 118 (65.1%) were female and the median (range) age was 36 (20 to 72) years. At baseline, E. coli nonsusceptible to SXT was isolated from 23 (53.5%) of 43 patients in group A, 25 (67.6%) of 37 patients in group B, and 37 (64.9%) of 57 patients in group C. The odds ratios (P value) for SXT nonsusceptibility in group C at weeks 1, 2, 4, and 24 compared with baseline were 3.4 (0.013), 3.0 (0.019), 2.9 (0.030), and 1.5 (0.515), respectively. SXT nonsusceptibility was associated with nonsusceptibility to ampicillin, chloramphenicol, ciprofloxacin, and nalidixic acid (P = 0.006).

Conclusion: In Tanzania, carriage of fecal E. coli nonsusceptible to SXT is common before SXT prophylaxis. Initiation of SXT leads to further loss of susceptibility to SXT and to other antimicrobials.

Key Words: antibiotic prophylaxis, Escherichia coli, feces, HIV, Tanzania, trimethoprim-sulfamethoxazole combination (J Acquir Immune Defic Syndr 2008;47:585–591)

Trimethoprim-sulfamethoxazole (SXT) has been shown to reduce morbidity and mortality among persons living with HIV in Africa.1–4 Based on the results of clinical trials from the West African country of Côte d’Ivoire,1,4 in 2000, the World Health Organization (WHO) and the Joint United Nations Program on AIDS (UNAIDS) recommended the use of SXT prophylaxis for persons with symptomatic HIV disease or with CD4 T-lymphocyte counts (CD4 counts) <500 cells/mm³ in Africa.5 At the time, uncertainty was expressed about whether clinical benefits seen in Côte d’Ivoire, where the prevalence of resistance to SXT is relatively low,6 would also be seen in East Africa and southern Africa, where the prevalence of resistance is higher.7,8 In addition, concern was raised that the widespread use of SXT may substantially increase the prevalence of antimicrobial resistance in common community-acquired pathogens.

Inexpensive and relatively safe, SXT and the related compound sulfadoxine-pyrimethamine play central roles in the management of common clinical syndromes in Africa. These drugs are frequently used to treat dysentery, lower respiratory tract infection, and fever in which Shigella spp., non-Typhi serotypes of Salmonella enterica, Streptococcus pneumoniae, and Plasmodium spp., respectively, play major roles.9,10 It follows that increases in resistance to SXT among these pathogens could reduce the effectiveness of empiric treatment strategies, leading to more illness and death. Large,
randomized, community-based cohort studies would be required to investigate the role that SXT prophylaxis plays in the emergence of antimicrobial resistance among isolates from patients with these specific infections at the community level. Randomized studies of SXT prophylaxis are no longer acceptable, however, because of the established benefit of SXT on morbidity and mortality.

To understand the role that SXT might play in driving antimicrobial resistance, we selected fecal Escherichia coli as an indicator organism for enteric pathogens. We then examined the hypothesis that initiation of SXT prophylaxis in HIV-infected individuals would lead to rapid and widespread resistance of fecal E. coli to SXT compared with HIV-infected and HIV-uninfected persons not receiving SXT.

METHODS

Study Design and Participants

We designed a 3-group prospective observational cohort study of persons aged ≥18 years who had recently received HIV voluntary counseling and testing (VCT) at VCT centers in Moshi, Tanzania, between August 2004 and December 2005. Some patients were co-enrolled in another study that evaluated the role of simple clinical and laboratory evaluations to identify HIV-infected patients with CD4 counts <200 cells/mm$^3$.

Clinical Procedures

VCT centers referred HIV-infected and HIV-uninfected subjects to the Infectious Diseases Clinic (IDC) at Kilimanjaro Christian Medical Centre (KCMC) for management of HIV infection and for study enrollment. After providing written informed consent, a standardized clinical history and physical examination were done. HIV-infected patients not yet on SXT were staged according to the WHO system. In accordance with WHO/UNAIDS recommendations, HIV-uninfected patients (group A) and those with asymptomatic HIV infection (WHO stage 1; group B) were not offered SXT. Patients with symptomatic HIV infection (WHO stage 2, 3, or 4; group C) were offered free SXT prophylaxis in the form of 2 single-strength tablets, each containing 80 mg of trimethoprim and 400 mg of sulfamethoxazole, daily. Pregnancy in women of reproductive age was excluded at each visit using a menstrual history and urine pregnancy test. Although women in the first trimester of pregnancy were not included in the study, those in the second or third trimester were included. Whole stool was collected at the baseline visit and before the first dose of SXT for all subjects. Subjects then returned to the KCMC IDC 1, 2, 4, and 24 weeks after the baseline visit. At each visit, whole stool was collected and the standardized clinical history and physical examination were repeated. Adherence to SXT prophylaxis was assessed at each follow-up visit by patient self-report using a standardized questionnaire. Patients who entered the study in WHO stage 1 and progressed to WHO stages 2 through 4 or those who were found to have CD4 counts <500 cells/mm$^3$ were allowed to move between study groups. This study spanned a period of transition from the availability of antiretroviral therapy (ART) to patients in Tanzania who could afford it to the provision of free therapy.

Laboratory Procedures

Whole stool was inoculated to MacConkey agar with an SXT disk and incubated for 24 hours at 37°C. Plates were examined for the presence of flat, dry, lactose-utilizing colonies consistent with E. coli. The presence or absence of presumptive E. coli was recorded. If colonies consistent with E. coli were not seen within <16 mm of the SXT disk, the stool was classified as having susceptible E. coli. If colonies consistent with E. coli were seen within <16 mm of the SXT disk, the stool was classified as having nonsusceptible E. coli and the colony nearest to the disk was picked and subcultured to sheep blood agar. The inoculated sheep blood agar plate was then incubated for 24 hours at 37°C, and the spot indole test was performed on the resulting growth. Indole-positive isolates were stored on nutrient agar at room temperature. All SXT-nonsusceptible E. coli isolates and a sample of susceptible isolates were shipped to the Duke University Medical Center Clinical Microbiology Laboratory (DUMC CMB) for further evaluation.

At the DUMC CMB, isolates were subcultured to sheep blood agar and MacConkey agar and were confirmed as E. coli using oxidase and indole tests. Isolates without classic E. coli colony morphology were identified using the MicroScan WalkAway system panelNEG Combo type 32 (Dade MicroScan, West Sacramento, CA). Susceptibility testing for ceftriaxone, nalidixic acid, ampicillin, ciprofloxacin, chloramphenicol, and azithromycin was done using the Kirby-Bauer disk diffusion method to Clinical Laboratory Standards Institute (CLSI) standards. Staphylococcus aureus interpretive criteria were used to evaluate zone sizes for azithromycin. Minimum inhibitory concentration (MIC) to SXT was determined using the E-test (AB BIODISK, Solna, Sweden).

Statistical Analysis

Prespecified analyses included descriptive analyses of the cohort by study group, comparison of changes in the proportion of E. coli nonsusceptible to SXT by study arm over time, and assessment of the effect of SXT use on coselection of nonsusceptibility to other antimicrobial agents. The characteristics of study subjects and E. coli antimicrobial susceptibility testing results were calculated as medians, ranges, and proportions. Antimicrobial susceptibility patterns were modeled using general estimating equations to account for repeated measures on individuals. Within-group differences over time were assessed in a pooled model with interactions between study group and visit type. All analyses were done using STATA, version 9.2 (Stata Corporation, College Station, TX).

Research Ethics

The protocol for this study was approved by the KCMC Research Ethics Committee, the Tanzania National Institute for Medical Research National Research Ethics Coordinating Committee, and an Institutional Review Board of Duke University Medical Center.

RESULTS

Baseline Characteristics

One hundred eighty-one subjects were seen at baseline. Of these, 118 (65.1%) were female and the median (range) age
was 36 (20 to 72) years. Of the 181 subjects, 54 (29.8%) were in group A, 53 (29.3%) were in group B, and 74 (40.9%) were in group C. A greater proportion of subjects in groups B and C had primary education or less than subjects in group A. All patients in group B were in WHO stage 1. All patients in group C were in WHO stage 2, 3, or 4, but 58 (78.4%) of 74 patients in group C were in WHO stage 3 or 4. The median (range) CD4 count at baseline was 297 (56 to 1200) cells/mm³ in group B compared with 187 (2 to 1322) cells/mm³ in group C. Other baseline characteristics of subjects are shown in Table 1.

Use of SXT and Related Antimicrobials

The proportions of subjects in group C reporting 100% adherence to SXT at study weeks 1, 2, 4, and 24 were 79.0%, 80.0%, 73.1%, and 75.0%, respectively, in group C. Two subjects in group A and 7 subjects in group B took short courses of SXT during the study. Three subjects in group A and 1 subject in group B took short courses of sulfadoxine-pyrimethamine during the study. SXT was discontinued by 1 (1.4%) subject in group C because of rash. No patient developed Stevens-Johnson syndrome.

Susceptibility of Fecal Escherichia coli to SXT at Follow-Up

Of 181 study subjects, 158 (87.3%) were retained in follow-up for the week 1 visit, 138 (76.2%) at week 2, 132 (72.9%) at week 4, and 91 (50.3%) at week 24. Subject retention by study group is shown in Figure 1A. There was no difference in subject retention between the 3 groups by study week, except at week 24, when more subjects were retained in group A compared with group C (P = 0.004). Of persons retained to follow-up, E. coli was isolated from 137 (75.7%) persons at baseline, 137 (86.7%) at week 1, 126 (91.3%) at week 2, 115 (87.1%) at week 4, and 81 (89.0%) at week 24. Of baseline stool samples, SXT-nonsusceptible E. coli was isolated from 23 (53.5%) of 43 group A patients, 25 (67.6%) of 37 group B patients, and 37 (64.9%) of 57 group C patients. Baseline proportions of E. coli nonsusceptible to SXT were not significantly different between the 3 groups (P = 0.365). By week 1, SXT nonsusceptibility was present in E. coli from 17 (43.6%) of 39 subjects in group A, 21 (72.5%) of 29 subjects in group B, and 50 (86.2%) of 58 subjects in group C. Changes in the proportion of E. coli isolates in these and all subsequent study groups and study weeks are illustrated in Figure 1B. A comparison of the proportions of E. coli isolates nonsusceptible to SXT across study groups by study week yielded significantly higher proportions nonsusceptible in group C relative to group A at weeks 1, 2, and 4 (P < 0.001, P < 0.001, and P = 0.006, respectively) and in group B relative to group A at weeks 1 and 2 (P = 0.009 and P = 0.031, respectively). The differences between group C and group B were not statistically significant at conventional levels (P > 0.092). In a generalized estimating equation model, the odds ratios (ORs) for resistance in group C at study weeks 1, 2, 4, and 24 compared with baseline were 3.4 (P = 0.013), 3.0 (P = 0.019), 2.9 (P = 0.030), and 1.5 (P = 0.515), respectively. No significant differences in the odds of SXT resistance were seen in group A or B compared with baseline (Table 2).

Escherichia coli SXT Nonsusceptibility and Other Antimicrobials

Coselection of antimicrobial nonsusceptibility was assessed among 419 fecal E. coli isolates. SXT nonsusceptibility was associated with nonsusceptibility to ampicillin (OR = 10.2; P < 0.001), chloramphenicol (OR = 7.8; P < 0.001), ciprofloxacin (OR = 17.1; P = 0.006), and nalidixic acid (OR = 26.4; P = 0.001) but not with nonsusceptibility to azithromycin (OR = 1.2; P = 0.545) (Table 3). All fecal E. coli isolates were susceptible to ceftriaxone.

DISCUSSION

We demonstrate that in northern Tanzania, carriage of fecal E. coli nonsusceptible to SXT is common among HIV-uninfected persons and among HIV-infected patients before the commencement of SXT prophylaxis. Furthermore, the

| Table 1. Sociodemographic and Clinical Characteristics of Study Subjects at Baseline Visit, KCMC, 2004 to 2005 |

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV Uninfected (Group A)</th>
<th>HIV Infected, No SXT (Group B)</th>
<th>HIV Infected, SXT (Group C)</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/n (% or min, max)</td>
<td>n/n (% or min, max)</td>
<td>n/n (% or min, max)</td>
<td>n/n (% or min, max)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>29/54 (53.7)</td>
<td>34/53 (64.2)</td>
<td>55/74 (74.3)</td>
<td>118/181 (65.2)</td>
</tr>
<tr>
<td>Median age, y (min, max)</td>
<td>36 (20, 72)</td>
<td>34 (21, 63)</td>
<td>39 (20, 65)</td>
<td>36 (20, 72)</td>
</tr>
<tr>
<td>Primary education or less, n (%)</td>
<td>7/54 (12.9)</td>
<td>13/53 (24.5)</td>
<td>16/74 (21.6)</td>
<td>36/181 (19.9)</td>
</tr>
<tr>
<td>Urban, n (%)</td>
<td>26/54 (48.1)</td>
<td>21/53 (39.6)</td>
<td>24/74 (32.4)</td>
<td>71/181 (39.2)</td>
</tr>
<tr>
<td>WHO stage, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>26/54 (12.9)</td>
<td>13/53 (24.5)</td>
<td>16/74 (21.6)</td>
<td>53/127 (41.7)</td>
</tr>
<tr>
<td>3</td>
<td>29/54 (53.7)</td>
<td>53/53 (100.0)</td>
<td>0/74 (0.0)</td>
<td>53/127 (41.7)</td>
</tr>
<tr>
<td>4</td>
<td>29/54 (53.7)</td>
<td>0/53 (0.0)</td>
<td>8/74 (11.7)</td>
<td>53/127 (41.7)</td>
</tr>
<tr>
<td>Median CD4 count, cells/mm³ (min, max)</td>
<td>297 (56, 1200)</td>
<td>187 (2, 1322)</td>
<td>211 (2, 1322)</td>
<td></td>
</tr>
<tr>
<td>Median body mass index (range)</td>
<td>21.8 (15.8, 39.5)</td>
<td>22.0 (17.8, 35.3)</td>
<td>19.0 (11.7, 40.5)</td>
<td>21.2 (11.7, 40.5)</td>
</tr>
</tbody>
</table>

max indicates maximum; min, minimum; NA, not applicable.
initiation of SXT prophylaxis rapidly leads to further loss of susceptibility not only to SXT but to other important antimicrobial agents. These findings provide valuable insights into the possible negative consequences of widespread use of life-extending SXT for HIV-infected individuals in Africa.

The large proportion of subjects found to be carrying SXT-nonsusceptible E. coli before commencement of SXT prophylaxis was consistent with reports showing SXT nonsusceptibility to be common among other Enterobacteriaceae from patients in East Africa and southern Africa.8,16,17 Concern about the impact of SXT nonsusceptibility among key HIV bacterial copathogens on the efficacy of SXT prophylaxis has been raised.18 Although large observational studies done in East Africa and southern Africa have shown that SXT prophylaxis significantly reduces morbidity and mortality in people with HIV, the potential for development of resistance to SXT and other antimicrobials is a concern.

### TABLE 2. Changes in the Risk of E. coli Nonsusceptibility to SXT by Study Week Relative to Baseline, KCMC, 2004 to 2005

<table>
<thead>
<tr>
<th>Follow-Up Week</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.67 (0.30, 1.5)</td>
<td>0.324</td>
<td>1.3 (0.49, 3.2)</td>
<td>0.645</td>
<td>3.4 (1.3, 8.9)</td>
<td>0.013</td>
</tr>
<tr>
<td>2</td>
<td>0.92 (0.40, 2.1)</td>
<td>0.846</td>
<td>1.4 (0.54, 3.8)</td>
<td>0.465</td>
<td>3.0 (1.2, 7.3)</td>
<td>0.018</td>
</tr>
<tr>
<td>4</td>
<td>1.14 (0.44, 2.9)</td>
<td>0.779</td>
<td>1.3 (0.43, 3.6)</td>
<td>0.680</td>
<td>2.9 (1.1, 7.7)</td>
<td>0.030</td>
</tr>
<tr>
<td>24</td>
<td>2.04 (0.75, 5.5)</td>
<td>0.161</td>
<td>1.3 (0.44, 3.8)</td>
<td>0.637</td>
<td>1.5 (0.46, 4.6)</td>
<td>0.515</td>
</tr>
</tbody>
</table>

ORs and 95% confidence intervals (CIs) were calculated on the basis of parameter estimates and standard errors from a general estimating equation model with interactions between study group and visit type.

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Table 3. Antimicrobial Susceptibility of SXT-Susceptible and Nonsusceptible Fecal E. coli, KCMC, 2004 to 2005

<table>
<thead>
<tr>
<th>Proportion Nonsusceptible to Other Antimicrobials n (%)</th>
<th>SXT Susceptibility</th>
<th>Amoxicillin n (%)</th>
<th>Azithromycin n (%)</th>
<th>Chloramphenicol n (%)</th>
<th>Ciprofloxacin n (%)</th>
<th>Nalidixic Acid n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SXT susceptible (n = 180)</td>
<td>25 (13.9)</td>
<td>146 (80.7)</td>
<td>5 (2.8)</td>
<td>1 (0.6)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td>SXT nonsusceptible (n = 239)</td>
<td>153 (64.6)</td>
<td>202 (84.5)</td>
<td>44 (18.4)</td>
<td>20 (8.4)</td>
<td>26 (10.9)</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>10.2</td>
<td>1.2</td>
<td>7.8</td>
<td>17.1</td>
<td>26.4</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>5.9 to 17.8</td>
<td>0.71 to 1.9</td>
<td>3.0 to 20.2</td>
<td>2.3 to 127.7</td>
<td>3.6 to 194.5</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.545</td>
<td>&lt;0.005</td>
<td>0.006</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

ORs and 95% CIs were calculated using general estimating equation models predicting nonsusceptibility to each antimicrobial.

found dfr or sul gene-containing integrons present in 59% of isolates. Analysis of the regional distribution of these integrons indicated that horizontal gene transfer was the main mechanism of resistance spread rather than clonal expansion.24 These studies suggest that coselection of resistance by means of mobile genetic elements in fecal E. coli attributable to SXT use is likely to occur and that these genetic elements can disseminate from fecal flora to bacterial enteric pathogens.

Azithromycin has been proposed as a possible alternative to SXT for prophylaxis among HIV-infected persons in Africa.4 Azithromycin might provide a replacement antimicrobial for patients with sulfa drug sensitivity or in populations in which SXT nonsusceptibility among important human pathogens becomes sufficiently common so as to impair its efficacy for prophylaxis or treatment. In addition, its spectrum of in vitro activity includes a number of important HIV copathogens such as Streptococcus pneumoniae, Pneumocystis jiroveci, Toxoplasma gondii, and Plasmodium spp.

Azithromycin has also been demonstrated to be useful in the treatment of typhoid fever and shigellosis.25,26 Its efficacy in the treatment of typhoid fever suggests that it may also be active against non-Typhi Salmonella. Although we found that the development of SXT-associated rash was uncommon and was consistent with study findings from elsewhere in Africa, suggesting that SXT is well tolerated,1,4,27,28 SXT nonsusceptibility was common among E. coli isolates in our study. Unlike other antimicrobials studied, we found that azithromycin nonsusceptibility did not seem to be coselected by SXT use. In contrast to other studies that have found azithromycin resistance to be uncommon among gram-negative organisms,8,25 however, the proportion of E. coli isolates that were not susceptible to azithromycin in our study exceeded 80%. Comparing azithromycin antimicrobial susceptibility testing results for gram-negative organisms across studies is hampered by the lack of established interpretive criteria for zone sizes for the Kirby-Bauer disk diffusion method and by the occurrence of the dual-zone phenomenon.29 Although we arbitrarily used interpretive criteria for S. aureus4 and read the zone of complete inhibition rather than the zone of partial inhibition on the disk diffusion test, other investigators may have selected different interpretive criteria leading to quite different reported rates of resistance. Nonetheless, the high proportion of E. coli isolates that were not susceptible to azithromycin in our area casts doubt on
whether it would be useful to prevent or treat infections caused by gram-negative organisms in our setting.

Our study has a number of limitations. Because of the established efficacy of SXT in preventing morbidity and mortality in HIV-infected patients, our study was of an observational rather than randomized design. This limitation was addressed to some extent by obtaining baseline stool samples from patients in each study group to establish differences in SXT nonsusceptibility before SXT use. The high baseline proportion of E. coli isolates nonsusceptible to SXT limited the number of individuals who could switch from carrying SXT-susceptible E. coli to carrying SXT nonsusceptible E. coli. Despite this limitation, we were able to demonstrate rapid and statistically significant changes in antimicrobial resistance of the fecal indicator organism. Although our loss to follow-up rate was consistent with projections, loss to follow-up may have introduced bias into our results if there were differences in rates of SXT nonsusceptibility between retained and lost patients. Two factors may have diluted the observed effect of SXT on E. coli antimicrobial susceptibility: reported adherence <100% occurred in a quarter of patients in group C, and group A and B subjects were contaminated by the use of short courses of SXT and sulfadoxine-pyrimethamine for intermittent illness. Finally, our interpretation of the impact of the effect of SXT prophylaxis on antimicrobial resistance in key human pathogens is, by necessity, an extrapolation from observations made on the indicator organism, fecal E. coli. Fecal E. coli has a long track record of use as an indicator organism for resistance among enteric pathogens, and there is ample evidence that resistance genes are freely shared between fecal flora such as E. coli and clinically important enteric pathogens. Our study demonstrates that fecal E. coli, an indicator organism for enteric pathogens, rapidly develops resistance to SXT and a number of other clinically important antimicrobials after initiation of SXT prophylaxis. Furthermore, it is likely that mobile genetic elements would facilitate the movement of the selected resistance genes between fecal flora and enteric pathogens. These data suggest that while the substantial benefits of SXT prophylaxis are realized in Africa, surveillance for its ongoing efficacy for prophylaxis against HIV coinfections and the emerging management of diarrheal fever, and pneumonia syndromes should be established and maintained. Larger long-term studies are needed to evaluate the impact of widespread use of SXT prophylaxis on these clinical outcomes. In addition, efforts to monitor the prevalence of resistance to other antimicrobials that are coselected by SXT use among important pathogens and research to evaluate alternative effective and inexpensive antimicrobial agents are warranted.

ACKNOWLEDGMENTS

The authors are grateful to the staff of Kikundi cha Wamawake Kilimanjaro Kapambana na UKMWI (KIWAKKUKI; Women Against AIDS in Kilimanjaro), Angaza, and the Rainbow Centre for referring recent VCT clients and to Dr. Mark E. Swai, Director of Hospital Services, KCMC, for making space available for patient follow-up for the study. Anna Mchaki, Praxed Mosh, Rhoda Mremi, Robert Shugubu, Helen Y. Chu, L. Brett Caram, Cynthia A. Myoian, Susanna Naggie, and Keren Z. Landman assisted with operations of the follow-up clinic. The authors thank Richard Tarino and Aloyce Ole Sudali for performing stool screening and isolating E. coli isolates at the KCMC Clinical Laboratory; Anne B. Morrissey for assistance with shipping of isolates; Dolores Calley and Hina Patel at the DUMC CMB for assisting with confirmation of organism identification and antimicrobial susceptibility testing; and Francis P. Karia and Stanley Mirrett for administrative support for the study.

REFERENCES


5.15. Molecular epidemiology and transmission dynamics of *Mycobacterium tuberculosis* in rural Africa


**CONTRIBUTION**

My position as third author reflects my role as primary implementer of the research. I led data collection in the field, day-to-day logistics, and data entry. Wilkinson conceived the study idea and obtained funding. Pillay conducted molecular subtyping under the supervision of Sturm. Lombard led statistical analysis. Davies advised on field activities. Wilkinson wrote the first draft of the manuscript. All authors contributed to revisions of the manuscript.

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Molecular epidemiology and transmission dynamics of *Mycobacterium tuberculosis* in rural Africa

David Wilkinson,1 Manormoney Pillay,1 John Crump,1 Carl Lombard,1 Geraint R. Davies1 and A. Willem Sturm2

1 Centre for Epidemiological Research in South Africa, South African Medical Research Council, Hlabisa, South Africa
2 Hlabisa Hospital, Hlabisa, South Africa
3 Department of Medical Microbiology, University of Natal, Durban, South Africa

Summary

The relative contribution of reactivated and recently acquired tuberculosis to the disease burden in developing countries is unknown, as are the settings within which most transmission occurs. In an attempt to answer these questions, we combined molecular techniques (restriction fragment length polymorphism analysis) and conventional epidemiology (risk factor analysis and contact tracing) to study 246 consecutive cases of smear-positive tuberculosis in rural South Africa. We estimate that 29–43% of the cases were recently acquired, as they were clustered. We were unable to identify firm transmission links between 73% of clustered cases. Our findings suggest that most smear-positive tuberculosis in rural Africa is both recently acquired and casually transmitted. Tuberculosis control may therefore depend more on promoting early presentation, rapid diagnosis and vaccine development than on chemotherapy.

Keywords tuberculosis, molecular epidemiology, transmission, South Africa

Correspondence David Wilkinson, Centre for Epidemiological Research in South Africa, South African Medical Research Council, PO Box 658, Hlabisa 3937, South Africa

Introduction

Restriction fragment length polymorphism (RFLP) analysis offers new insights into the epidemiology and transmission dynamics of tuberculosis (Small & van Embden 1994). Unexpected chains of transmission (Kline et al. 1995) and rapid progression of newly acquired infection in HIV-infected patients have been documented (Daley et al. 1992). Reactivation of latent infection can be distinguished from new infection (Godfrey-Faussett & Stoker 1992).

Molecular epidemiology has been combined with conventional epidemiology and clinical data in developed countries (Alland et al. 1994; Small et al. 1994). In San Francisco, around a third of new cases of tuberculosis were probably the result of recent infection but few of the clusters of cases identified by RFLP analysis were fully explained by conventional contact tracing (Small et al. 1994), suggesting the possibility of casual transmission. Our knowledge of the molecular epidemiology of tuberculosis in developing countries, where the burden of disease and the risk of infection is greatest, is limited. While recent infection accounts for some disease, reactivation of latent infection is believed to account for most (Enarson & Rouillon 1994). Knowledge of the relative importance of each is important for disease control.

Transmission of tuberculosis is more likely following prolonged and intimate contact with an active case (Enarson & Rouillon 1994). However, it was estimated that less than 1% of infections in children in a high prevalence setting were secondary to known active cases (Madico et al. 1995). There may be an apparent paradox here and it is reasonable to ask: how frequent is transmission of tuberculosis following casual contact?
The aim of this study was to describe the molecular epidemiology of Mycobacterium tuberculosis from a population perspective in rural South Africa, to interpret these findings using epidemiological and clinical data, and to attempt to identify the settings within which transmission occurs. This is the first study of this scope and magnitude from such a setting.

Methods

Setting

The Hlabisa health district of KwaZulu/Natal, South Africa, has a population estimated at 205,463. The area is rural and people live in widely scattered kraals, depending on pension remittances, migrant labour and subsistence farming for money and food. Details of the tuberculosis control programme have been described before (Wilkinson 1994). Briefly, all patients with suspected tuberculosis are admitted to the district hospital for diagnosis and initiation of therapy, and all patients with tuberculosis in the district are managed through the Hlabisa control programme. Cases diagnosed in neighbouring districts but resident in the Hlabisa district are referred to the Hlabisa control programme for treatment. Cases diagnosed in Hlabisa but resident in other districts are referred to their own district hospital for treatment and were excluded from this study. The prevalence of HIV infection in adults with tuberculosis was 58% in 1993 (Davies et al. 1996).

All patients are eligible for community-based directly observed therapy for their tuberculosis and in 1994, 90% received it; the completion of treatment rate is 85% (Wilkinson 1996). Since 1991 a computerised tuberculosis register containing detailed demographic and clinical data has been prospectively maintained.

Collection of Mycobacterium tuberculosis isolates and RFLP analysis

All smear-positive sputum specimens identified in Hlabisa were further processed in the Department of Medical Microbiology, Durban. Isolates, grown on Lowenstein-Jensen slopes, were fingerprinted according to standardized methodology (van Embden et al. 1993) as follows: chromosomal DNA was cleaved with PvulI, electrophorised, and vacuum-blotted onto a Hybond N+ membrane. After hybridization with a PCR-generated 245 bp fragment of IS6110 labelled with horseradish peroxidase, the restriction fragment length polymorphisms (RFLPs) were detected by enhanced chemiluminescence. The resulting fingerprints containing 5 copies of IS6110 were compared by the GelCompar computer software (Applied Maths, Kortrijk, Belgium). All lanes that were found by GelCompar to have similar banding patterns were compared visually and classified as matched if the banding patterns were 100% identical (in number and molecular weight). Isolates with less than 5 bands were further analysed using a PGRS probe (van Soolingen et al. 1994). Thus clusters of genetically identical isolates were identified. One RFLP was done per patient.

Epidemiological investigations

We only traced clustered patients. For logistical reasons, field work was done in May and June 1995 before all clusters had been fully constructed. After giving consent, a detailed questionnaire was completed by each patient. Each was asked if he knew the other members of his cluster, and if so, in what context and to what degree of intimacy. Patients were asked in detail about place of residence (past and present), schooling, work, social activities, medical history, regular travel routes, and attendance at community gatherings.

Contact scoring system

To categorize the degree of contact between members of each cluster, a simple and pragmatic scoring system was developed.

0 patients reported not knowing each other and no possible point of contact could be identified;

1 patients who reported not knowing each other but for whom casual contact was possible (e.g. sharing a transport route regularly or living in the same subdistrict);

2 patients who reported knowing each other by name, but acquaintance was only casual;

3 patients who knew each other well and had prolonged or intimate contact (e.g. family members, friends).

We compared the frequency distribution of scores for contact between patients in clusters with the frequency distribution of scores for contact between nonclustered
patients, and with the frequency distribution of scores for contact between clustered and nonclustered patients. To do this, in addition to the traced patients in clusters, we asked 20 consecutively diagnosed nonclustered patients about their contact with one another, and their contact with each member of each cluster.

Statistical analysis

A cluster was defined as two or more patients with 100% identical RFLP patterns; those with nonidentical patterns were nonclustered. Possible risk factors for clustering (age, sex, HIV status, residence and occupation) were investigated by comparing these variables for clustered with nonclustered patients. Data was analysed using Epilinfo 6.02. Students t-test and the Chi-square test were used to test for risk factors for being in a cluster. A matrix was formed from the contact scores and the frequency distributions of contact scores for nonclustered and for clustered patients were compared using the chi-square test. Patients with missing contact information were necessarily excluded from this analysis. If the cluster size was reduced to one member due to missing data, the entire cluster was ignored in the analysis.

Results

Patients and RFLP patterns

Between May 1993 and March 1994, 339 consecutive adult cases of smear-positive pulmonary tuberculosis were diagnosed in Hlabisa district. Specimens from 31 (15%) were either not sent to Durban (34) or were mislaid there (17). Of the remaining 288, 42 (15%) either showed no initial growth or failed to grow after freeze-storage. Isolates from 246 (73%) patients were available for analysis. The characteristics of the patients from whom isolates were not available did not differ from the analysed group.

RFLP analysis revealed 175 distinct patterns, 136 of which were present in only one patient (Fig. 1). There were therefore 39 clustered patterns, 34 clusters defined by IS6110 alone and 5 (containing 15 patients) defined in conjunction with PGRS. The 39 clusters contained 110 patients, 45% of the total studied (Table 1). Assuming that each cluster contained patients between whom transmission had occurred, including an index case

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**Table 1** Cluster size and number of patients per cluster

<table>
<thead>
<tr>
<th>Cluster size (number of patients)</th>
<th>Number of clusters</th>
<th>Total number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>110</td>
</tr>
</tbody>
</table>

(Small et al. 1994), there remain n-1 patients who acquired disease recently in each cluster. It can thus be estimated that at least 71 (110-39) of the 246 patients (29%) acquired infection recently, and that infection progressed to disease rapidly.

Risk factors for clustering

Age (n = 242), gender (n = 246) and HIV status (n = 218) were known for most patients. There were no significant differences in these parameters between the clustered and nonclustered groups (Table 2). Neither was clustering associated with place of residence or occupational history.

Epidemiological investigation of clusters

In all, 64 of the 110 clustered patients (58%) were traced. For 10 clusters, no members were traced; for nine clusters, all members were traced, leaving 20

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**Table 2** Age, gender and HIV distribution in clustered and nonclustered patients

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>All patients</th>
<th>Clustered</th>
<th>Nonclustered</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean (sd)</td>
<td>35.5 (14.2)</td>
<td>35.5 (14.1)</td>
<td>35.4 (14.4)</td>
<td>0.94</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>63%</td>
<td>59%</td>
<td>63%</td>
<td>0.44</td>
</tr>
<tr>
<td>female</td>
<td>38%</td>
<td>41%</td>
<td>35%</td>
<td></td>
</tr>
<tr>
<td>HIV (n, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>65 (186)</td>
<td>52 (159)</td>
<td>33 (34)</td>
<td>0.48</td>
</tr>
<tr>
<td>Negative</td>
<td>153 (62)</td>
<td>68 (62)</td>
<td>85 (63)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>28 (12)</td>
<td>10 (9)</td>
<td>18 (13)</td>
<td></td>
</tr>
</tbody>
</table>
D. Wilkinson et al. Epidemiology and transmission dynamics of *Mycobacterium tuberculosis*

Figure I Restriction fragment length polymorphism analysis of selected isolates of *Mycobacterium tuberculosis*

partially traced clusters. Tracing occurred before clusters were fully constructed and thus no attempt was made to trace members of seven clusters. A total of 81% of the patients in clusters at the time of the field work were traced. The epidemiological and clinical characteristics of the traced and untraced groups were similar.

Only 4 clusters – each with 2 members – were fully explained by prolonged or intimate contact (score = 3). For only 14 patients (12%) was prolonged or intimate contact identified. The 14 constituted 7 pairs in 7 different clusters: 3 pairs were siblings, 1 pair was an uncle and a niece, 1 pair cohabited, 1 pair shared the same hostel accommodation at work, and 1 pair was explained by nosocomial transmission. This is the first case of nosocomial transmission of tuberculosis in Africa detected by RFLP analysis (Wilkinson et al. 1997).

A further 5 patients (5%; one pair and one set of 3), in 2 clusters, were casually acquainted (score = 2). Six patients (7%; three pairs), in 3 clusters, could have had contact (score = 1). Thus, for most clustered patients (67%; 73%) no link could be identified.

Contact score analysis

The first analysis included all available contact scores. Patients in clusters were more likely to have higher
Table 3: Frequency distributions of contact scores for clustered and nonclustered patients (all scores included)

<table>
<thead>
<tr>
<th>Score</th>
<th>Clustered patients</th>
<th>Nonclustered patients</th>
<th>Between nonclustered and clustered</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34 (70)</td>
<td>159 (84)</td>
<td>974 (89)</td>
</tr>
<tr>
<td>1</td>
<td>3 (6)</td>
<td>25 (13)</td>
<td>92 (8)</td>
</tr>
<tr>
<td>2</td>
<td>6 (12)</td>
<td>6 (3)</td>
<td>33 (3)</td>
</tr>
<tr>
<td>3</td>
<td>6 (12)</td>
<td>0 (0)</td>
<td>6 (0)</td>
</tr>
<tr>
<td>Totals</td>
<td>49 (100)</td>
<td>190 (100)</td>
<td>1106 (100)</td>
</tr>
</tbody>
</table>

χ² test for difference between frequency distributions a and b; P < 0.0001. χ² test for difference between frequency distributions b and c; P = 0.2. *100 is the total number of contacts scored from the matrix. Totals for columns 'a' and 'b' are the number of contact scores derived from patient contacts.

Contact scores than patients not in clusters (Table 3): 24% of clustered patients had a contact score of 2 or more, compared to 3% of nonclustered patients (P < 0.0001). The distribution of scores for contact between nonclustered and clustered patients was similar to the distribution of scores for contact between nonclustered patients only (P = 0.18). This suggests that clustered patients did not have higher contact scores simply because they were more likely to be in contact with other people than nonclustered patients.

The second analysis included only patients with contact scores between zero and two, eliminating patients who had prolonged or intimate contact (score = 3). The aim of this analysis was to determine if clustering was associated with more casual contact. The distribution of contact scores for clustered and nonclustered patients remained significantly different (Table 4), with a higher frequency of scores = 2 in the clustered patients (17% vs. 3% P = 0.008). The distribution of scores for contact between clustered and nonclustered patients was again similar to the distribution of scores for contact for nonclustered patients (P = 0.06).

Discussion

Our data suggest that at least 29% of diagnosed cases of smear-positive pulmonary tuberculosis in this rural South African health district are due to recent transmission. Also, most of this transmission seems to follow interpersonal contact that is difficult to detect with standard contact tracing techniques. These findings raise interesting questions about tuberculosis transmission dynamics in this setting and have potentially important implications for tuberculosis control.

We have systematically described, for the first time at the population-level, the molecular epidemiology of tuberculosis in a rural African setting. RFLP analysis was done on isolates from 73% of consecutive smear-positive adults resident in a defined geographical area. The marked strain diversity (136 distinct patterns) implies considerable reactivation of latent infection. However, we also identified 39 clusters that contained 43% of the cases studied, and such clustering is generally recognized as indicating recent transmission (Small & van Embden 1994; Small et al. 1994). Previous studies assumed that each cluster contains an index case and therefore (n-1) newly acquired cases (Small et al. 1994); using this approach we estimated that 29% of cases were recently transmitted. However, as we identified an index case in only 14% of clusters, it is possible that as many as 43% of the cases were due to recent transmission (14% × 39 = 5.5; (110–5.5)/246 = 43%). It seems then, that while most disease was due to reactivation, much (29%–43%) was recently acquired. None of the risk factors studied...
(Table 2) were associated with clustering and this may reflect the intensity of ongoing transmission, or a larger sample size may be needed to measure any associations.

In an attempt to identify the settings within which transmission might be occurring, conventional contact tracing was done to complement the molecular studies. Contact tracing completely explained only 4 clusters, and prolonged or intimate contact was identified between only 15% of clustered patients. Our contact tracing was incomplete, and while the characteristics of the traced patients did not differ from those not traced, this does make identification of contacts less likely, particularly in the large clusters. A study of this nature will inevitably be incomplete, and while we are unable to identify bias due to these gaps, our interpretation of possible transmission dynamics is necessarily cautious.

What possible explanation is there for the lack of index cases and clear transmission links in most clusters? The fact that we did not identify links within clusters does not mean that such links do not exist. It is possible that the untraced cases (unidentified common sources) do explain the transmission; the ‘missing links’ may also have been diagnosed in other hospitals. Also, smear-negative cases that we were unable to study may have contributed. However, it is also possible that a significant proportion of the clustering was due to transmission through casual contact – contact that is not easy to detect. There is some supporting evidence for this, as defined casual contact (score = 2) was more frequent among clustered patients (Table 3). We did not measure contact between members of different clusters but this would have been useful information and should be included in future studies. It seems reasonable to consider then that a considerable proportion of the disease transmission observed may have occurred through casual contact.

Most of the close links that were established by contact tracing were between family members. Only the case of nosocomial transmission was unusual: this is unlikely to be due to contamination, as the specimens were collected and cultured months apart and were processed on different gels. Small et al. (1994) used outbreak investigation to validate the association between identical RFLP patterns and recent transmission, and considered the possibility that contact need be neither prolonged nor intense in cluster members not identified by conventional contact tracing. Our findings support these observations, and suggest that casual transmission may be particularly common in high prevalence settings.

Our findings also support conventional wisdom that prolonged or intimate contact with an active case is a risk factor for transmission (Grzybowski et al. 1975; Kumar et al. 1984), as all identified episodes of intimate contact were in clustered patients (Table 3). However, we were unable to identify such contact between most clustered patients. Our findings also support Madico et al. (1995) who failed to identify an index case amongst the majority of infected children in their study. Kenyon et al. (1996) reported tuberculosis transmission during an aeroplane flight, demonstrating that transmission need be neither intimate nor prolonged, but that risk of infection is higher in close contacts. Is it possible that while prolonged or intimate contact increases the risk of transmission of Mycobacterium tuberculosis, a considerable proportion of – perhaps most – transmission is casual in nature?

If the rate of recent transmission is high and much of it is casual, why do some strains cluster at all? Possibilities for further study include risk factors such as variation in strain-specific virulence and human genetic susceptibility. Although previous studies (Alland et al. 1994; Small et al. 1994) indicate that clustering equates with recent transmission, perhaps this is not truly so in high prevalence settings: there is much more to learn about tuberculosis transmission dynamics in Africa.

In Hlabisa, 83% of patients complete treatment (Wilkinson et al. 1996), yet, as many as 43% of cases may have been due to recent transmission. Is chemotherapy not preventing transmission? Perhaps most transmission is occurring before therapy can be started. If chemotherapy delivered under direct observation is an effective means of reducing transmission, a smaller proportion of patients would be expected to become clustered over time and we are studying this. While the current emphasis on the effective treatment of infectious cases is appropriate, more attention may need to be given to promoting early presentation with disease, rapid and accurate diagnosis, and vaccine development if tuberculosis is to be effectively controlled.

References

Epidemiology and transmission dynamics of Mycobacterium tuberculosis

fingerprinting and conventional epidemiologic methods. 
5.16. Nosocomial transmission of tuberculosis in Africa documented by restriction fragment length polymorphism


CONTRIBUTION

My position as second author reflects my role as primary implementer of the research. I conducted all aspects of the fieldwork, data collection, and data analysis for this project. I wrote the first draft of the manuscript with my mentor, Dr. Wilkinson. Pillay and Sturm conducted laboratory assessments, including molecular subtyping. All authors contributed to revisions of the manuscript.

CITATIONS

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2.162
Nosocomial transmission of tuberculosis in Africa documented by restriction fragment length polymorphism

David Wilkinson1,2, John Crump1, Manormoney Pillay3 and A. Willem Sturm3 1Centre for Epidemiological Research in Southern Africa, South African Medical Research Council, P.O. Box 658, Hlabisa, 3937, South Africa; 2Hlabisa Hospital, Hlabisa 3937, South Africa; 3Department of Medical Microbiology, University of Natal, Durban, South Africa

Keywords: tuberculosis, Mycobacterium tuberculosis, nosocomial transmission, restriction fragment length polymorphism

Introduction
That nosocomial transmission of tuberculosis is a risk, and can occur, is recognized (CDC, 1994). While health workers in Africa are assumed to be similarly at risk, documenting transmission from patient to health worker depends on a combination of molecular techniques and conventional contact tracing. A MedLine search and contact with researchers active in the field revealed no report of such events in a developing country. We therefore report the first case in Africa of transmission of tuberculosis from patient to nurse, documented by restriction fragment length polymorphism (RFLP) analysis.

Case report
In early 1993, a 24 years old nurse working in our district hospital in Hlabisa, South Africa presented with a history of chronic cough and weight loss. Chest X-ray was compatible with tuberculosis, sputum smears showed acid-fast bacilli, and culture was positive for Mycobacterium tuberculosis. She declined testing for human immunodeficiency virus (HIV). Since the initiation of a comprehensive tuberculosis register in 1991, this was the first case of tuberculosis in a nurse in our hospital. We considered the possibility of nosocomial transmission.

From 1993 we have done RFLP analysis on all incident cases of tuberculosis diagnosed in Hlabisa, and from the data set of the first 246 specimens we identified one other patient whose isolate had an identical RFLP banding pattern to that of the nurse (Figure). This HIV infected patient had been admitted with smear-positive tuberculosis 3 months before the onset of symptoms in our nurse. The nurse recalled the patient, and duty records confirmed that she was allocated at the time of the admission to the section of the ward in which the patient was accommodated. The patient was admitted for 13 d, and the nurse was on duty for 9 of those days. The patient later died, and the nurse completed treatment through the Hlabisa tuberculosis control programme (WILKINSON, 1994). This sequence of events is highly suggestive of transmission of infection from patient to nurse and subsequent rapid disease progression in the nurse.

Discussion
There was no case of tuberculosis in health workers in Hlabisa hospital in 1991 or 1992, at a time when the prevalence of HIV in women attending antenatal clinics was 4-2% (WILKINSON, 1992). Between 1993 and 1995, we recorded 15 cases amongst nurses, and the prevalence of HIV infection in antenatal attenders had increased to 14% (unpublished data). As many of our staff with tuberculosis decline HIV testing we are unable to attribute this increase directly to HIV infection; furthermore, until we complete our longitudinal RFLP studies we will be unable to estimate accurately the relative importance of nosocomial and community-acquired infection. However, with increasing numbers of health workers in Africa infected with HIV (BUIE et al., 1994), and with the high prevalence of tuberculosis, nosocomial transmission seems inevitable. This has important implications for staff health, for employers' legal obligation to provide a safe working environment, and for the health system's ability to attract and retain health workers.

Comprehensive strategies to control nosocomial transmission in developed countries (CDC, 1994) are beyond the reach of developing countries, financially and logistically. Simple measures such as educating patients how to cough and dispose of sputum safely, opening windows, and wearing masks is almost all that can be done. Provision of preventive therapy is another possibility, although this would require HIV testing of health workers and regular surveys of local drug susceptibility patterns. With the increasing spread of multi-drug-resistant tuberculosis throughout the world, the development of strategies to control nosocomial transmission of tuberculosis becomes even more important.

Acknowledgement
We thank Dr S. B. Squire for thoughtful comments.

References


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5.17. TB or not TB?


CONTRIBUTION

My position as second author reflects my role as a major contributor to development of the content and writing of the manuscript. I collected the data for one of the patients with a cryptic form of tuberculosis presented in this symposium and paper, with my colleagues Taheri and Samarasinghe. Weir provided mentorship. All authors contributed to revisions of the manuscript.

CITATIONS

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JOURNAL IMPACT FACTOR

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Clinical case reports

TB or not TB?

Shahrad Taheri, John Crump, Dunisha Samarasinghe and William Weir 
Coppetts Wood Hospital, Coppetts Road, Muswell Hill, London, N10 1JN, UK

Abstract

These 3 cases illustrate what we believe to be unusual presentations of tuberculosis. In no case was there conclusive proof of infection with Mycobacterium tuberculosis using histological, microbiological or radiological techniques. All were treated empirically with anti-tuberculous medication, with complete recovery. With the re-emergence of tuberculosis, there may be a rise in such cases, and the importance of their recognition and empirical treatment is discussed.

Keywords: tuberculosis, Mycobacterium tuberculosis, unusual presentation

Introduction

There were at least 7·5 million cases of tuberculosis world-wide in 1990 (ANONYMOUS, 1993); 4·2% of these cases were estimated to be associated with human immunodeficiency virus (HIV) infection. It is predicted that the percentage of tuberculosis cases associated with HIV will increase to 13·8 by the year 2000, by which time there will be more than 10 million cases of tuberculosis world-wide. Although HIV has played some role in the resurgence of tuberculosis, the main factors appear to be poverty, overcrowding and poor public health services (PORTER & MCDAM, 1994). We present case histories of 3 patients whom we believed to have tuberculosis despite lack of classical presentations and despite lack of microbiological proof from relevant specimens. These cases illustrated the importance of considering tuberculosis in patients who may present in an unusual fashion, especially at a time when the increasing incidence of tuberculosis is likely to lead to more frequent clinical encounters of this type.

Case histories

Case no. 1

A 27 years old white female gymnastic worker was referred to Coppetts Wood Hospital, London, UK from a district general hospital in July 1994. In February 1994 she had attended the casualty department of the referring hospital 3 times within one week and she had finally been admitted under the surgical team for observation. She complained of a week of abdominal pain, vomiting and constipation. She had last been well in August 1993 when she had begun to have episodes of arthralgia, night sweats and lethargy. She had also developed secondary amenorrhoea. Of possible note was the fact that she had been scratched by a cat in September 1993. Her past medical history was unremarkable apart from a negative laparoscopy in 1991 for abdominal pain. She had received bacillus Calmette-Guerin (BCG) vaccination and had had no contact with tuberculosis. Her last travel had been to Italy in 1993. Investigations showed a haemoglobin level of 10·7 g/dL, a white blood cell count of 16·7x10^9/L (neutrophilia) and a platelet count of 545x10^9/L. Her biochemical profile was within normal limits, as was a plain abdominal X-ray. During this admission, she continued to have fevers, abdominal pain and vomiting, prompting a laparotomy to be carried out on 4 March 1994.

Address for correspondence: Dr William Weir, Consultant Physician, Coppetts Wood Hospital, Muswell Hill, London, N10 1JN, UK.

At laparotomy, massively enlarged small bowel mesenteric nodes were noted. All nodes sampled had abscess centres. Histological examination showed granulomatous inflammation with central suppuration. The differential diagnosis at the time included yersiniosis, lymphogranuloma venereum, tuberculosis, and cat scratch disease. A genitourinary opinion excluded lymphogranuloma venereum on clinical grounds. In March 1994, she was started on a 6 weeks course of tetracycline for a presumptive diagnosis of cat scratch disease, without any response. She was also given an 8 weeks trial of antituberculous therapy which was discontinued once culture of relevant specimens was negative for mycobacteria. Initial response to antituberculous therapy resulted in some resolution of her symptoms, but she continued to have occasional night sweats, abdominal pain and arthralgias. On discontinuation of antituberculous medications, her continuous fevers returned, she became anaemic requiring blood transfusion, her erythrocyte sedimentation rate increased, and she developed thrombocytosis and abnormal liver function tests. Further investigations were carried out. A liver biopsy showed lobular hepatitis while bone marrow biopsy was normal. Ultrasound examination of her abdomen revealed appearances consistent with splenic micro-abscesses.

The patient was transferred to Coppetts Wood Hospital on 29 July 1994. Her weight had decreased from 58 kg to 55 kg in 3 months. Apart from non-invasive investigations, a liver biopsy was suggested to confirm the presence of micro-abscesses.

Fig. 1. Computerized tomography scan of the abdomen of case no. 1, showing multiple rounded lesions within the spleen, consistent with the appearance of abscesses.
specific abdominal tenderness and pyrexia, there was no other remarkable physical finding. Therapy was started with rifampicin, isoniazid and pyrazinamide while she was further investigated. Her haemoglobin level was 13·5 g/dL, her white blood cell count was 12·2×10⁹/L (neutrophilia), and her platelet count was 477×10⁹/L. Her erythrocyte sedimentation rate was 86 mm in the first hour. Her liver function tests were abnormal, with raised alkaline phosphatase and mildly raised transaminases. Clotting and autoimmune profiles were normal. The following serologies were negative: syphilis, yersiniosis, Clostridiosis, lymphogranuloma venereum, cat scratch disease, bartonellosis, amebiasis, brucellosis, melioidosis, toxoplasmosis, hepatitis B and C, and tularemia. A Mantoux test was negative despite previous BCG immunization but, apart from general debility, there was no evidence to suggest immunodeficiency and serology for HIV was negative. Early morning urines, gastric washings and bone marrow cultures were negative for mycobacteria. Chest X-ray was normal. A computerized tomography (CT) scan of her abdomen confirmed splenic microabcesses (Fig. 1).

The patient’s progress on antituberculous chemotherapy was initially poor with continuing abdominal pain, swinging pyrexia, night sweats and weight loss. Her haemoglobin level decreased while her white blood cell and platelet counts began to rise, as did her alkaline phosphatase level. On 11 August 1994, she began to have joint pains and developed erythema nodosum (Fig. 2). Her worsening condition was interpreted as a possible allergic reaction to antituberculous medication or heightened immune response to mycobacteria with initiation of therapy. Rifampicin and isoniazid were discontinued while she was started on ciprofloxacin and continued on pyrazinamide. Her symptoms subsided, but returned once isoniazid was restarted. Isoniazid was then permanently discontinued while rifampicin was restarted. On therapy with ciprofloxacin, pyrazinamide and rifampicin, there was resolution of her abdominal pain, fevers, night sweats, arthralgia and erythema nodosum. She began to gain weight, while her blood tests became normal. A CT scan carried out in December 1995 showed complete resolution of her splenic microabcesses.

Case no. 2

A 62 years old female of Asian descent, born in Tanzania, was referred to Coppetts Wood Hospital by endocrinologists with 5 months’ history of weight loss and 2 weeks’ history of night sweats and diarrhoea. Diabetic control had become poor on her usual oral hypoglycaemics and she had become insulin-requiring. Her weight loss was approximately 10 kg and her diarrhoea, which occurred 3 or 4 times daily, was loose with occasional dark blood. Although there was a family history of non-insulin dependent diabetes, there was no history of tuberculosis or inflammatory bowel disease. She did not suffer from any cough, musculoskeletal complaint or rash.

On examination, she was pale and emaciated without any palpable lymphadenopathy. Her weight was 36·6 kg and she was febrile at 39°C. She was not tachycardic and she was normotensive. Cardiovascular and respiratory examinations were unremarkable. Abdominal examination revealed tenderness in the left iliac fossa; rectal examination was normal. Initial investigations showed microcytic anaemia with a haemoglobin level of 6·5 g/dL; a white blood cell count of 17·7×10⁹/L with a neutrophilia of 14·1×10⁹/L, and an erythrocyte sedimentation rate of 86 mm in the first hour. Biochemical investigations were normal. Her ferritin level was raised and haemoglobin electrophoresis revealed an A+A pattern. Blood cultures were negative. Chest X-ray was normal, but a plain abdominal X-ray revealed a thick-walled loop of sigmoid colon. Her Mantoux test was strongly positive with one unit of purified protein derivative, but multiple stool specimens were negative for mycobacteria, ova, cysts or parasites. Early morning urines and gastric washings did not grow mycobacteria.

Further investigations were carried out. Sigmoidoscopy was unhelpful and abdominal ultrasound examination was normal. Colonoscopy showed loss of vascular pattern, granular mucosa, and ulceration that spared the rectum and caecum. Histological evaluation of colonic biopsies revealed chronic active colitis, but did not reveal its aetiology.

The patient was started on antituberculous medications for a presumptive diagnosis of tuberculous colitis, with isoniazid, rifampicin and pyrazinamide. On this regimen, her fever settled but her diarrhoea took longer to resolve. At follow-up 9 months later, she was well, free of diarrhoea, with good appetite, and she had regained her 10 kg weight loss. Her diabetes was no longer insulin-requiring and her erythrocyte sedimentation rate was 34 mm/h. It was decided to add a fourth antituberculous drug should her symptoms fail to improve, but this proved unnecessary. She continues treatment with rifampicin and isoniazid.

Case no. 3

A 54 years old white male greengrocer was admitted to Coppetts Wood Hospital in October 1995 complaining of 4 months’ history of fevers, rigors, weight loss and loss of appetite. He had travelled to northern Cyprus and Israel in July 1995 and Cyprus again in September 1995. He was heterosexual and had no history of intravenous drug abuse. A previous HIV test had been negative. Marital problems had contributed to excessive alcohol intake, although the patient was not prepared to acknowledge this. On examination, he was febrile (38·8°C) and had a tachycardia of 120 beats/min. On percussion, there was dulness in the left upper zone, although on auscultation the chest was found to be clear. Preliminary tests revealed a haemoglobin level of 7·6 g/dL (microcytic anaemia), a white blood cell count of 15·8×10⁹/L with neutrophilia of 13·4×10⁹/L, and a platelet count of 778×10⁹/L. The erythrocyte sedimentation rate was 130 mm in the first hour. Chest X-ray was normal. The differential diagnosis at presentation included tuberculosis, brucellosis and atypical pneumonia.

One week following his admission, the patient continued to be unwell with fevers and night sweats. He was empirically started on antituberculous treatment with rifampicin, isoniazid, pyrazinamide and ethambutol. Further investigations were carried out. A CT scan of his chest revealed loss of volume in both apices with pleural thickening and fibrosis; several possible granulomas were noted. A gallium scan showed diffuse uptake in the upper part of both lungs, at the right hilum and in the spleen, suggestive of tuberculosis (Fig. 3). Liver biopsy...
Table 1. Summary of Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Presentation</th>
<th>Medi­cations</th>
<th>Out­come</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colonic</td>
<td>Abdominal</td>
<td>Rifampicin</td>
<td>Resolved</td>
</tr>
<tr>
<td>2</td>
<td>Mesenteric</td>
<td>Fluid collection</td>
<td>Ethambutol</td>
<td>Resolved</td>
</tr>
<tr>
<td>3</td>
<td>Spleen</td>
<td>Pain, fever</td>
<td>Streptomycin</td>
<td>Resolved</td>
</tr>
</tbody>
</table>

Discussion

In none of the 3 cases presented was the presentation one of classical tuberculosis, and no acid fast bacillus was isolated from relevant specimens. In the first case, the history of being scratched by a cat and granulomas in the mesenteric nodes and microabcesses in the spleen suggested a diagnosis of cat scratch disease (Dolan et al., 1993). However, not only were the putative organisms not cultured, but serology was negative. There was also failure to respond to a 6 weeks' course of tetracycline. The 8 weeks' trial with antituberculous therapy was most probably unsuccessful due to a possible drug reaction to isoniazid (as demonstrated by stopping and restarting isoniazid), or failure of persistence with medications by the clinicians when absolute symptomatic control was not achieved and microbiological investigations for mycobacteria proved negative.

The first and second cases were abdominal presentations of tuberculosis. Abdominal presentations are common in some ethnic groups and are much better recognized in Africa, Asia and South America. With the global re-emergence of tuberculosis and the rise in extrapulmonary disease due to HIV, such presentations may become more common (Elder, 1992; Jayanthi et al., 1993; Marshall, 1993).

A review of several series of abdominal tuberculosis cases from different countries (Marshall, 1993) showed that half were classified as peritoneal disease and the rest were intraluminal, with a small proportion classified as mesenteric lymphadenitis. Colonic disease, as in the second case presented here, accounted for 6% of cases of abdominal tuberculosis. The pathogenesis of abdominal tuberculosis involves swallowing acid-fast bacilli by patients with pulmonary disease, ingestion of contagious milk or food, haematogenous spread, or direct extension. Cases of enteric tuberculosis often present in a non-specific manner (Al Karawi et al., 1995); the majority report fever, abdominal pain, anorexia and change in bowel habit and weight loss (Singh et al., 1996). Occasionally, the presentation is that of an acute abdomen.

The diagnosis of abdominal tuberculosis can be challenging and the differential diagnosis broad. Classically, one expects to find caseating granulomas. However, tuberculosis may not present as a granuloma and, even if this is the case, the granuloma may be non-caseating. Histological appearances may be very non-specific. The differential diagnosis for granulomas is vast and can include tuberculosis, sarcoidosis, syphils, brucellosis, berylliosis, cat scratch disease and fungal infections. Ill-formed granulomas were noted in the liver biopsy of the third case presented and in the mesenteric nodes of the first case. The histological appearance, as in leprosy, represents various host reactions to mycobacteria which have been described as reactive and areactive (Proudfoot, 1971). Similarly the clinical response once antituberculous medication is started may reflect variable immune responsiveness. Radiography, Mantoux testing, smears and cultures for organisms may be equally unhelpful. The chest X-ray may show active pulmonary disease in fewer than 50% of cases of abdominal tuberculosis (Jayanthi et al., 1993).

Before the description of Crohn's disease in 1932, tuberculosis was considered high on the list of the differential diagnosis of colonic disease. Now that tuberculosis is re-emerging, it is important to recognize colonic tuberculosis, as giving immunosuppressive medications can be risky (Burke & Zafar, 1975). Asians, diabetics and immunocompromised persons appear to be particularly at risk of enteric tuberculosis (Jayanthi et al., 1996). Colonoscopic findings in tuberculosis include mucosal nodules and ulcers, stricture with nodules and ulcerations, and mucosal nodules with or without pseudopolypoid folds (Bharadwaj et al., 1992). Segmental colonic involvement is not uncommon (Shah et al., 1992), and lesions may also mimic...
cancerous (SINGH et al., 1996). The possibility of resistant organisms must always be borne in mind when treating immigrants. The diagnosis of tuberculosis requires a high index of suspicion and alert clinical judgement (LINGENFELSER et al., 1993). Recognizing the limitations of diagnostic tools, a therapeutic trial with antituberculous medication has an important role in dealing with these patients.

PROUDFOOT (1971), in his classic review of cryptic disseminated tuberculosis, described its various presentations, with abnormal blood pictures, raised erythrocyte sedimentation rates, abnormal liver function tests (particularly alkaline phosphatase), liver and marrow abnormalities, and miliary mottling on the chest X-ray. He pointed out that, in such cases, no definitive proof of the existence of mycobacteria may emerge. The cases he was particularly concerned with in his review were the elderly. The cases we have described were younger, and such cases may be encountered more frequently in the future. Our objective in presenting these cases has been to alert clinicians to what may occur in the light of the rise in the incidence of tuberculosis. We realize that alternative diagnostic possibilities exist for all the cases presented, but we believe that, with our pragmatic approach, we have managed to treat 3 patients who suffered from serious disease. In the third case presented, the response to initial therapy was not satisfactory and alterations were made in the treatment to deal with atypical mycobacterial infection. The gallium scan in the third case was most suggestive of tuberculosis; some workers have suggested that this investigation is useful in the detection of occult tuberculosis (YANG et al., 1992). Whether the polymerase chain reaction will take on a greater role in the diagnosis of difficult tuberculosis cases remains to be seen.

Acknowledgements
We thank Dr Owen Epstein (Director of Endoscopy, Royal Free Hospital, London, UK) for providing the video of the colonoscopy of the second case for presentation at the meeting, Dr Paul Dhillon (Department of Histopathology, Royal Free Hospital, London, UK) for providing histology slides, and Professor Sebastian Lucas (St Thomas’s Hospital, London, UK) for kindly reviewing the relevant histology and contributing to the meeting. Finally, we thank the Royal Society of Tropical Medicine and Hygiene for providing the opportunity to present our cases and to benefit from the lively discussion of each case.

References

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5.18. Miliary tuberculosis with paradoxical expansion of intracranial tuberculomas complicating HIV infection in a patient receiving highly active antiretroviral therapy


CONTRIBUTION

I conceived the report, collected the data, and wrote the manuscript. Tyrer, Lloyd-Owen, and Han participated with me in managing the patient and conceptualizing the paper. Lipman and Johnson provided mentorship. All authors contributed to revisions of the manuscript.

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results of the remainder of his biochemical profile, including levels of serum amylase and red-cell transketolase, were normal. Electrocardiography showed atrial fibrillation with a rapid ventricular rate (170 beats); echocardiography showed global restrictive left ventricular dysfunction with an ejection fraction of 36%. EIA for antibodies to CMV showed a strong reaction to IgM and a weak reaction to IgG. Other serological studies for evidence of hepatitides A, B, and C; HIV infection; Epstein-Barr virus infection; Q fever; cryptococcosis; legionellosis; parainfluenza; influenza; mycoplasma; Lyme disease; mumps; chlamydia; leptospirosis; brucellosis; and toxoplasmosis; and the Weil-Felix reaction were all negative. Blood, sputum, and urine cultures were negative. Tests for anti-ENA and DNA antibodies were negative. Findings on ultrasonography of the abdomen were normal, apart from hepatomegaly.

Because of the severity of his clinical and echocardiographic findings, the patient began receiving ganciclovir (5 mg/kg q12h), and he completed a 14-day course. He became afibrile within 3 days. In addition, he was treated with digoxin, amiodarone, furosemide, and perindopril, responded well to this therapy, and converted to sinus rhythm. His symptoms abated, with the exception of some ongoing lethargy. All of his liver function indices improved, although his alkaline phosphatase level peaked at 1,076 U/L. Pulmonary infiltrates evident on a chest radiograph resolved. Serial echocardiograms showed progressive improvement, with reversion to complete normality and an ejection fraction of 66% within 2 weeks. Therapy with digoxin, amiodarone, and furosemide was discontinued; treatment with perindopril was maintained for 6 months. Repeated serology for CMV showed an increase in the IgG reactivity and a decrease in the IgM reactivity within 2 weeks. A urine culture yielded CMV; a cytopathic effect was observed, and the infection was confirmed by immunofluorescence. He returned to work within 2 months.

The spectrum of illness caused by CMV is well documented in certain immunocompromised risk groups (e.g., transplant recipients, patients with AIDS or malignancy, and neonates). However, among immunocompetent hosts, acquisition of infection usually goes undiagnosed. A recent report and review of severe CMV infection in immunocompetent hosts described a total of 34 cases [1]. In these patients, the most commonly identified infection sites were the liver (17 cases), CNS (17 cases), and lungs (9 cases).

Seven of the 34 patients were treated with either ganciclovir or foscamet. Fifteen patients died, 14 of whom had not received either of these agents. The four cases in which there was cardiac involvement were in patients aged 14, 28, 37, and 43 years [2–5], all of whom had involvement in other organs as well. None of these patients received antiviral therapy; three died of their infections.

The patient we describe in this report was seriously ill with atrial fibrillation and a rapid ventricular rate, hypotension, and significant myocardial impairment evident on echocardiography. He also had significant involvement of his liver and lungs. Ganciclovir therapy was associated with complete resolution of his myocardiitis and pneumonitis within 2 weeks and a slower resolution of his hepatitis. Perhaps his illness would have resolved without ganciclovir; however, at the time that therapy was begun, his clinical condition was deteriorating, and a marked improvement was observed within days. The major concern, which prompted the initiation of ganciclovir, was his worsening clinical and echocardiographic status. We suggest that these should be the major factors in the consideration of antiviral therapy.

This unusual case illustrates that although CMV infection in immunocompetent hosts is usually self-limiting, severe disease can occur, and specific antiviral therapy can be beneficial in such cases.

Joseph G. McCormack, Simon D. Bowler, J. Elizabeth Donnelly, and Charles Steadman
Department of Medicine, University of Queensland, and Mater Medical Centre, Mater Adult Hospital, South Brisbane, Queensland, Australia

References

Miliary Tuberculosis with Paradoxical Expansion of Intracranial Tuberculomas Complicating Human Immunodeficiency Virus Infection in a Patient Receiving Highly Active Antiretroviral Therapy

The paradoxical expansion of tuberculomas during the course of tuberculous chemotherapy has been reported occasionally [1].

To our knowledge, the phenomenon has been reported only once in the context of HIV infection [2], and in that case it occurred after the patient’s therapy had been switched to isoniazid prophylaxis. We know of no case other than the one we describe in which expansion of tuberculomas occurred concurrently to the induction of highly active antiretroviral therapy (HAART). A 35-year-old male tested HIV positive in September 1996 (blood CD4 T cell count, 210 × 10^3/L; HIV viral load, 325,000 copies/mL). He was enrolled in a clinical trial in December in which zidovudine, 250 mg b.d., lamivudine, 150 mg b.d., and nelfinavir/placebo were administered.

Five weeks later, his HIV viral load had decreased to <400 copies/mL. However, he complained of lethargy, sweats, and weight loss. Physical examination demonstrated an 11-kg weight loss since the time of diagnosis, and there was a firm, 3 × 4 cm, nontender lymph node palpable in the right anterior cervical
triangle. The patient was afebrile, findings on a chest examination were normal, and there was no hepatosplenomegaly or rash. A chest radiograph was obtained that showed widespread miliary shadowing. He was admitted to the hospital for further investigation.

Biopsy of the lymph node was performed; histopathologic examination showed caseating giant cell granulomas containing moderate numbers of acid-fast bacilli. Cultures of lymph-node biopsy material and early morning urine yielded Mycobacterium tuberculosis, which was fully susceptible to first-line antituberculous agents.

The patient started receiving therapy with rifampin, isoniazid, ethambutol, pyrazinamide, and pyridoxine. Within 4 days he developed a low-grade fever, which resolved over the subsequent 2 weeks. He was discharged to his home.

Results of outpatient laboratory studies revealed an increased blood CD4 T cell count, and the HIV viral load remained <400 copies/mL. The cervical lymph nodes expanded and overnight skin broke down.

Five months after starting antituberculous therapy, the patient had a grand mal seizure. A CT scan of the brain was obtained, followed by an MRI. The MRI showed enhancing lesions with surrounding vasogenic edema, compatible with tuberculosis. The diagnosis of tuberculosis was confirmed by stereotactic biopsy, which demonstrated no viable mycobacteria. A course of oral prednisolone was started. The patient continued to receive this therapeutic regimen, and abatement of the intracranial lesions was monitored radiologically.

It has been suggested that the growth of tuberculomas during treatment may be related to an immunologic process involving altered cell-mediated responsiveness in the context of mycobacterial killing during chemotherapy [1, 3]. Certainly, clinicians have long recognized that enlargement of regional lymph nodes occurs during chemotherapy [4].

The recent advent of widespread use of HAART in HIV disease has produced encouraging results in terms of reduction in HIV viral loads and some degree of immune reconstitution reflected by rising CD4 T lymphocyte counts and other indices [5]. Various speculations have been made about the clinical effects of such immunologic changes [6].

Biliary Aspiration After Administration of Intravenous Cholecystokinin for the Diagnosis of Hepatobiliary Fascioliasis

Hepatobiliary fascioliasis is a zoonosis that is usually acquired after the ingestion of uncooked wild watercress. The acute stage is characterized by fever, urticarial rash, arthralgias, abdominal pain, hepatomegaly, and eosinophilia. The chronic stage is characterized by cholestasis and persistent eosinophilia. Both nodular and tunnel-like branching hypodense lesions may be found on abdominal CT scans. Indirect immunofluorescence and ELISA are the methods currently used for diagnosis confirmation [1].

Detection of eggs or parasites by microscopic analysis of bile samples obtained via upper endoscopy after intravenous infusion of cholecystokinin may be a new method for the diagnosis of hepatobiliary fascioliasis. Cholecystokinin promotes gallbladder contraction, a relaxation of Oddi’s sphincter, and release of bile into the duodenum a few minutes after infusion. We describe a patient with hepatobiliary fascioliasis that was diagnosed with use of this new technique.

A 67-year-old woman was admitted to the hospital because of peripheral eosinophilia. Five months before admission, she had developed fever (temperature, 38°C), an urticarial rash on the lower extremities, and arthralgias in the ankles and knees. The arthralgias had resolved in 15 days. One month before admission, she became asymptomatic. She had never had diarrhea or abdominal pain. She...
5.19. Two decades of disseminated tuberculosis at a university medical center: the expanding role of mycobacterial blood culture


CONTRIBUTION

I conceived the research idea, developed the protocol, collected, entered, and analyzed the data, and wrote the manuscript. Reller provided mentorship for the project and sought and obtained funding. All authors contributed to revisions of the manuscript.

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Two Decades of Disseminated Tuberculosis at a University Medical Center: The Expanding Role of Mycobacterial Blood Culture

John A. Crump1,2 and L. Barth Reller1,3

1Clinical Microbiology Laboratory, Duke University Medical Center, and Departments of 2Pathology and 3Medicine, Duke University School of Medicine, Durham, North Carolina

We describe the clinical presentation, predisposing conditions, diagnostic approach, and outcome for 52 patients with disseminated tuberculosis who presented at Duke University Medical Center (Durham, NC) from 1980 through 1999. The mean age of the patients was 52 years (range, 2–93 years). Fever and weight loss were common at presentation, and delays in the initiation of therapy often occurred. Predisposing conditions included human immunodeficiency virus infection (46% of patients), immunosuppressive therapy (21%), alcoholism (12%), diabetes mellitus (12%), and hematologic disorders (8%); 17% of patients had no disorder of immunity detected. Examination of biopsy specimens from sites of localized disease, especially lymph nodes, had a high diagnostic yield. In this study, mycobacterial blood culture appeared to be as sensitive as bone marrow culture in diagnosing disseminated tuberculosis (sensitivity, 58% vs. 54%). To diagnose disseminated tuberculosis, a search for sites of localized disease should be undertaken, and samples from these sites should be obtained. Mycobacterial blood culture can play an increasing role in the diagnosis of disseminated tuberculosis when localized disease is not found.

Disseminated tuberculosis is increasingly recognized to be an important cause of morbidity and mortality in developing countries among patients who are also infected with HIV [1–3]. In areas in which both tuberculosis and HIV infection are endemic, bacteremic disseminated tuberculosis is among the leading causes of bloodstream infection in hospitalized patients [4] and is a neglected presentation of the disease, despite its substantial contribution to AIDS-related deaths. In industrialized countries, HIV infection [5] and chronic noninfectious diseases and immunosuppressive drugs and treatments [6] predispose to disseminated tuberculosis. Furthermore, the treatment of tuberculosis, particularly disseminated disease, may be complicated in patients who are also eligible for HAART, as a result of drug interactions and immuneconstitution syndromes [7, 8]. The population at risk for disseminated tuberculosis has been increasing worldwide with the expansion of the HIV pandemic [9] and the wider application of medical therapies that result in immunosuppression. Diagnosis of disseminated tuberculosis is difficult [10, 11], because the clinical presentation may be nonspecific. Modern diagnostic tests may play a key role in diagnosis but may not be available to countries where the burden of disease is greatest.

Here, we review a series of patients with disseminated tuberculosis who presented at Duke University Medical Center (DUMC; Durham, NC) from 1980 through 1999. During this period, 497 patients received a diagnosis of tuberculosis (at any site): 282 during the
1980s and 215 during the 1990s. Several events occurred during this period that might have had an impact on the incidence, presentation, and diagnosis of disseminated tuberculosis. These include the emergence of the HIV pandemic, increasing use of immunosuppressive therapies to treat organ transplant recipients and patients with cancer, and improvements in mycobacterial culture systems. This study had several purposes: (1) to describe the clinical features and diagnostic test results for a series of patients with disseminated tuberculosis, (2) to describe the predisposing conditions for disseminated tuberculosis in the study population, (3) to define the changing role of available diagnostic microbiologic tests in the diagnosis of disseminated tuberculosis, (4) to compare bacteremic and nonbacteremic disseminated tuberculosis, and (5) to identify areas in which further research is needed.

METHODS

Our study was a retrospective review of medical and laboratory records from 1980 through 1999 at DUMC.

Case definition. The strict case definition for disseminated tuberculosis described by Iseman [12] was used. "Disseminated tuberculosis" was defined as isolation of Mycobacterium tuberculosis from blood or bone marrow, from a liver biopsy specimen, or from specimens from ≥2 noncontiguous organs in a single patient. Because the radiologic appearance of miliary shadowing has relatively low sensitivity [13] and specificity [14] for detection of disseminated tuberculosis, patients with a single culture positive for M. tuberculosis of a specimen from any site (except blood, bone marrow, or liver biopsy specimens) and a chest radiograph report by a radiologist of miliary shadowing were not included in this study.

Case finding. Because our case definition was based on strict microbiologic criteria, cases were found by reviewing laboratory records. The Mycobacteriology Section of the Clinical Microbiology Laboratory (CMB) at DUMC has maintained a searchable database of all cultures positive for Mycobacterium species since 1977.

Mycobacterial culture methods used by the CMB changed during the study period. For mycobacterial culture of blood, the Isolator 10 (Wampole Laboratories) lysis-centrifugation method, plated to Middlebrook 7H10 or 7H11 medium, was used from 1980 through 1990. The Bactec 460 system with 13A medium (Becton Dickinson) was used from 1991 through 1999. The BacT/ALERT Classic or 3D system with MB medium (bioMérieux) and the Bactec 9240 system with MYCO/F-Lytic medium (Becton Dickinson) were used from 1999 onward. Respiratory and other samples were cultured on Middlebrook 7H10 and Lowenstein-Jensen media from 1980 through 1989 and in Bactec 12B medium (Becton Dickinson), Middlebrook 7H11 agar, and Middlebrook 7H11 selective agar (with polymyxin B, carbenicillin, amphotericin B, and trimethoprim) from 1989 onward.

Data collection and analysis. We systematically sought several types of data for persons who met the case definition for disseminated tuberculosis. Clinical data were determined by review of medical records and the DUMC clinical information database. The results of hematologic and biochemical tests and radiologic investigation were also obtained by review of medical records and the DUMC clinical information database. Histopathologic findings were sought by review of medical records, the DUMC clinical information database, and the DUMC histopathologic information database. Microbiologic findings were sought by review of the CMB-DUMC mycobacteriology database and workbooks. Data were compiled and analyzed using Epi Info, version 6.04 (Centers for Disease Control and Prevention), and SAS System for Windows, release 8.0 (SAS Institute). P < .05 was considered to be statistically significant.

RESULTS

Clinical features. A total of 52 patients met the case definition for disseminated tuberculosis. Of these, 18 patients received the diagnosis during the 1980s, compared with 34 during the 1990s (figure 1). The mean age of patients was 52 years (range, 2–93 years). Twenty-eight patients (54%) were female. Forty-nine patients (94%) had temperatures >38.5°C within 24 h of admission, and 32 (62%) had experienced weight loss of >10% of body weight before admission. Eleven patients (21%) are known to have died within 1 month after presentation. Antituberculous chemotherapy was started before death in 44 patients (85%). Among patients who received such therapy, the mean time from presentation to initiation of therapy was 4.5 days (range, 1–43 days). The mean time between presentation and initiation of therapy was longer during the 1980s than during the 1990s (5.9 vs. 3.8 days), but the difference was not statistically significant (P > .05).

Underlying sources of immunosuppression are summarized in table 1. Of the 52 patients with disseminated tuberculosis, 24 (46%) were coinfected with HIV. Of those coinfected with HIV, the mean CD4+ T lymphocyte count was 67 cells/mm³ (range, 0–224 cells/mm³). Immunosuppressive therapy, including glucocorticosteroid treatment and cancer chemotherapy, was being received by 11 patients (21%). Six patients (12%) were found to have current alcohol dependence. Diabetes mellitus was present in 6 patients (12%), 5 of whom had insulin-requiring diabetes mellitus. Hematologic disorders were present in 4 patients (8%), 2 of whom had acute leukemia. Connective tissue diseases were present in 2 (4%). No immune disorder was detected in 9 patients (17%) with disseminated tuberculosis. HIV was an underlying source of immunosuppression.
for 6 (33%) of the 18 patients in whom disseminated tuberculosis was diagnosed during the 1980s and for 18 (53%) of the 34 patients in whom disseminated tuberculosis was diagnosed during the 1990s. This difference was not statistically significant.

**Hematologic and biochemical tests.** Hematologic and biochemical findings are summarized in table 2. Median values for patients with disseminated tuberculosis were outside normal ranges for hemoglobin concentration (10.1 g/dL for male patients and 9.1 g/dL for female patients), erythrocyte sedimentation rate (101 mm/h), lactate dehydrogenase concentration (779 U/L), and albumin concentration (2.4 g/dL). Median platelet and WBC counts were within normal ranges.

**Chest radiography.** Chest radiographs showed parenchymal opacity for 43 patients (83%); bilateral parenchymal opacity was present for 33 (65%). Predominant upper lobe disease was present for 15 patients (29%). Miliary shadowing was present for 15 (29%). Pleural effusion was present for 13 (25%) and intrathoracic lymphadenopathy for 12 (23%). Cavitation was noted for 2 (4%). The findings of chest radiography were reported to be normal for 7 patients (13%); of these 7 patients, sputum samples were not submitted for mycobacterial culture for 3 (43%). Of the 4 patients for whom sputum samples were submitted for mycobacterial culture, *M. tuberculosis* was isolated from samples from 3 (75%).

**Histopathologic examination.** The results of histopathologic examination of biopsy specimens are summarized in table 3. The most common biopsy sites were bone marrow, followed by lymph node, viscera (including stomach, gallbladder, ileocecum, colon, and appendix), transbronchial lung, bone and joint, "open" lung (lung tissue obtained by thoracotomy), peritoneum, pericardium, and testis. No liver or pleural biopsy specimens were obtained from our patients. Lymph node, peritoneal, and testicular biopsy specimens most often had features consistent with tuberculosis, followed by "open" lung, transbronchial lung, bone marrow, viscera, bone and joint, and pericardium specimens. Overall, 26 (50%) of 52 biopsy specimens had histopathologic features consistent with tuberculosis. Granulomas were present in 26 specimens (50%), caseating granulomas were present in 14 (27%), and acid-fast bacilli were seen in 10 (19%).

**Microbiologic investigations.** Specimens submitted for mycobacteriologic studies and the frequency with which specimens tested positive are summarized in table 4. Data are ag-

![Graph](image)

**Figure 1.** No. of patients with disseminated tuberculosis and role of blood culture in diagnosis at Duke University Medical Center (Durham, NC), in the 1980s and 1990s. Filled areas, positive mycobacterial blood culture contributed to diagnosis; open areas, diagnosis was made with tests other than mycobacterial blood culture.

<table>
<thead>
<tr>
<th>Source of immunosuppression</th>
<th>No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV infection</td>
<td>24 (48)</td>
</tr>
<tr>
<td>Receipt of immunosuppressive drugs</td>
<td>11 (21)</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Hematologic disorder</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>2 (4)</td>
</tr>
<tr>
<td>No apparent immune disorder</td>
<td>9 (17)</td>
</tr>
</tbody>
</table>

* Mean CD4+ cell count, 67 cells/mm²; range, 0-224 cells/mm².
* Includes 9 patients receiving glucocorticosteroids and 2 patients receiving cancer chemotherapy.
* Includes 5 patients with insulin-requiring diabetes mellitus and 1 patient with non-insulin-requiring diabetes mellitus.
* Includes 2 patients with acute leukemia, 1 patient with multiple myeloma, 1 patient with myelodysplastic syndrome, and 1 patient with β-thalassemia.
* Includes 1 patient with systemic lupus erythematosus and 1 patient with a mixed connective tissue disorder.
Table 2. Hematologic and biochemical findings for patients with disseminated tuberculosis.

<table>
<thead>
<tr>
<th>Test (no. of patients tested)</th>
<th>Normal range</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin concentration, g/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male patients (24)</td>
<td>13.7–17.3</td>
<td>10.1 (6.3–13.8)</td>
</tr>
<tr>
<td>Female patients (28)</td>
<td>12.0–15.5</td>
<td>9.1 (6.8–12.5)</td>
</tr>
<tr>
<td>Platelet count, platelets × 10⁹/L (49)</td>
<td>150–450</td>
<td>208 (14–822)</td>
</tr>
<tr>
<td>Total WBC count, cells × 10⁹/L (52)</td>
<td>3.2–9.8</td>
<td>6.4 (1.3–22.7)</td>
</tr>
<tr>
<td>Neutrophil count, neutrophils × 10⁹/L (46)</td>
<td>1.2–7.8</td>
<td>4.8 (0.9–21.7)</td>
</tr>
<tr>
<td>Lymphocyte count, lymphocytes × 10⁹/L (46)</td>
<td>0.3–4.9</td>
<td>0.6 (0.2–3.0)</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate, mm/h (16)</td>
<td>1–15</td>
<td>101 (16–125)</td>
</tr>
<tr>
<td>Alamine aminotransferase level, U/L (45)</td>
<td>10–60</td>
<td>31 (3–378)</td>
</tr>
<tr>
<td>Alkaline phosphatase level, U/L (50)</td>
<td>30–135</td>
<td>128 (33–1635)</td>
</tr>
<tr>
<td>Lactate dehydrogenase concentration, U/L (37)</td>
<td>260–600</td>
<td>779 (198–2848)</td>
</tr>
<tr>
<td>Albumin concentration, g/dL (39)</td>
<td>3.9–5.0</td>
<td>2.4 (1.2–3.7)</td>
</tr>
</tbody>
</table>

Mycobacterial blood culture played a larger role in the diagnosis of disseminated tuberculosis during the 1990s than during the 1980s (figure 1). The proportion of persons in whom disseminated tuberculosis was diagnosed based on the positive results of blood culture increased significantly, from 3 (17%) of 18 patients tested during the 1980s to 16 (47%) of 34 during the 1990s (P = .030).

In addition, we identified 6 patients with disseminated tuberculosis for whom both bone marrow and blood were cultured for mycobacteria. In each case, the bone and bone marrow samples were collected within a period of 2 weeks and before the initiation of antituberculous therapy. Four patients had immunocompromise resulting from HIV infection, 1 patient had immunosuppression associated with oral prednisone therapy, and 1 had no apparent immunosuppression. All 6 patients had positive results of blood cultures for M. tuberculosis, but the results of culture of bone marrow aspirate were positive for only 4 (table 5).

Comparison of bacteremic and nonbacteremic disseminated tuberculosis. Patients with bacteremic disseminated tuberculosis were less likely (risk ratio, 0.32; P = .037) to have miliary shadowing on a chest radiograph and were more likely to die (risk ratio, 5.60; P = .035) within 1 month after admission to DUMC than were patients with nonbacteremic disseminated tuberculosis. There were no significant differences in the results of hematologic and biochemical tests, chest radiographic findings, histopathologic findings, and mycobacterial culture results for patients with bacteremic and patients with nonbacteremic disseminated tuberculosis.

DISCUSSION

We describe a series of patients with disseminated tuberculosis at DUMC from 1980 through 1999 who were selected on the basis of a strict case definition. Because of the retrospective design of our study, we were not able to capture complete clinical and laboratory information and could assess only a limited range of patient outcomes. Nonetheless, the results highlight several important findings.

The number of patients meeting the case definition for disseminated tuberculosis at DUMC during the 1990s was twice that during the 1980s, despite a decrease in the number of patients in whom tuberculosis of any site was diagnosed at DUMC during the same period. There are several possible explanations for this increase [15]. One is that a larger immunosuppressed population at risk for disseminated tuberculosis presented to DUMC. This population includes patients infected with HIV and patients who require aggressive medical therapies that result in immunosuppression. Although our data show that the proportion of patients with disseminated tuberculosis who were coinfected with HIV was larger during the 1990s than during the 1980s, the difference was not statistically significant. Recent improvements in clinical awareness of disseminated tuberculosis could lead to the correct diagnosis for more patients. Advances in mycobacterial culture systems offer another ex-
Table 3. Histopathologic findings for 52 patients with disseminated tuberculosis.

<table>
<thead>
<tr>
<th>Biopsy site</th>
<th>No. (%) of specimens examined</th>
<th>No. (%) of positive specimens</th>
<th>No. (%) of specimens with indicated finding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Granuloma</td>
<td>Casing granuloma</td>
</tr>
<tr>
<td>Lymph node</td>
<td>6 (12)</td>
<td>6 (12)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>2 (4)</td>
<td>2 (4)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Testis</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>“Open” lung</td>
<td>4 (8)</td>
<td>3 (6)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Transbronchial lung</td>
<td>5 (10)</td>
<td>3 (6)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>23 (44)</td>
<td>8 (15)</td>
<td>8 (36)</td>
</tr>
<tr>
<td>Visceraa</td>
<td>6 (12)</td>
<td>2 (4)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Bone/joint</td>
<td>4 (8)</td>
<td>1 (2)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Pericardium</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Liver</td>
<td>0 (0)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pleura</td>
<td>0 (0)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Total</td>
<td>52 (100)</td>
<td>26 (50)</td>
<td>26 (50)</td>
</tr>
</tbody>
</table>

NOTE. AFB, acid-fast bacilli; NA, not available.
*a* includes stomach, gallbladder, ileocecum, colon, and appendix.

planation. The liquid and continuously monitored systems that were introduced during the late 1980s and the 1990s may be more sensitive [16] and have shorter detection times [17] than older systems. Furthermore, mycobacterial blood culture has become established and widely used as the test of choice for the detection of disseminated *Mycobacterium avium* complex disease in HIV-infected individuals [18]. Disseminated tuberculosis may present in a similar fashion in this patient population and thus may be detected by chance.

Patients in this study presented with undifferentiated fever and weight loss. The clinical signs and symptoms and derangements of basic hematologic and biochemical tests are not specific for the diagnosis of disseminated tuberculosis. This underscores the need for clinicians to maintain a high degree of clinical suspicion for the disease, particularly in caring for patients with underlying sources of immunosuppression, and to look for broad patterns of clinical and laboratory features.

Miliary shadowing occurred in only a minority (29%) of patients in our study, which emphasizes that this sign cannot be relied on to rule out the diagnosis of disseminated tuberculosis [19]. Because miliary shadowing occurs in association with a variety of other diseases, it also lacks specificity for disseminated tuberculosis [14]. Furthermore, a substantial proportion (13%) of our patients had normal findings of chest radiography. Sputum culture did not contribute to the diagnosis in more than one-half of such patients in our study, either because this test was not performed or because the results were negative. The problem of diagnosing disseminated tuberculosis in patients who have normal findings of chest radiography is compounded in contexts in which operational and resource constraints are greater [2, 20, 21]. In such instances, the clinician must rely heavily on interpretation of a constellation of clinical features [22].

Our series underscores the problems associated with delays in diagnosis and treatment of persons with disseminated tuberculosis. The mean time from clinical presentation to initiation of antituberculous chemotherapy was 4.5 days (range, 1–43 days), and treatment was not initiated for 15% of patients before they died. Such delays in the initiation of therapy could be avoided by an increase in awareness among clinicians, more-frequent use of empirical therapy, and more-rapid and sensitive diagnostic tests for tuberculosis [23].

We illustrate the value of searching for localized disease in patients with undifferentiated febrile illnesses who might have disseminated tuberculosis. Thorough clinical examination and radiographic studies may detect sites amenable to mycobacterial

Table 4. Mycobacterial culture results for specimens from 52 patients with disseminated tuberculosis.

<table>
<thead>
<tr>
<th>Sample used in culture</th>
<th>No. (%) of specimens tested</th>
<th>No. (%) of positive specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>44 (85)</td>
<td>42 (81)</td>
</tr>
<tr>
<td>Tissue</td>
<td>29 (58)</td>
<td>25 (48)</td>
</tr>
<tr>
<td>Urine</td>
<td>23 (44)</td>
<td>16 (31)</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>10 (19)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Blood</td>
<td>33 (63)</td>
<td>19 (37)</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>13 (25)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>Stool</td>
<td>9 (17)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>CSF</td>
<td>15 (29)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>5 (10)</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

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Table 5. Culture of peripheral blood versus bone marrow for diagnosis of disseminated Mycobacterium tuberculosis infection.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Year</th>
<th>Predisposing condition</th>
<th>Peripheral blood culture result</th>
<th>Bone marrow culture result</th>
<th>Mycobacterial culture system used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1988</td>
<td>HIV infection; CD4⁺ cell count of 224 cells/mm³</td>
<td>Positive</td>
<td>Positive</td>
<td>Isolator lysis-centrifugation; plated to Middlebrook 7H10 solid medium</td>
</tr>
<tr>
<td>2</td>
<td>1988</td>
<td>None</td>
<td>Positive</td>
<td>Negative</td>
<td>Isolator lysis-centrifugation; plated to Middlebrook 7H10 solid medium</td>
</tr>
<tr>
<td>3</td>
<td>1990</td>
<td>HIV infection; CD4⁺ cell count of 15 cells/mm³</td>
<td>Positive</td>
<td>Positive</td>
<td>Isolator lysis-centrifugation; plated to Middlebrook 7H11 solid medium</td>
</tr>
<tr>
<td>4</td>
<td>1992</td>
<td>Receipt of prednisone</td>
<td>Positive</td>
<td>Positive</td>
<td>Bactec 460 with 13A medium for peripheral blood and 12B medium for bone marrow</td>
</tr>
<tr>
<td>5</td>
<td>1995</td>
<td>HIV infection; CD4⁺ cell count of 12 cells/mm³</td>
<td>Positive</td>
<td>Positive</td>
<td>Bactec 460 with 13A medium for peripheral blood and 12B medium for bone marrow</td>
</tr>
<tr>
<td>6</td>
<td>1997</td>
<td>HIV infection; CD4⁺ cell count of 78 cells/mm³</td>
<td>Positive</td>
<td>Negative</td>
<td>Bactec 460 with 13A medium for peripheral blood and 12B medium for bone marrow</td>
</tr>
</tbody>
</table>

NOTE. Isolator was manufactured by Wampole Laboratories; the Bactec 460 system was manufactured by BD Biosciences.

Our data highlight the emerging role of mycobacterial blood culture in the diagnosis of disseminated tuberculosis (figure 1). We show that mycobacterial blood culture contributed to the diagnosis of disseminated tuberculosis in 17% of cases during the 1980s and that this increased to 47% during the 1990s (P = .030).

We report the first (to our knowledge) patient series in which the sensitivity of blood culture and bone marrow culture for the diagnosis of disseminated tuberculosis are compared. Blood cultures were positive for M. tuberculosis as often as bone marrow cultures (19 [58%] of 33 vs. 7 [54%] of 13 cultures, respectively); the difference between the 2 types of culture did not reach statistical significance (P > .05). Furthermore, evidence from 6 patients for whom both blood cultures and bone marrow cultures for mycobacteria were done suggests that culture of peripheral blood performed using modern systems may have a sensitivity comparable to that of culture of bone marrow for the diagnosis of disseminated tuberculosis. These findings are consistent with those for disseminated M. avium complex disease, in which peripheral blood culture has been demonstrated to have sensitivity comparable to that of culture of bone marrow in HIV-infected individuals [18, 25]. For disseminated tuberculosis, the possible equivalence of sensitivity may be due to a combination of improvements in mycobacterial blood culture systems and increased frequency and magnitude of bacteremia due to immunosuppression. Blood culture may be safer, less invasive, and cheaper than either bone marrow examination or liver biopsy and, therefore, is an attractive investigation when disseminated tuberculosis is suspected. On the other hand, a bone marrow aspirate can be examined for acid-fast bacilli and other organisms. However, acid-fast bacilli were seen on bone marrow smear for only a minority of our patients.

Data on the relative sensitivity of blood culture and bone marrow culture require validation in a prospective comparative study. However, a multiple-center design or inclusion of a setting in which the prevalence of disseminated tuberculosis is known to be high would be needed to accumulate sufficient cases. Our data support the role of mycobacterial blood culture in cases of suspected disseminated tuberculosis in which accessible end-organ disease is not present.

Our data show important differences between persons with bacteremic and those with nonbacteremic disseminated tuberculosis. We found that patients with bacteremic disseminated tuberculosis were less likely to have miliary shadowing on chest radiographs than were nonbacteremic patients. This may be due to the profound impairment of cell-mediated immunity that may accompany bacteremic disease; this impairment results in insufficient inflammatory response, causing a radiographically apparent miliary appearance. This finding is consistent with that of Long et al. [19]. Furthermore, we found that patients with bacteremic tuberculosis were 5 times more likely to die within 1 month after presentation than were those with nonbacteremic disseminated tuberculosis. The cryptic presentation and high mortality associated with bacteremic disseminated tuberculosis underscore the need for even greater vigilance for the subgroup of persons with that disease.

Disseminated tuberculosis is an increasing problem among patients presenting to DUMC and worldwide [2]. A high level of clinical suspicion should be maintained, especially in patients with certain sources of immunosuppression. Classic findings, such as miliary shadowing on chest radiographs, may be absent, especially in patients with mycobacteremia. When localized disease is present, these sites should be the focus of diagnostic tests. When localized disease is not present, increasing evidence...
supports the central diagnostic and prognostic role of mycobacterial blood cultures. Studies are needed to further clarify the role of mycobacterial blood culture, to develop and assess rapid diagnostic tests, and to determine the value of empirical therapy in disseminated tuberculosis.

References
5.20.  Controlled comparison of BACTEC 13A, MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems for detection of mycobacteremia


CONTRIBUTION

I conceived the research idea, designed the study, wrote the protocol, implemented the research, participated in laboratory evaluations, entered and analyzed the data, and wrote the manuscript. Tanner coordinated activities at the Carolinas Medical Center and Mirrett and McKnight coordinated activities at Duke University Medical Center. Reller sought and obtained funding, coordinated interactions between study sights, and provided mentorship and critical input to all aspects of the research. All authors contributed to revisions of the manuscript.

CITATIONS

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JOURNAL IMPACT FACTOR

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4.153
Controlled Comparison of BACTEC 13A, MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 Systems for Detection of Mycobacteremia

John A. Crump,1,2 David C. Tanner,3 Stanley Mirrett,1 Celeste M. McKnight,1 and L. Barth Reller1,2,4*

Clinical Microbiology Laboratory, Duke University Medical Center1 and Departments of Pathology2 and Medicine,2 Duke University School of Medicine, Durham, North Carolina 27710, and Clinical Microbiology Laboratory, Carolinas Medical Center, Charlotte, North Carolina 282033

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To compare the performance of the BACTEC 13A (Becton Dickinson, Sparks, Md.), BACTEC MYCO/F LYTIC (Becton Dickinson), BacT/ALERT MB (bioMérieux, Durham, N.C.), and ISOLATOR 10 (Wampole Laboratories, Cranbury, N.J.) systems for detection of mycobacteremia in adults, we inoculated 5-ml aliquots of blood from patients with suspected mycobacteremia into the bottle or tube required for each system. Of 600 sets tested, 85 (14%) yielded Mycobacterium avium complex (MAC) and 9 (2%) yielded other species of mycobacteria. Of 26 complete (three bottles and one tube) adequately filled (5 ± 1 ml) sets from which MAC was recovered, BACTEC 13A was positive for 19 (73%), BACTEC MYCO/F LYTIC was positive for 21 (81%), BacT/ALERT MB was positive for 22 (85%), and ISOLATOR 10 was positive for 21 (81%). Of the six possible two-way comparisons, the mean time to detection for the recovery of MAC from each bottle in positive adequately paired sets was 15.3 days for BACTEC 13A versus 12.8 days for MYCO/F LYTIC for 33 of 340 pairs, 14.1 days for BACTEC 13A versus 11.6 days for BacT/ALERT MB for 38 of 380 pairs, 12.6 days for BACTEC 13A versus 20.0 days for ISOLATOR 10 for 26 of 261 pairs, 12.8 days for BACTEC MYCO/F LYTIC versus 11.0 days for BacT/ALERT MB for 33 of 340 pairs, 13.2 days for BACTEC MYCO/F LYTIC versus 20.4 days for ISOLATOR 10 for 24 of 230 pairs, and 9.9 days for BacT/ALERT MB versus 19.0 days for ISOLATOR 10 for 24 of 257 pairs. There were no significant differences in yields between the systems. However, the mean time to detection differed significantly among the systems. The time to detection was shortest for BacT/ALERT MB, followed by BACTEC MYCO/F LYTIC and BACTEC 13A and then ISOLATOR 10. Although the numbers were too small for statistical comparison, the time to detection was substantially shorter for MAC than for Mycobacterium tuberculosis complex in the liquid systems. The continuously monitored systems (BACTEC MYCO/F LYTIC and BacT/ALERT MB) were as sensitive and, on balance, faster for the detection of MAC bacteremia than were the heretofore standard manual ISOLATOR 10 and radiometric BACTEC 13A systems.

Disseminated Mycobacterium avium complex (MAC) infection is a common opportunistic infection in patients with advanced human immunodeficiency virus (HIV) disease that is associated with a reduced probability of survival (7). Treatment with appropriate antimicrobial therapy can significantly improve patient survival (4). Although advances in HIV management such as prophylactic antimicrobial therapy (e.g., with clarithromycin) (12) and highly active antiretroviral therapy (3) have led to reduced incidences of disseminated MAC infection among patients with access to care, timely and accurate diagnosis of the disease remains an important function of the clinical microbiology laboratory. The clinical manifestations of disseminated MAC infection are nonspecific, so clinicians rely on laboratory confirmation to secure the diagnosis. Mycobacterial blood culture is the test of first choice for the diagnosis of disseminated MAC disease (15).

Mycobacterial blood culture methods in common use include visual inspection of processed blood inoculated on a solic medium (e.g., the ISOLATOR 10 system), intermittent radiometric detection in liquid medium inoculated with blood (e.g., the BACTEC 13A system), and now, continuous nonradiometric detection in liquid medium inoculated with blood (e.g., the BACTEC MYCO/F LYTIC or BacT/ALERT MB system). We conducted a multicenter controlled study to compare the performances of the BACTEC 13A, BACTEC MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems for the detection of mycobacteremia in adults.

(This study was presented in part at the 102nd General Meeting of the American Society for Microbiology, Salt Lake City, Utah, 20 May 2002 [J. A. Crump, D. C. Tanner, S. Mirrett, C. M. McKnight, and L. B. Reller, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. C-46, 2002].)

MATERIALS AND METHODS

Blood culture and collection. Samples for blood cultures were collected from patients hospitalized or seen as outpatients at the Duke University Medical Center and the Carolinas Medical Center from November 1999 through March 2002. All patients were suspected of having disseminated mycobacterial infection, determined by consultation with the infectious diseases and/or medical microbiology service. Blood cultures were performed as part of routine patient care. Venipuncture sites were disinfected with alcohol and then povidone iodine and were allowed to dry. Twenty milliliters of blood was obtained with a sterile
The BACTEC 13A system was positive for 19 (73%), the BACTEC MYCO/F LYTIC system was positive for 21 (81%), the BacT/ALERT MB system was positive for 22 (85%), and the ISOLATOR 10 system was positive for 21 (81%). Dimorphic fungi and yeasts were successfully recovered from all four blood culture systems, but the sample size for this study was too small to permit comparisons of sensitivity or mean times to detection for these isolates.

Table 2 shows the yields of MAC for all six two-way comparisons of adequately filled pairs of bottles. There were no significant differences between any two bottles compared. Of the six possible two-way comparisons of times to detection for adequate pairs of positive blood cultures, the mean time to detection was the shortest for the BacT/ALERT MB system, followed by the BACTEC MYCO/F LYTIC and BACTEC 13A systems and then the ISOLATOR 10 system (Table 3). There were no significant differences in the times to detection for the BACTEC 13A and BacT/ALERT MB bottles between bottles collected at the Duke University Medical Center and those collected at the Carolinas Medical Center.

The 94 mycobacteria recovered, 9 (10%) were of species other than MAC. M. tuberculosis complex bloodstream infections predominated among the non-MAC isolates (Table 1). Although the numbers are small, the mean time to detection was substantially shorter for MAC (14.0 days) than for the M. tuberculosis complex (23.8 days) in the liquid systems. Of the five sets yielding M. tuberculosis complex, M. tuberculosis was recovered from 4 (80%) BACTEC 13A bottles, 3 (60%) BACTEC MYCO/F LYTIC bottles, 2 (40%) BacT/ALERT MB bottles, and 4 (80%) ISOLATOR 10 tubes. Of the adequately filled individual bottles or tubes, the mean time to detection for each system for the M. tuberculosis complex was 28.0 days (range, 16 to 40 days) for the BACTEC 13A system, 26.5 days (range, 18 to 42 days) for the BACTEC MYCO/F LYTIC system, 25.0 days (range, 24 to 26 days) for the BacT/ALERT MB system, and 22.8 days (range, 19 to 31 days) for the ISOLATOR 10 system.

**RESULTS**

A total of 600 sets of blood cultures were processed; 85 (14%) sets yielded MAC, 5 (1%) sets yielded Mycobacterium tuberculosis complex (including one Mycobacterium bovis isolate), 3 (1%) sets yielded Mycobacterium chelonae, 1 set yielded Mycobacterium kansasii, 9 (2%) sets yielded Cryptococcus neoformans, 5 (1%) sets yielded Histoplasma capsulatum, 4 (1%) sets yielded Candida spp., and 1 set yielded Candida glabrata (Table 1). Of 26 complete (three bottles and one tube) adequately filled (5 ± 1 ml) sets from which MAC was recovered, the BACTEC 13A system was positive for 19 (73%), the BACTEC MYCO/F LYTIC system was positive for 21 (81%), the BacT/ALERT MB system was positive for 22 (85%), and the ISOLATOR 10 system was positive for 21 (81%).

**DISCUSSION**

In this controlled comparison of the BACTEC 13A, BACTEC MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems for the detection of mycobacteremia, the continuously monitored systems (BACTEC MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10) were significantly more sensitive than the BACTEC 13A system for the detection of mycobacteremia.
BacT/ALERT MB) were as sensitive and, on balance, faster for the detection of MAC bacteremia than the heretofore standard manual ISOLATOR 10 system (6) and radiometric BACTEC 13A system (14). Although we are not aware of previous studies that simultaneously compared the BACTEC 13A, BACTEC MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems, our results are consistent with those of comparisons of the BACTEC MYCO/F LYTIC system with a second commercial system for the detection of mycobacteria (5, 16). However, previous studies indicate that the equivalent sensitivities of the BacT/ALERT MB system and the BACTEC 460 system and the shorter recovery times of the BacT/ALERT MB system compared with that of the BACTEC 460 system for MAC from blood cultures does not imply superior performance for the recovery of the M. tuberculosis complex from respiratory specimens (13).

The reasons for the faster detection times for the continuously monitored systems (BACTEC MYCO/F LYTIC, BacT/ALERT MB) could include their frequency of examination compared with the twice-weekly examination of the radiometric BACTEC 13A broth system and the weekly examination of plates inoculated with sediment from the ISOLATOR 10 system. More frequent examination of the manual systems may have led to the faster detection of growth. However, the differences observed were greater than those that could be accounted for by weekly examination. Moreover, it was our intention to assess the systems as they would be applied in routine use in the clinical microbiology laboratory, and as such, our results reflect a practical difference in time to detection of growth.

To standardize volumes, we inoculated 5 ml of blood into each system. The ISOLATOR 10 system is designed for 10 ml of blood. It is possible that the increased concentrations of lytic agent and anticoagulant in the ISOLATOR 10 system relative to the lower volume of blood obtained may have inhibited mycobacterial growth (18). However, ISOLATOR 10 tubes were processed within 8 h of collection. Furthermore, to avoid the inhibition noted with inoculation into BACTEC 12B broth, sediment from the ISOLATOR 10 tubes was plated onto solid medium (17). It should be noted that despite the longer detection times demonstrated in our study, the ISOLATOR 10 system does have the advantage of providing isolated colonies for the purposes of mycobacterial identification and antimicrobial susceptibility testing. Agitation of the continuously monitored systems may also contribute to the shorter time to positivity for the BACTEC MYCO/F LYTIC and BacT/ALERT MB systems (8).

Mycobacteria other than MAC accounted for a large proportion (10%) of the sources of mycobacterial bloodstream infections in this study. However, M. tuberculosis complex isolates predominated among the non-MAC isolates as sources of bloodstream infections. The substantially longer mean time to detection for M. tuberculosis complex than for MAC in the liquid systems is of concern because mycobacterial bloodstream infection carries a high mortality rate (1, 2) and is an important HIV-associated opportunistic infection in sub-Saharan Africa and Southeast Asia (9). Further studies in areas of endemicity will help to define the roles of these systems for the detection of other mycobacterial species.

In conclusion, clinical microbiology laboratories may shorten the times for the recovery of MAC from blood cultures without compromising the yield by changing from the more manual methods with the ISOLATOR 10 and BACTEC 13A systems to one of the continuously monitored systems studied, namely, the MYCO/F LYTIC or BacT/ALERT MB system.

ACKNOWLEDGMENTS

This study was supported in part by bioMérieux. We gratefully acknowledge the assistance of the laboratory staff of the Clinical Microbiology Laboratories at the Duke University Medical Center and the Carolinas Medical Center, particularly Mary Rutledge.

REFERENCES


### TABLE 3. Comparative Times to Detection of MAC from BACTEC 13A, MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 Systems

<table>
<thead>
<tr>
<th>Bottle pair (bottle 1, bottle 2)</th>
<th>Mean (range) time (days) to positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bottle 1</td>
</tr>
<tr>
<td>BACTEC 13A, MYCO/F LYTIC</td>
<td>15.3 (6–42)</td>
</tr>
<tr>
<td>BACTEC 13A, BacT/ALERT MB</td>
<td>14.1 (6–39)</td>
</tr>
<tr>
<td>BACTEC 13A, ISOLATOR 10</td>
<td>12.6 (6–32)</td>
</tr>
<tr>
<td>MYCO/F LYTIC, BacT/ALERT MB</td>
<td>12.8 (7–23)</td>
</tr>
<tr>
<td>MYCO/F LYTIC, ISOLATOR 10</td>
<td>13.2 (7–23)</td>
</tr>
<tr>
<td>BacT/ALERT MB, ISOLATOR 10</td>
<td>9.9 (1–17)</td>
</tr>
</tbody>
</table>

*NS, not significant.
5.21. Community acquired bloodstream infections in Africa: a systematic review and meta-analysis


CONTRIBUTION

My position as last author reflects my role as senior, supervising author on the paper. I conceived the research idea, designed the study, and supervised the research including the study team members responsible for the systematic review and analysis. I mentored the fellow Dr. Reddy and the medical student, Ms. Shaw, who worked on this project. Reddy and Shaw searched electronic databases, screened papers, and sought and reviewed full-length papers. I served as tiebreaker reviewer and guided the work on a day-to-day basis. Reddy wrote the first draft of the manuscript. All authors contributed to revisions of the manuscript.

CITATIONS

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Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis

Elizabeth A Reddy, Andrea V Shaw, John A Crump

Data on the prevalence and causes of community-acquired bloodstream infections in Africa are scarce. We searched three databases for studies that prospectively studied patients admitted to hospital with at least a blood culture, and found 22 eligible studies describing 58,296 patients, of whom 2051 (13·5%) of 15,166 adults and 3527 (8·2%) of 43,130 children had bloodstream infections. 1643 (29·1%) non-malaria bloodstream infections were due to Salmonella enterica (58·4% of these non-typhoidal Salmonella), the most prevalent isolate overall and in adults, and 1031 (18·3% overall) were due to Streptococcus pneumoniae, the most common isolate in children. Other common isolates included Staphylococcus aureus (531 infections; 9·5%) and Escherichia coli (412; 7·3%). Mycobacterium tuberculosis complex accounted for 166 (30·7%) of 539 isolates in seven studies that used mycobacterial culture techniques. HIV infection was associated with any bloodstream infection, particularly with S enterica and M tuberculosis complex bacteremia. Where recorded, patients with bloodstream infections had an in-hospital case fatality of 18·1%. Our results show that bloodstream infections are common and associated with high mortality. Improved clinical microbiology services and reassessment of empirical treatment guidelines that account for the epidemiology of bloodstream infections might contribute to better outcomes.

Introduction

Febrile illness is a leading reason for admission to hospital in Africa, and rates of febrile illness are fuelled by the HIV epidemic. Despite the major contribution of infectious diseases to hospital admission, the availability of diagnostic microbiology services for bloodstream infections other than malaria is often limited by cost, infrastructure, and personnel constraints. Consequently, health-care workers must often rely on syndrome-oriented empirical approaches to treatment and might underestimate or overestimate the likelihood of certain diseases, risking poor clinical outcomes and the promotion of antimicrobial resistance. Understanding the causes and prevalence of community-acquired bloodstream infection, which is associated with high risk of death, can inform efforts to improve health outcomes in Africa and promote the meeting of millennium development goals for the reduction of child mortality and HIV/AIDS, malaria, and tuberculosis.

Early studies of bloodstream infections in children admitted to African hospitals suggest that the prevalence of bacterial bloodstream infections among inpatients with fever or clinical sepsis exceeds that described in wealthier regions and that bacteremia is a common cause of illness both in areas of high and low malaria prevalence. Gram-negative organisms, particularly Salmonella enterica, rival or exceed Gram-positive organisms in importance in several published reports on bloodstream infections in both adults and children from African countries. In recent years, use of blood culture to assess seriously ill patients infected with HIV has led to a growing understanding of their increased risk of a range of invasive bacterial and fungal diseases, including Streptococcus pneumoniae, disseminated tuberculosis, cryptococcosis, and Salmonella bacteraemia caused by non-typhoidal Salmonella.

We sought to review studies that used blood culture to identify non-malaria bloodstream infections among prospectively sampled adults and children with predefined, replicable, inclusion criteria admitted to hospitals in Africa, and then to aggregate these data to better quantify the prevalence of bloodstream infections and document the most commonly isolated organisms overall and among different subgroups. We postulated that bloodstream infections would be identified among many patients admitted to hospital in Africa, S enterica would be among the most commonly isolated pathogens, and that age, presence of HIV infection, and features of illness would affect the prevalence of bloodstream infections and predominant organisms isolated.

Methods

Search strategy and selection criteria

We searched two major scientific databases (PubMed and Embase) and one topical database (African Healthline) with terms defined with the assistance of a library science technologist (Megan Von Isenburg). PubMed was searched with the search string: “Africa and (fever or fevers or bacteremia or bacteraemias or septicemia or septicemias) limit humans”. Embase was searched by use of the terms: “Africa” (explored to all subheadings) and “fever” or “fevers.mp or fevers.ip or bacteremia/ or bacteriemias.mp or bacteremia.mp or septicemia/ or septicemia.mp or septicemias.mp or septicemias.ip”. Results were limited to humans. African Healthline was searched by use of the string: “Africa and fever or bacteremia or septicemia” limit humans and scholarly (peer reviewed) journals. Abstracts and titles from all years and in all languages—translated though services provided by the search engines as needed—were compiled in Endnote (Thomson Reuters) and reviewed individually by two investigators.
Included studies were required to be prospective, to recruit systematically or consecutively sampled paediatric or adult hospital admissions, and to evaluate all admissions, all febrile admissions, or all febrile admissions without a focus of infection using, at least, an aerobic blood culture. Studies specifying use of predefined criteria that included afebrile patients with suspected infections in addition to febrile patients were also included.

During abstract review, we excluded articles that investigated a single or narrow cause of febrile illness; studies with the primary goal of investigating diagnostics or treatments; and review articles, editorials, policy statements, and behavioural research. We also excluded articles describing illness in people living outside the African continent (eg, returned travellers), articles that only assessed bloodstream infections in specific risk groups (eg, sickle-cell anaemia), non-hospital-based studies, retrospective analyses, case reports, studies focused on nosocomial infections, and studies during an epidemic or outbreak.

During full-text review, we excluded studies that examined specific risk populations or used retrospective inclusion techniques (eg, bacteraemia as the trigger for study entry), and those that used subjective or poorly defined inclusion criteria. Articles in which separation of outpatient and inpatient cohorts was not possible, and those that reported on patient cohorts already included in another more comprehensive study were excluded. We also excluded articles that did not distinguish between community-acquired and nosocomial infections. All articles were required to quantify the total number of blood cultures obtained and the total number of pathogenic isolates, as well as to identify the three most commonly isolated pathogens and detail the numbers of patients from whom these pathogens were isolated. If incomplete data or unclear methods precluded article inclusion, authors were contacted through the contact information provided by the article or obtained through internet search engines. Only studies meeting our minimum requirements for data completeness described above were included.

**Validity assessment**

Study validity was established by use of the selection criteria described above, thereby excluding studies that were thought likely to have invalid results or whose results could not be compared with studies included in the analysis. We expected variability to exist between microbiological techniques and interpretation of culture results, and that such variability probably reflects realities of studies of this type done in resource-limited settings. Therefore studies were not excluded on the basis of having used media and transport techniques that might have limited the isolation of fastidious pathogens. Studies were also not excluded on the basis of lack of detailed reporting of contaminants; however, the prevalence of possible contaminants among the...
<table>
<thead>
<tr>
<th>Location; study dates</th>
<th>Hospital type</th>
<th>Age (population type)</th>
<th>Primary eligibility criteria</th>
<th>Other criteria</th>
<th>Temperature (oral/rectal)</th>
<th>Non-malarial RSI</th>
<th>Malarial RSI</th>
<th>Mycobacterial culture</th>
<th>Patients infected with HIV (proportion of patients tested)*</th>
<th>Most common isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afifi et al*</td>
<td>Multiple locations, Egypt: 1999-2003</td>
<td>Public infectious disease hospitals</td>
<td>&gt;4 years (primarily adults)</td>
<td>Fever without localising signs</td>
<td>38-5°C (oral, 38°C (rectal))</td>
<td>1005 (10.2%)</td>
<td>446 (9.9%)</td>
<td>No</td>
<td>-</td>
<td>Salmonella enterica serotype Typhi, Brucella spp, Staphylococcus aureus</td>
</tr>
<tr>
<td>Akeede et al*</td>
<td>Benin City, Nigeria, October 1988 to October 1989</td>
<td>Urban teaching hospital</td>
<td>1 month to 5 years</td>
<td>Fever without localising signs</td>
<td>38°C (rectal)</td>
<td>67 (10.4%)</td>
<td>446 (9.9%)</td>
<td>No</td>
<td>-</td>
<td>Staphylococcus aureus, Enterobacteriacea (75% untyped), Citrobacter spp</td>
</tr>
<tr>
<td>Archibald et al*</td>
<td>Dar es Salaam, Tanzania, February to April 1995</td>
<td>Urban referral and teaching hospital</td>
<td>&lt;15 years (primarily adults)</td>
<td>Fever</td>
<td>37.5°C (axillary)</td>
<td>145 (28.9%)</td>
<td>49 (9.8%)</td>
<td>Yes</td>
<td>282 (56.2%)</td>
<td>Mycobacterium tuberculosis complex, non-typhoidal Salmonella, S enterica</td>
</tr>
<tr>
<td>Archibald et al*</td>
<td>Lilongwe, Malawi, March to April 1998</td>
<td>Urban government hospital</td>
<td>&gt;13 years (primarily children)</td>
<td>Fever</td>
<td>37.5°C (axillary)</td>
<td>12 (35.0%)</td>
<td>15 (45.0%)</td>
<td>Yes</td>
<td>25 (31.3%)</td>
<td>Non-typhoidal Salmonella, Escherichia coli, S enterica serotype Typhi, S aureus</td>
</tr>
<tr>
<td>Archibald et al*</td>
<td>Lilongwe, Malawi, July to August 1998</td>
<td>Urban government hospital</td>
<td>1 month to 13 years (primarily children)</td>
<td>Fever</td>
<td>35 (35.0%)</td>
<td>35 (57.0%)</td>
<td>Yes</td>
<td>63 (28.0%)</td>
<td>Non-typhoidal Salmonella, E coli</td>
<td></td>
</tr>
<tr>
<td>Ayoola et al*</td>
<td>Ibadan, Nigeria, June to November 1998</td>
<td>Urban referral and teaching hospital</td>
<td>1-12 months (primarily children)</td>
<td>Fever</td>
<td>38°C (rectal)</td>
<td>39 (38.2%)</td>
<td>47 (46.1%)</td>
<td>No</td>
<td>-</td>
<td>E coli, S aureus, Klebsiella spp</td>
</tr>
<tr>
<td>Bahwere et al*</td>
<td>Lwi a, DR Congo, January 1989 to December 1990</td>
<td>Rural children’s hospital</td>
<td>Unspecified (primarily children)</td>
<td>All hospital or ward admissions, irrespective of fever</td>
<td>--</td>
<td>124 (15.9%)</td>
<td>182 (28.0%)</td>
<td>No</td>
<td>-</td>
<td>Non-typhoidal Salmonella, E coli, Citrobacter spp</td>
</tr>
<tr>
<td>Bell et al*</td>
<td>Lilongwe, Malawi, March to May 1998</td>
<td>Urban government hospital</td>
<td>&gt;14 years (primarily adults)</td>
<td>Fever</td>
<td>37.5°C (axillary)</td>
<td>67 (28.2%)</td>
<td>72 (31.2%)</td>
<td>Yes</td>
<td>173 (72.7%)</td>
<td>Non-typhoidal Salmonella, M tuberculosis complex, Cryptococcus spp</td>
</tr>
<tr>
<td>Berkeley et al*</td>
<td>Kilifi, Kenya, August 1999 to July 2002</td>
<td>Rural district referral hospital</td>
<td>&lt;13 years (primarily children)</td>
<td>All hospital or ward admissions, irrespective of fever</td>
<td>--</td>
<td>3064 (6.6%)</td>
<td>173 (9.4%)</td>
<td>No</td>
<td>243 (11.7%)</td>
<td>Staphylococcus pneumoniae, non-typhoidal Salmonella, Haemophilus influenzae</td>
</tr>
<tr>
<td>Brent et al*</td>
<td>Kilifi, Kenya, July to October 2003</td>
<td>Rural district referral hospital</td>
<td>0-5 years (primarily children)</td>
<td>All hospital or ward admissions, irrespective of fever</td>
<td>--</td>
<td>9 (4.2%)</td>
<td>0 (0.0%)</td>
<td>No</td>
<td>-</td>
<td>S pneumoniae, S aureus</td>
</tr>
<tr>
<td>Dougle et al*</td>
<td>Mumias, Kenya, July to October 1994</td>
<td>Rural missionary hospital</td>
<td>#5 years (primarily adult)</td>
<td>Fever</td>
<td>38.0°C (axillary)</td>
<td>51 (22.3%)</td>
<td>25 (11.0%)</td>
<td>No</td>
<td>51 (22.5%)</td>
<td>S enterica serotype Typhi, non-typhoidal Salmonella, S pneumoniae</td>
</tr>
</tbody>
</table>

(Continues on next page)
organisms described as pathogenic was recorded and its possible effect on the prevalence of bloodstream infections is discussed. Heterogeneity was controlled primarily by specifying the use of predefined inclusion criteria. However, since the goal of the review was to broadly assess prevalence and type of community-acquired bloodstream infections across Africa, it was important that included articles encompassed expected diversity in different hospital communities (eg, academic, government, rural, urban) without focusing too narrowly on specific patient groups at risk for bloodstream infections (eg, patients in whom treatment for malaria had already failed). Subgroup analyses were done to explore the effect of heterogeneity on the overall prevalence or predominant types of bloodstream infections. Publication bias was not systematically

<table>
<thead>
<tr>
<th>Location, study dates</th>
<th>Hospital type</th>
<th>Age (population type)</th>
<th>Primary eligibility criteria</th>
<th>Other criteria</th>
<th>Temperature</th>
<th>Non-malarial BSI</th>
<th>Malarial BSI</th>
<th>Mycobacterial culture</th>
<th>Patients infected with HIV (proportion of patients tested)*</th>
<th>Most common isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enwere et al&lt;sup&gt;13&lt;/sup&gt;</td>
<td>Bansang, Gambia; August, 2000, to April, 2004</td>
<td>Rural hospital</td>
<td>2-29 months (primarily children)</td>
<td>Fever</td>
<td>38.0°C</td>
<td>248 (8.7%)</td>
<td>-</td>
<td>No</td>
<td>-</td>
<td>S pneumoniae, S enterica, H influenzae</td>
</tr>
<tr>
<td>Falade et al&lt;sup&gt;14&lt;/sup&gt;</td>
<td>Badain, Nigeria; February, 2005, to January, 2006</td>
<td>Urban teaching, maternity, and state hospitals</td>
<td>2-59 months (primarily children)</td>
<td>Suspect pneumococcal disease</td>
<td>-</td>
<td>222 (18.3%)</td>
<td>No</td>
<td>-</td>
<td>S aureus, Klebsiella spp, S enterica</td>
<td></td>
</tr>
<tr>
<td>Gordon et al&lt;sup&gt;15&lt;/sup&gt;</td>
<td>Blantyre, Malawi; December, 1992, to November, 1998</td>
<td>Urban government referral and teaching hospital</td>
<td>14-76 years (primarily adults)</td>
<td>Fever</td>
<td>37.5°C</td>
<td>449 (16.1%)</td>
<td>No</td>
<td>-</td>
<td>Non-typhoidal Salmonella, S pneumoniae, E coli</td>
<td></td>
</tr>
<tr>
<td>Hyams et al&lt;sup&gt;16&lt;/sup&gt;</td>
<td>Port Sudan, Sudan; January, 1994</td>
<td>Urban government hospital</td>
<td>&gt;12 years (primarily adults)</td>
<td>Fever without localising signs</td>
<td>37.5°C</td>
<td>22 (22.0%)</td>
<td>13 (13.0%)</td>
<td>No</td>
<td>-</td>
<td>S enterica serotype Typhi, S enterica serotype Paratyphi A, Shigella flexneri</td>
</tr>
<tr>
<td>Nathoo et al&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Harare, Zimbabwe; June, 1993, to July, 1994</td>
<td>Urban referral hospital for municipal clinics</td>
<td>0-8 years</td>
<td>Fever</td>
<td>38.0°C (axillary)</td>
<td>95 (30.7%)</td>
<td>No</td>
<td>168 (54.4%)</td>
<td>Staphylococcus epidermidis, S aureus, S pneumoniae</td>
<td></td>
</tr>
<tr>
<td>Okwara et al&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Nairobi, Kenya; January to March 2001</td>
<td>Urban public teaching hospital</td>
<td>3 months to 12 years</td>
<td>Fever without localising signs</td>
<td>37.5°C</td>
<td>32 (12.1%)</td>
<td>358 (59.8%)</td>
<td>No</td>
<td>-</td>
<td>Non-typhoidal Salmonella, Citrobacter spp, S aureus, Enterococcus spp</td>
</tr>
<tr>
<td>Peters et al&lt;sup&gt;19&lt;/sup&gt;</td>
<td>Blantyre, Malawi; February to May, 2000</td>
<td>Urban government referral and teaching hospital</td>
<td>&gt;14 years (primarily adults)</td>
<td>Fever</td>
<td>37.4°C (axillary)</td>
<td>128 (36.4%)</td>
<td>69 (19.6%)</td>
<td>Yes</td>
<td>291 (82.7%)</td>
<td>M tuberculosis complex, non-typhoidal Salmonella, S pneumoniae</td>
</tr>
<tr>
<td>Petit et al&lt;sup&gt;20&lt;/sup&gt;</td>
<td>Mukuru, Kenya (study II); 1987</td>
<td>Rural hospital</td>
<td>&gt;8 years (primarily adults)</td>
<td>Fever</td>
<td>38.0°C</td>
<td>25 (7.4%)</td>
<td>104 (31.0%)</td>
<td>No</td>
<td>12 (3.6%)</td>
<td>S enterica, Acinetobacter spp, Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Petit et al&lt;sup&gt;21&lt;/sup&gt;</td>
<td>Rural Western Kenya (study II); 1987</td>
<td>Rural hospital</td>
<td>&gt;8 years (primarily adults)</td>
<td>Fever without localising signs</td>
<td>38.0°C</td>
<td>33 (29.2%)</td>
<td>22 (27.5%)</td>
<td>No</td>
<td>-</td>
<td>Proteus mirabilis, S enterica, S aureus</td>
</tr>
</tbody>
</table>

(Continues on next page)
calculated, since negative studies would be extremely unlikely and no standard for measuring expected prevalence of bloodstream infections in Africa exists. However, the possibility of publication bias in exaggerating the prevalence of bloodstream infections is discussed qualitatively.  

Data extraction

Descriptive and quantitative data from each paper were extracted individually by two investigators (EAR and AVS) and entered into a Microsoft Excel 2007 spreadsheet; this included hospital setting and location, region of Africa according to regional groupings detailed within the UNAIDS epidemic update 2007, study time frame, specific inclusion or exclusion criteria, and culture techniques. Quantitative data collected included number of patients, age range, pathogens and contaminants isolated, antimicrobial susceptibilities, and use of additional tests (eg, for HIV). Paediatric studies were defined as those in which all included patients were younger than 15 years; studies with mixed populations of adult and children were analysed as adult studies. Inconsistencies between investigators after data extraction were resolved by return to the original papers.

Statistical analysis

Data on individual patients were compiled and analysed in aggregate to compare prevalence of bloodstream infections across studies and among different subpopulations. Analyses of associations between types of patients or clinical conditions (eg, HIV) and specific bloodstream infections were only done for studies in which full data were available for both the pathogens and factors being assessed; no data were imputed for any analyses. The $\chi^2$ test or Fisher’s exact test for frequencies less than five was used to establish the

Table 1: Summary of 22 studies of community-acquired bloodstream infection (BSI) among patients admitted to hospital in Africa, 1984-2006

<table>
<thead>
<tr>
<th>Location, study dates</th>
<th>Hospital type</th>
<th>Age (population type)</th>
<th>Primary eligibility criteria</th>
<th>Other criteria</th>
<th>Temperature</th>
<th>Non-malarial BSI</th>
<th>Malarial BSI</th>
<th>Mycobacterial culture</th>
<th>Patients infected with HIV (proportion of patients tested)*</th>
<th>Most common isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigauque et al (^a)</td>
<td>Manhija, Mozambique; May, 2001, to April, 2006</td>
<td>Rural district referral hospital</td>
<td>&lt;15 years</td>
<td>All hospital or ward admissions, irrespective of fever</td>
<td>38°C (orally)</td>
<td>1551 (7.8%); 17354 (61.6%); No</td>
<td>-</td>
<td>-</td>
<td>92 (75.3%)</td>
<td>Non-typhoidal Salmonella, S pneumoniae, S enterica</td>
</tr>
<tr>
<td>Siali et al (^a)</td>
<td>Kampala, Uganda; June to April, 1997</td>
<td>Urban public teaching hospital</td>
<td>15-65 years (primarily adults)</td>
<td>Fever</td>
<td>38°C (orally)</td>
<td>56 (16-9%); Yes</td>
<td>202 (53.3%)</td>
<td>54 (16-9%)</td>
<td>Yes</td>
<td>S enterica, M tuberculosis complex, E coli</td>
</tr>
<tr>
<td>Vugia et al (^a)</td>
<td>Abidjan, Côte d'Ivoire; May to June, 1991</td>
<td>Urban referral hospital</td>
<td>Unspecified (primarily adults)</td>
<td>All hospital or ward admissions, irrespective of fever</td>
<td>Inclusion: admitted to hospital ID service</td>
<td>56 (16-9%); Yes</td>
<td>202 (53.3%)</td>
<td>54 (16-9%)</td>
<td>Yes</td>
<td>S enterica, M tuberculosis complex, E coli</td>
</tr>
</tbody>
</table>

*Archibald et al \(^a\) included 30 infants <18 months seropositive for HIV alone; Nathoo et al \(^a\) included 61 infants <18 months seropositive for HIV with clinical immunosuppression; Vugia et al \(^a\) included 139 patients infected with HIV-1, 22 infected with HIV-2, and 45 co-infected HIV-1 and HIV-2. Originally reported as 100%.

Figure 2: Study locations and summary findings including prevalent pathogens

Mycobacterium tuberculosis was excluded because adequate comparison of its relative importance could not be made since several study sites did not use culture media capable of isolating mycobacteria.

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significance of associations made to bloodstream infections or specific causative organisms; values were expressed as odds ratios (ORs) calculated with JMP statistical software version 7.0.

Results
The online database search done on June 11, 2009, yielded 10,412 articles, 7,596 of which were unique articles located in at least one of the three databases (figure 1): 3,366 were unique to PubMed, 1,244 unique to Embase, and 380 unique to African Healthline. Most of these articles were excluded on the basis of a primary topical focus other than assessment of suspected infection in patients admitted to hospital; others were excluded because of the type of study or population. 87 full-text articles (two in French, the rest in English) were obtained for more detailed evaluation; one of these had been identified through a review of references. 65 articles were excluded during detailed screening.

Among the excluded articles 20 were excluded because they described analysis of patient cohorts with specific risks for bloodstream infections, and 11 for subjective, non-replicable, inclusion criteria. 22 selected articles, representing 23 unique patient cohorts, were eligible for systemic review. One article described results from two distinct cohorts, and one article was included after provision of additional data from the authors (Enwere G, WHO, Geneva, Switzerland, personal communication). For other articles excluded because of incomplete data, attempts to contact the authors were unsuccessful or data were not available.

The 22 eligible studies were done in 34 locations between 1984 and 2006 (table 1), and included 6,137 patients from southern (23,893 patients; 39.0%), east (21,317; 34.8%), north (10,230; 16.7%), and west and central (5,887; 9.6%) Africa. 45,899 patients (74.6%) were from exclusively paediatric cohorts. Figure 2 shows the study locations and the three leading causes of non-typhoidal bloodstream infection. Of the

<table>
<thead>
<tr>
<th>Enterobacteriaceae</th>
<th>Number of isolates (proportion of total isolates)</th>
<th>Number of isolates in adults (proportion of isolates in adults)</th>
<th>Number of isolates in children (proportion of isolates in children)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella entoria</td>
<td>1643 (29.1%)</td>
<td>878 (42.3%)</td>
<td>765 (21.4%)</td>
</tr>
<tr>
<td>Non-typhoidal Salmonella</td>
<td>960 (17.0%)</td>
<td>291 (14.0%)</td>
<td>669 (18.7%)</td>
</tr>
<tr>
<td>S. enterica serotype Typhimurium</td>
<td>460 (8.5%)</td>
<td>185 (8.9%)</td>
<td>275 (7.7%)</td>
</tr>
<tr>
<td>S. enterica serotype Enteritidis</td>
<td>234 (4.3%)</td>
<td>77 (3.7%)</td>
<td>157 (4.4%)</td>
</tr>
<tr>
<td>S. enterica serotype Paratyphi A</td>
<td>9 (&lt;1.0%)</td>
<td>5 (&lt;1.0%)</td>
<td>4 (&lt;1.0%)</td>
</tr>
<tr>
<td>S. enterica serotype Choleraesuis</td>
<td>3 (&lt;0.6%)</td>
<td>0 (0.0%)</td>
<td>3 (&lt;1.0%)</td>
</tr>
<tr>
<td>S. enterica serotype Typhi</td>
<td>560 (9.9%)</td>
<td>553 (26.6%)</td>
<td>7 (&lt;1.0%)</td>
</tr>
</tbody>
</table>

(Continues on next page)
61,372 patients, 43,741 (71.3%) were recruited from rural district or missionary hospitals, 7,456 (12.2%) from urban hospitals and referral centres, and 10,130 (16.5%) from a public infectious disease hospital system in rural and urban areas. Basic inclusion criterion was hospital or ward admission for 40,228 patients (65.6%), fever without apparent focus of infection for 11,249 (18.3%), and fever or specific signs of infection for 9,589 (15.6%).

All studies described the microbiological techniques used, although culture media and methods of identification of organisms varied between studies. Minimum acceptable culture volumes ranged from 1 mL to 3 mL in paediatric studies and from 5 mL to 18 mL in adult studies that provided this information.

Only two paediatric studies detailed the actual volume of specimens received from patients. Blood culture results were available for 58,296 (95.1%) of the 61,372 patients across all studies, and 5,578 (9.6%, range 4.2–38.2%) had bacterial or fungal bloodstream infections. Of patients from seven studies that used both aerobic and mycobacterial culture techniques 513 (25.3%) of 2,025 patients had bacterial or fungal bloodstream infections. A total of 5,647 non-malaria pathogenic isolates were recovered across all studies: 3,286 (58.2%) were Gram negative and 1,885 (33.4%) were Gram positive (table 2). S. enterica serotypes were the most commonly isolated pathogens, accounting for 1,643 (29.1%) of isolates recovered overall, and 878 (42.3%) of pathogenic isolates among adults. S. enterica serotypes were the second most prevalent organisms cultured from children, constituting 765 (21.4%) of 3,569 pathogenic isolates in children. Across the whole sample, non-typhoidal serotypes (960 isolates; 17.0%) were more commonly isolated than were

<table>
<thead>
<tr>
<th>Number of Isolates (proportion of total isolates)</th>
<th>Number of Isolates in adults (proportion of isolates in adults)</th>
<th>Number of Isolates in children (proportion of isolates in children)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive organisms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other streptococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A streptococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B streptococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Gram-positives</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Unspecified Gram positives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Yeasts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mycobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium tuberculosis complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other mycobacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium avium complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of undescrbed isolates**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of polymicrobial infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of isolates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Blood culture isolates from 22 studies, Africa, 1984-2006
S. enterica serotype Typhi (569; 9.9%), and 507 (90.5%) of the S. enterica serotype Typhi isolates were from patients in the two north African studies. Among 960 non-typhoidal Salmonella, 706 (73.5%) were serotyped; S. enterica serotype Typhimurium was the most prevalent non-typhoidal Salmonella accounting for 460 (65.2%) of serotyped isolates, then S. enterica serotype Enteritidis with 234 (33.1%). 688 (12.2%) of the isolates were non-Salmonella Enterobacteriaceae, 412 (59.9%) of which were Escherichia coli. Haemophilus influenzae type b, Staphylococcus epidermidis, and 21 Gram-negative rods of the genera Enterobacteriaceae, Aeromonas, Flavimonas, Flavibacterium, and Xanthomonas.

Among prevalent Gram-positive organisms, Streptococcus pneumoniae accounted for 1031 (18.3%) of the isolates, and was the most common isolate in children, constituting 833 (23.3%) of the paediatric pathogens. Staphylococcus aureus constituted 537 (9.5%) of the total isolates across the age spectrum. Fungi accounted for 40 (0.7%) of the isolates. Most fungal isolates were Cryptococcus spp (29 Cryptococcus neoformans and one Cryptococcus laurentiae).

Of all included children, 3527 (8.2%) of 43,330 had bloodstream infections, compared with 2051 (13.5%) of 15,166 adults (OR 0.60; p<0.0001). HIV infection was diagnosed in 499 (18.8%) of 2695 tested patients in paediatric studies compared with 1217 (53.5%) of 2273 tested patients in adult studies (OR 0.20; p<0.0001). Compared with adults, bacteraemia in children was more likely to be caused by Gram-positive organisms (OR 1.6; p<0.0001), including S. pneumoniae (OR 1.5; p<0.0001), S. aureus (OR 1.4; p<0.0001), and group A streptococci (OR 10.8; p<0.0001), or by non-Salmonella Enterobacteriaceae (OR 1.4; p<0.0001). H. influenzae infection was found almost exclusively in children (OR 101.2; p<0.0001). S. enterica (OR 3.4; p<0.0001), mycobacteria (OR 34.6; p<0.0001), and yeasts (OR 111.2; p<0.0001) predominated among adults (table 2).

Among the five adult studies that included mycobacterial blood-culture techniques, M. tuberculosis complex was the most common isolate, accounting for 166 (33.8%) of 491 isolates from 1716 patients. Four of these studies detailed data on polymicrobial bloodstream infections; 13 (8.5%) of 153 patients with M. tuberculosis bacteremia also had bacteraemia with another organism, most commonly non-typhoidal Salmonella (5; 39%) or S. pneumoniae (4; 31%). In the two paediatric studies which assessed for mycobacteraemia, one child had Mycobacterium avium complex bacteremia, but M. tuberculosis was not recovered from any child, despite bloodstream infections with other pathogens being identified in 12 (15.0%) of 80 and 35 (15.3%) of 229 included patients. All of the children in these studies had previously received a BCG vaccination; cultures were obtained by drawing 3 mL of blood into BACTEC MYCO/F LYTIC blood culture bottles (Becton Dickinson Inc).

Organisms thought to be skin-flora contaminants were explicitly reported to have been excluded from the analyses in 17 studies; in 11 studies providing full data, contaminants were isolated from 5448 (13.3%) of 41,443 paediatric blood cultures versus 47 (3.5%) of 1355 adult blood cultures (OR 3.8; p<0.0001). In addition to the organisms reported to have been excluded, 137 (7.3%) of 1885 Gram-positive and 21 (0.6%) of 3286 of Gram-negative contaminants were reported as pathogens justified by clinical scenarios, but could have been classified as contaminants in other studies. These included 27 Staphylococcus epidermidis isolates, 109 Streptococcus viridans or unspecified streptococci, one Bacillus cereus, and 21 Gram-negative rods of the genera Burkholderia, Aeromonas, Flavimonas, Flavibacterium, and Xanthomonas. 132 (83.5%) of 158 possible contaminants were identified in paediatric studies. Additionally, Falade and colleagues"
reported that 18 of 78 isolates of *S. aureus* isolated from their cohort were sent to a reference laboratory for confirmation (the same laboratory used by Enwere and colleagues) in their analysis included in this study), but only one of the 18 was confirmed to be *S. aureus*; the remaining 17 isolates were coagulase negative staphylococci. Confirmation for the remaining 60 isolates was not available.

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Proportion of susceptible isolates (range of susceptibility)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>220</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>195</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>208</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>213</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>23</td>
</tr>
<tr>
<td>MetiCillin</td>
<td>35</td>
</tr>
<tr>
<td><em>Chloramphenicol</em></td>
<td></td>
</tr>
<tr>
<td><em>Ceftriaxone</em></td>
<td></td>
</tr>
<tr>
<td><em>Ciprofloxacin</em></td>
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</tr>
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<td><em>Gentamicin</em></td>
<td></td>
</tr>
<tr>
<td><em>Erythromycin</em></td>
<td></td>
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<tr>
<td><em>MetiCillin</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Proportion of susceptible isolates (range of susceptibility)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
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<tr>
<td>Ampicillin</td>
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</tr>
<tr>
<td>Chloramphenicol</td>
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</tr>
<tr>
<td>Ceftriaxone</td>
<td>71</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>22</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>207</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>45</td>
</tr>
</tbody>
</table>

*Cumulative number of isolates across cited studies with susceptibilities reported; not all studies tested susceptibilities to all listed antibiotics.

<table>
<thead>
<tr>
<th>Table 6: Antimicrobial susceptibilities of common bloodstream isolates in seven reporting studies, Africa, 1993-2006</th>
</tr>
</thead>
</table>

13 studies reported the prevalence of malaria parasitaemia identified by thick or thin smear,\(^a,b,c,e,f,g,h,i,j,k,l,m\) of 21131 patients for whom malaria film results were reported 11 914 (56.4%, range 50.7–61.7%) were parasitaemic; in five adult and two paediatric cohorts, prevalence of bacterial or fungal bloodstream infections was greater than the prevalence of malaria parasitaemia.\(^a,d,e,f,g,h,i,j,k,l,m\) Among two adult and seven paediatric studies, 769 (6-5%) of 11814 patients with parasitaemia also had fungal or bacterial bloodstream infections.\(^a,d,e,f,g,h,i,j,k,l,m\)

Archibald and colleagues\(^h\) in Dar es Salaam, Tanzania, and Bell and colleagues\(^i\) in Lilongwe, Malawi, assessed the prevalence of malaria among febrile study patients compared with asymptomatic outpatients or people admitted to hospital with non-febrile disorders. Among patients from Lilongwe, parasitaemia was significantly more common (relative risk 2.3, \(p=0.0021\)) among study patients (72 [31%] of 231) compared with randomly selected afebrile outpatients (10 [14%] of 73), but there was no difference (\(p=0.575\)) in prevalence of parasitaemia between study patients (49 [9.5%] of 517) and afebrile patients admitted to the orthopaedic ward in Dar es Salaam (12 [8.0%] of 150). In a rural western Kenya cohort described by Dougle,\(^j\) 197 (86.0%) of 229 study patients were smear negative for malaria, yet 137 (59.8%) of all 229 study patients had a primary diagnosis of malaria. Bloodstream infections were identified in 51 (22.3%) of the patients in this study, 35 (69%) of whom were diagnosed with malaria on admission.

In addition to blood culture, Afifi and colleagues\(^i\) tested serum samples for *Brucella* spp by use of slide or tube agglutination with *Brucella abortus* antigens. Agglutination testing had a specificity of 79% and a sensitivity of 89% compared with growth in biphasic blood-culture media (PML Microbiologicals, Wilsonville, OR, USA). A positive serum agglutination test for *Brucella* spp in the absence of isolation in blood culture was identified in one of 336 patients in Petit and
Lethargy or restlessness
- Four paediatric studies \(^{12,15,16,86}\)
  OR 2.4-4.4 \(^{12,15,16}\)
- Oral candidiasis
  Two paediatric studies \(^{12,15}\)
  OR 1.8-7.2 \(^{12,15}\)
  Two adult studies \(^{86}\)
Jaundice
- One paediatric study \(^{12}\)
  OR 2.3-7.8 \(^{12}\)
- Two adult studies \(^{86}\)
Splenomegaly
- One paediatric study \(^{12}\)
  OR 1.7-2.5 \(^{12}\)
- One adult study \(^{86}\)
Hepatomegaly
- Two paediatric studies \(^{12,15}\)
  OR 2.0-5.0 \(^{12,15}\)
Malnutrition or wasting
- Four paediatric studies \(^{12,15,16,86}\)
  OR 1.8-7.3 \(^{12,15,16,86}\)
- Two adult studies \(^{86}\)
Fever >38.5°C
- Two paediatric studies \(^{12,15}\)
  OR 2.3-4.4 \(^{12,15}\)
- Two adult studies \(^{86}\)
Anaemia
- Any BSI: two paediatric studies \(^{12}\)
  Any BSI: OR 2.0 \(^{12}\)
- One adult study \(^{86}\)
  OR 3.7 \(^{86}\)
- Mycobacterial BSI: two adult studies \(^{12}\)
  OR 9.5 \(^{12}\)
Leucopenia
- White blood cells <5000 cells per ml; one adult study \(^{86}\)
  OR 2.1 \(^{86}\)
- White blood cells <1000 cells per ml; one adult study \(^{86}\)
  OR 1.3 \(^{86}\)
Leucocytosis
- >15 000 cells per ml
  Two paediatric studies \(^{12,15}\)
  OR 2.4 \(^{12,15}\)

**Table 5: Clinical and laboratory associations with non-malaria bloodstream infection from 12 studies, Africa, 1991-2006**

**Reported positive association (p<0.05)**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Reported positive association</th>
<th>OR range, where reported*</th>
<th>Reported negative or no association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethargy or restlessness</td>
<td>Four paediatric studies (^{12,15,16})</td>
<td>OR 2.4-4.4 (^{12,15,16})</td>
<td>-</td>
</tr>
<tr>
<td>Oral candidiasis</td>
<td>Two paediatric studies (^{12,15})</td>
<td>OR 1.8-7.2 (^{12,15})</td>
<td>-</td>
</tr>
<tr>
<td>Jaundice</td>
<td>One paediatric study (^{12})</td>
<td>OR 2.3-7.8 (^{12})</td>
<td>-</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>One paediatric study (^{12})</td>
<td>OR 1.7-2.5 (^{12})</td>
<td>-</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>Two paediatric studies (^{12,15})</td>
<td>OR 2.0-5.0 (^{12,15})</td>
<td>-</td>
</tr>
<tr>
<td>Malnutrition or wasting</td>
<td>Four paediatric studies (^{12,15,16,86})</td>
<td>OR 1.8-7.3 (^{12,15,16,86})</td>
<td>-</td>
</tr>
<tr>
<td>Fever &gt;38.5°C</td>
<td>Two paediatric studies (^{12,15})</td>
<td>OR 2.3-4.4 (^{12,15})</td>
<td>-</td>
</tr>
<tr>
<td>Anaemia</td>
<td>Any BSI: two paediatric studies (^{12})</td>
<td>Any BSI: OR 2.0 (^{12})</td>
<td>-</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>White blood cells &lt;5000 cells per ml; one adult study (^{86})</td>
<td>OR 2.1 (^{86})</td>
<td>-</td>
</tr>
<tr>
<td>Leucocytosis</td>
<td>&gt;15 000 cells per ml</td>
<td>Two paediatric studies (^{12,15})</td>
<td>OR 2.4 (^{12,15})</td>
</tr>
</tbody>
</table>

BSI=bloodstream infection. *Comparison of the prevalence of the following findings among patients with BSI versus those without BSI. Data from adult and paediatric studies are both presented in these ranges. Figueira et al\(^{12}\) found anaemia significant in multivariate, but not univariate analysis.

S. enterica serotype Typhi bacteraemia was identified in fewer patients infected with HIV than uninfected patients (OR 0.07, p<0.0001).\(^{12,15,86}\) *C. neoforans* (21 patients) and *Histoplasma capsulatum* (two patients) were found exclusively in patients infected with HIV in studies where pathogen-specific HIV serostatus was provided.\(^{12,15,86}\) Infection with *S. pneumoniae*\(^{12,15,86}\), *S. aureus*\(^{12,15,86}\), and *E. coli*\(^{12,15,86}\) were not associated with HIV infection among the studies in this Review.

Antimicrobial use before admission to hospital was common, ranging from 3% to 56% of patients across eight studies reporting such data.\(^{12,15,86}\) In four studies,\(^{12,15,86}\) receipt of antimicrobial drugs as an outpatient was not associated with blood culture results. One study showed an increased risk of bacteraemia and one study identified fewer bloodstream infections in patients previously treated with antimicrobial drugs than in those who were not.\(^{12,15,86}\)

13 studies, which included 3916 (69-3%) of the total pathogens isolated, reported results of antimicrobial susceptibility testing, all of which used disc diffusion or Etest methods.\(^{12,15,86}\) Five of these studies specified use of interpretive criteria according to National Committee on Clinical Laboratory Standards guidelines (now the Clinical Laboratories Standards Institute)\(^{12,15,86}\). Table 4 presents the major findings for prevalent isolates. Although 90-3% of *S. pneumoniae* and 89-1% of *S. enterica* serotype Typhi were classified as ampicillin susceptible, only 8-9% of *E. coli* isolates were ampicillin susceptible. Susceptibility of *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhimurium were 33-6% and 3-0% for ampicillin, 93-2% and 15-9% for co-trimoxazole, and 38-9% and 100% for chloramphenicol, respectively. More than 95% of Gram-negative organisms were susceptible to third-generation cephalosporins and ciprofloxacin.

Table 5 displays the clinical features associated with non-malaria bloodstream infections in at least two included cohorts. Clinical features significantly associated with bloodstream infections included lethargy, restlessness, oral candidiasis, and jaundice. Hepatomegaly and splenomegaly showed variable association with bloodstream infections across studies. Malnutrition, which was defined differently by different authors, correlated in some studies but not in others; four paediatric studies found an association between malnutrition and bloodstream infection;\(^{12,15,86}\) whereas two other paediatric and one adult study found no association or a negative association with malnutrition.\(^{12,15,86}\)

The proportion of patients with bloodstream infections was greater among cohorts that restricted enrolment to patients with fever or other signs of infection versus all admissions (15-4% vs 7-4%; OR 1·9, p<0.0001) and in cohorts that included fever with or without a focus over those that included patients with non-focal febrile illness alone (16-6% vs 10-5%; OR 1·7, p<0.0001). Fever higher than 38.9°C was associated
with bloodstream infections in four studies but showed no relation in one.

Mycobacteraemia was associated with chronic cough in two studies (OR 3.8-16.4, p<0.01). However, 23 (38%) of 60 patients and 13 (23%) of 57 patients with mycobacteraemia from two studies had no associated respiratory signs or symptoms. Three studies identified anaemia more often in patients with mycobacteraemia compared with all patients (OR 9.5, p<0.001), all patients infected with HIV (p<0.001), or all patients with bloodstream infections (p<0.05).

16 studies reported in-hospital fatality for all enrolled patients with and without bloodstream infections. 2,4,19,23,26,32,43,46,48,67,79,88,92 2,4,19,23,26,32,43,46,48,67,79,88,92 The common practice of diagnosing and treating fever as malaria was described in Kenyan and Malawian cohorts included in this Review, and was also shown by Reburn and colleagues in Tanzania, who found that most (2412; 53.9%) patients treated for malaria in hospital were slide-negative, and that 1571 (66%) of these patients did not receive antibacterial drugs. In the study by Reburn and colleagues, malaria slide-negative patients had a higher mortality than did slide-positive patients (6.9 vs 12.1%, p<0.001), suggesting that empirical treatment of malaria without subsequent treatment for other causes of febrile illness might have played a part in this excess mortality.

Our Review suggests that S enterica is one of the pathogens most commonly isolated throughout various settings in Africa and, like malaria, S enterica bacteraemia can cause undifferentiated fever and splenomegaly. We have also shown that M tuberculosis bacteraemia can present without focal signs, although this has only been proven in adults. Taken together, this information presents a strong case that empirical treatment of malaria alone in regions of both high and low prevalence will result in inadequate management of a substantial portion of patients with non-malaria bloodstream infections.

HIV infection is linked to the distribution, prevalence, and cause of community-acquired bloodstream infections in patients admitted to hospital in Africa. Non-malarial bloodstream infection—particularly with non-typhoidal Salmonella, M tuberculosis, or fungi—was more prevalent in patients infected with HIV than in those without HIV, and might have also influenced the prevalence and distribution of types of bloodstream infections between adults and children, given that HIV infection was present much more commonly among adults. Among the five studies in adults that used mycobacterial blood-culture techniques, M tuberculosis was the most common isolate, comprising just over one third of all bacterial and fungal bloodstream isolates. The vast majority of these patients were infected with HIV, and a substantial proportion of them did not present typical chest signs associated with tuberculosis disease.
chronic fever and wasting were common and many had abnormal chest radiographs even in the absence of chest symptoms. Knowledge of local HIV prevalence and routine HIV counselling and testing of patients admitted to hospital whose HIV status is unknown will assist health-care workers in formulating a differential diagnosis. Evidence suggests that even low levels of immune suppression can be associated with significantly increased risks of invasive bacterial disease. The influence of expanded access to antiretroviral therapy programmes on the epidemiology of bloodstream infections in Africa is yet to be established.

Of note, bloodstream infection with \( M \) tuberculosis was not identified among the Malawian children described by Archibald and colleagues, despite a high prevalence of HIV and other bloodstream pathogens. Blood-culture volume, effects of the BCG vaccine, or unique disease pathogenesis in adults and children might lead to this lack of association; this merits further investigation in other studies. Additionally, the inverse association between \( S \) enterica serotype Typhi bacteremia and HIV is striking and further research to substantiate and explore this might reveal important immunological or epidemiological differences between different serotypes of \( S \) enterica.

With the exception of \( E \) coli and non-typhoidal Salmonella, pathogens included in this Review tended to be susceptible to one or more locally available antimicrobial drugs. However, the studies in which antimicrobial susceptibility was assessed were done between 3 years and 16 years ago and might not reflect current local patterns. Few (<30%) non-typhoidal Salmonella isolated among studies in this Review were susceptible to ampicillin, most \( S \) enterica serotype Enteritidis were resistant to chloramphenicol, and most \( S \) enterica serotype Typhimurium were resistant to cotrimoxazole. Frequent isolation of non-typhoidal Salmonella resistant to commonly used antimicrobial drugs has been described by other investigators. Additionally, meticillin-resistant \( S \) aureus, penicillin-resistant \( S \) pneumoniae, and multidrug-resistant Enterobacteriaceae and \( M \) tuberculosis have been reported in Africa and present a threat to individual and public health. Most invasive bacterial isolates across all studies in this Review were susceptible to third-generation cephalosporins or fluoroquinolones. However, these drugs might be beyond the financial reach of patients and institutions, and their increased use as empirical treatment might promote antimicrobial resistance. Taken together, these data emphasise the complexity and potential pitfalls of attempting to treat common illnesses without improvement in directed diagnostics.

Vaccine-preventable diseases continue to cause a substantial burden of disease in Africa. \( S \) pneumoniae was the most commonly isolated paediatric pathogen among patients in our Review, and invasive \( H \) influenzae (the majority of which was documented to be the vaccine-preventable type B), was found in 286 (8.0%) children with bloodstream infections. Enhanced uptake of available vaccines and increased pathogen surveillance should be prioritised to optimise benefits from vaccines and to inform their further development. Given the prevalence of non-typhoidal Salmonella among patients in this Review, further research into vaccine development is crucial.

There are several important limitations to this study. We examined hospital-based cohorts to define community-acquired bloodstream infections, and thereby might have included patients with more severe illness and included only those who have access to health-care facilities. However, non-malaria bloodstream infection might be as common among certain groups of outpatients as in inpatients in Africa. Some important features related to the risks and clinical importance of bloodstream infections in different groups of patient (eg, infants or older children, patients with underlying clinical conditions other than HIV, patients with HIV across the spectrum of immune suppression) were not explored in this study. Enrolment and inclusion criteria varied by study, and studies spanned a period of more than 20 years; therefore overall prevalence of bloodstream infection and the types of organisms isolated should not be seen as a guide to diagnosis for individual clinical encounters, rather as an indication of the anticipated range of pathogens and to emphasise the need to consider the most locally appropriate treatment for a patient who might have a bloodstream infection. Aggregation of raw data resulted in weighting of large studies; the local importance of some pathogens isolated in smaller studies might have been underrepresented.

Some pathogens were either incompletely identified or incompletely reported (4.7% of all pathogens reported); these might have been bloodstream contaminants and might have resulted in an enhanced estimate of the true prevalence of bloodstream infections. Studies varied in how a blood-culture contaminant was defined, and as was noted by Falade and colleagues, the prevalence of \( S \) aureus as a pathogen and of bloodstream infection as a whole might have been overestimated by some laboratories incorrectly identifying coagulase-negative staphylococci as \( S \) aureus. Underestimation of bloodstream infections could have occurred as well, because the diagnostic sensitivity of the laboratory techniques used in various studies could not be rigorously assessed, and fastidious pathogens that need optimum culture and transport methods might not have been isolated in some laboratories. Commonly isolated organisms, such as \( S \) pneumoniae and \( H \) influenzae, might have been underestimated due to their fastidious nature. Bloodstream infection in children, in particular, might also have been underestimated as a result of
blood-culture volume inadequacy. On analysis of blood volume from 16 570 cultures from children, Berkley and colleagues\footnote{noted increased prevalence of bloodstream infection (7.9%) in 3 ml samples compared with 1 ml samples (5.6%, p=0.006).} Studies that found high prevalences of bloodstream infections might have been preferentially published over other studies. However, the exclusion of studies that enrolled patients who were subjectively defined as “septic” probably reduced the degree of bias presented in this Review. Patterns of vaccine use might have influenced the prevalence and types of pathogens isolated; the cohort presented by Enwere and colleagues\footnote{was part of a study to assess the efficacy of the pneumococcal vaccine.} was a strong case for the importance of considering non-malarial bloodstream infections in all patients admitted to hospital in Africa, particularly those with fever, known HIV infection, signs of impaired immunity, or lethargy. Our findings underscore the importance of continuous re-evaluation of WHO's Integrated Management of Childhood Illness and other empirical treatment algorithms for febrile illness in view of changing patterns of disease. When used, empirical treatment with antimicrobial drugs should include coverage for Gram-negative and Gram-positive organisms, and be effective against S enterica and S pneumoniae. Furthermore, our Review highlights that the prevalence of bacteraemia exceeds the prevalence of malaria parasitaemia among patients admitted to hospital in some regions, and that malaria parasitaemia and bacteraemia might coexist. Therefore, clinicians should be alert to local malaria transmission patterns and maintain a high index of suspicion for bacteraemia when proceeding through a diagnostic and treatment plan for an ill patient with a blood film showing malaria in a region of high prevalence. Public health leaders should prioritise the use of available vaccines, and consider means to alleviate the direct cost of antimicrobials to patients admitted to hospital. Further resources and research to define risk factors and prevention strategies, including vaccines, for non-typhoidal Salmonella in Africa are needed. Expanded and improved clinical microbiology services in Africa might contribute substantially to the improved management of community-acquired bloodstream infections. In the meantime, regular collection and dissemination of microbiological data from sentinel hospital studies, such as those included in the Review, are needed to inform empirical guidelines for management of patients with suspected community-acquired bloodstream infection.

Contributors
JAC conceived the idea for this study. EAR, AVS, and JAC designed the study. AVS and EAR searched published work, reviewed published papers, and made the primary selection of eligible papers. JAC resolved disagreements regarding the eligibility of papers. EAR and AVS compiled and analysed the data. EAR prepared the first draft of the paper. All authors contributed to the writing of the report and have seen and approved the final version.

Conflicts of interest
JAC has received honoraria from Abbott laboratories. EAR and AVS declare that they have no conflicts of interest.

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References
1. Petri PL, van GChecksum

Search strategy and selection criteria
These are detailed in the Methods section.


5.22. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania


CONTRIBUTION

I identified the research question, conceived the study, implemented and managed the research in Tanzania including establishment of a full service clinical microbiology laboratory, and wrote the manuscript. I mentored my junior Tanzanian colleague Dr. Habib Ramadhani. Dr. Ramadhani assisted with daytoday operations of the research until he began his PhD studies. Crump, Morrisey and Morpeth led laboratory aspects of the research. Saganda, Mwako, and Shaw coordinated research activities at the Mawenzi Regional Hospital site, and Njau, Shaw, Shao, and Maro at the Kilimanjaro Christian Medical Centre Site. Yang and Chow led statistical aspects of the research. Reyburn and Bartlett participated in study design. Diefenthal did radiologic assessments. Bartlett sought and obtained funding. Maro ensured that research activities were harmonized with patient care, communicated findings to clinicians, and provided critical feedback on drafts of the manuscript. All authors participated in revision of the manuscript.

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Invasive Bacterial and Fungal Infections Among Hospitalized HIV-Infected and HIV-Uninfected Adults and Adolescents in Northern Tanzania

John A. Crump,1,2,5 Habib O. Ramadhani,4,6 Anne B. Morrissey,1 Wilbrod Saganda,4 Mtumwa S. Mwako,6 Lan-Yan Yang,2,3 Shein-Chung Chow,2 Susan C. Morpeth,1 Hugh Reyburn,2 Boniface N. Njau,6 Andrea V. Shaw,1 Helmut C. Diefenthal,4,5 John F. Shao,4,5 John A. Bartlett,1,2,4,5 and Venance P. Mato4,5

1Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, 2Duke Global Health Institute, and 3Department of Biostatistics and Bioinformatics, Duke University, Durham, North Carolina; 4Kilimanjaro Christian Medical Centre, 5Kilimanjaro Christian Medical College, 6Mawenzi Regional Hospital, Moshi, Tanzania; 7National Cheng Kung University, Tainan, Taiwan, and 8London School of Hygiene and Tropical Medicine, London, United Kingdom

(See the editorial commentary by Levine and Farag, on pages 349–351.)

Background. Few studies describe patterns of human immunodeficiency virus (HIV) co-infections in African hospitals in the antiretroviral therapy (ART) era.

Methods. We enrolled consecutive admitted patients aged ≥13 years with oral temperature of ≥38.0°C during 1 year in Moshi, Tanzania. A standardized clinical history and physical examination was done and hospital outcome recorded. HIV antibody testing, aerobic and mycobacterial blood cultures, and malaria film were performed. HIV-infected patients also received serum cryptococcal antigen testing and CD4+ T lymphocyte count (CD4 cell count).

Results. Of 403 patients enrolled, the median age was 38 years (range, 14–96 years), 217 (53.8%) were female, and 157 (39.0%) were HIV-infected. Of HIV-infected patients, the median CD4 cell count was 98 cells/μL (range, 1–1,105 cells/μL), 20 (12.7%) were receiving ART, and 29 (18.5%) were receiving trimethoprim-sulfamethoxazole prophylaxis. There were 112 (27.7%) patients who had evidence of invasive disease, including 26 (23.2%) with Salmonella serotype Typhi infection, 24 (21.4%) with Streptococcus pneumoniae infection, 17 (15.2%) with Cryptococcus neoformans infection, 12 (10.7%) with Mycobacterium tuberculosis complex infection, 8 (7.1%) with Plasmodium falciparum infection, and 7 (6.3%) with Escherichia coli infection. HIV infection was associated with M. tuberculosis and C. neoformans bloodstream infection but not with E. coli, S. pneumoniae, or P. falciparum infection. HIV infection appeared to be protective against Salmonella. Typhi bloodstream infection (odds ratio,.12; $P = .001$).

Conclusions. While Salmonella Typhi and S. pneumoniae were the most common causes of invasive infection overall, M. tuberculosis and C. neoformans were the leading causes of bloodstream infection among HIV-infected inpatients in Tanzania in the ART era. We demonstrate a protective effect of HIV against Salmonella. Typhi bloodstream infection in this setting. HIV co-infections continue to account for a large proportion of febrile admissions in Tanzania.

Fever is a common symptom among patients presenting for hospitalization in Sub-Saharan Africa [1]. While malaria is often perceived to be a leading cause of fever, the importance of community-acquired bloodstream infections due to bacteria, mycobacteria, and fungi is increasingly appreciated [2]. The epidemiology of febrile illness varies geographically. The pattern of etiologies of fever can also be anticipated to change over time with, for example, efforts to control and effectively treat malaria [3], the emergence of the human immunodeficiency virus (HIV) infection pandemic and the increasing availability

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Correspondence: John A. Crump, MB, ChB, DTM&H, Associate Professor of Medicine, Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Box 100380, Durham, NC 27710 (crump017@mc.duke.edu).

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of free antiretroviral therapy (ART) [4], introduction of vaccines for Haemophilus influenzae type b [5] and Streptococcus pneumoniae [6], and changes in environmental risk factors.

Making an etiologic diagnosis in the febrile patient clinically is difficult, and clinical laboratory capacity is often limited in Sub-Saharan Africa [7, 8]. While the capacity to prepare and examine malaria films is widespread, the quality of such examinations may be poor [9], and facilities for blood culture are often absent. Consequently, sentinel hospital bloodstream infection studies using such methods have provided valuable information to inform empiric treatment guidelines and to help direct disease control efforts [2, 7, 10–15]. In order to understand the role of community-acquired bloodstream infections as causes of febrile illness among adults and adolescents in an area of low malaria transmission intensity during a period of increasing availability of free ART, we studied admissions to 2 hospitals in northern Tanzania.

MATERIALS AND METHODS

Setting

Moshi (population, >144,000) is the administrative center of the Kilimanjaro Region (population, >1.4 million) in northern Tanzania and is situated at an elevation of ~890 m above mean sea level. The climate is characterized by a long rainy period (March–May) and a short rainy period (October–December) [16]. Malaria transmission intensity is low [17]. Kilimanjaro Christian Medical Centre (KCMC) is a consultant referral hospital with 458 inpatient beds serving several regions in northern Tanzania, and Mawenzi Regional Hospital (MRH), with 300 beds, is the regional hospital for Kilimanjaro. Together KCMC and MRH serve as the main providers of hospital care in the Moshi area. In 2008, KCMC admitted 22,099 patients and MRH admitted 21,763 patients.

Participants

Participants were prospectively identified from among adult and adolescent inpatients at the KCMC and MRH in Moshi, Tanzania, from 17 September 2007 through 31 August 2008. All admitted patients aged ≥13 years and with oral temperatures of ≥38.0°C were invited to participate in the study. A standardized clinical history and physical examination were performed on consenting patients by a trained clinical officer who was a member of the study team. Provisional diagnoses by the hospital clinical team were recorded and coded using the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) codes. Following cleansing of the skin with isopropyl alcohol and povidone iodine, blood was drawn for aerobic blood culture (5 mL) and for mycobacterial blood culture (5 mL) as well as for complete blood count, examination for blood parasites, and HIV antibody testing. The case definition for attribution of febrile illness to malaria was a blood film with ≥500 asexual parasites per microliter [18]. Acute serum, plasma, and whole blood were archived on all participants. For patients found to be HIV seropositive, CD4+ T lymphocyte count (CD4 cell count) and serum cryptococcal antigen level were also measured. HIV-seronegative patients were screened for the presence of acute HIV infection by polymerase chain reaction (PCR) for HIV-1 RNA. Urine was collected as soon as possible after admission for detection of urine antimicrobial activity and for antigen detection for Histoplasma capsulatum, S. pneumoniae, and Legionella pneumophila serogroup 1. A chest radiograph was ordered for all patients and was reported using a standardized form by a radiologist (H.C.D.). A discharge form was completed at the time of discharge from the hospital that captured whether the patient died in hospital, the in-hospital management, and the discharge diagnoses coded using ICD-10 codes. The results of all study investigations were provided immediately to the hospital clinical team to inform patient management.

Laboratory Methods

Complete blood count and differential was performed using the CellDyn 3500 automated hematology analyzer (Abbott Laboratories). Thick and thin blood films stained with Giemsa were examined for blood parasites by oil immersion microscopy. Parasite density was determined by standard methods [19].

Blood culture bottles were assessed for volume adequacy by comparing the weight before and after inoculation with blood. Adequate volume was defined as the recommended volume ±20%. BacT/ALERT standard aerobic and mycobacterial bottles were loaded into the BacT/ALERT 3D Microbial Detection system (BioMérieux), where they were incubated for 5 and 42 days respectively. Standard methods were used for identifying bloodstream isolates. S. pneumoniae were serotyped by latex agglutination and the Quellung reaction. Nontypoidal Salmonella were serotyped according to the Kauffmann-White scheme [20]. Antimicrobial susceptibility testing was done according to the methods guidelines of the Clinical Laboratory Standards Institute (Wayne, PA), M100-S18, January 2008 [21].

HIV-1 antibody testing was done on whole blood using both the Capillus HIV-1/HIV-2 (Trinity Biotech) and Determine HIV-1/HIV-2 (Abbott Laboratories) rapid HIV antibody tests. The Capillus test was replaced with the SD Bioline HIV-1/HIV-2 test (version 3.0; Standard Diagnostics) on 4 March 2008 after a change in Tanzania Ministry of Health HIV testing guidelines. If rapid tests were discordant, the sample was tested using enzyme-linked immunosorbent assay (ELISA; Vironostika UniForm II plus O Abs; bioMérieux). If the ELISA was negative, no further testing was done. If the ELISA was positive, a Western blot (Genetic Systems HIV-1 Western blot kit; Bio-Rad) was done to confirm the result [22]. HIV-1 RNA PCR was done using the Abbott m2000 system RealTime HIV-1 assay (Abbott...
Laboratories) [23]. The CD4 cell count was measured using the FACSCalibur system (Becton Dickinson). Cryptococcal antigen level was measured using the Latex Cryptococcal Antigen Detection System assay (Immuno-Mycologics).

Urine was tested for *S. pneumoniae* and *L. pneumophila* serogroup 1 antigen using the Binax NOW *S. pneumoniae* antigen test and the Binax NOW Legionella urinary antigen test (Binax). Urine was tested for *H. capsulatum* antigen using the MView *H. capsulatum* quantitative antigen enzyme immunoassay (Miravista Diagnostics) [24]. Antimicrobial activity in urine was measured using a modification of the method described by Liu and others [25].

During the study, the laboratory participated successfully in relevant external quality assurance programs of the College of American Pathologists, the Viral Quality Assurance program of the AIDS Clinical Trials Group, and the United Kingdom National External Quality Assessment Service.

**Statistical Analysis**

Data were entered using the Cardiff Teleform system (Cardiff) into an Access database (Microsoft). For continuous responses, analysis of variance was used to assess treatment differences between groups. For categorical data and binary responses, the Cochran-Mantel-Haenszel test was performed to compare groups. Descriptive statistics for demographics and baseline patient characteristics were obtained for baseline comparability. A multivariate logistic regression analysis was performed to identify risk factors associated with various invasive infections. Backward stepwise logistic regression analysis was conducted for establishing a predictive model of various invasive infections with the selected relevant predictors. All statistical tests performed were 2-sided at the 5% level of significance. Statistical analyses were performed with SPSS software (version 12.0; SPSS).

**Research Ethics**

This study was approved by the KCMC Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an institutional review board of Duke University Medical Center.

**RESULTS**

Over the study period 6353 patients admitted to the medical services of KCMC and MRH were screened for eligibility. Of these, 666 (10.5%) met eligibility criteria and 403 (60.5%) could be enrolled into the study. The median age of study participants was 38 years (range, 14–96 years), and 217 (53.8%) were female. Of 403 standard aerobic and 326 mycobacterial blood culture bottles, 372 (92.3%) and 224 (68.7%) were classified as adequately filled, respectively. Of enrolled patients, 112 (27.7%) had evidence of an invasive bacterial or fungal infection, 68 (16.6%) on the basis of a clinically important organism isolated from blood culture. Twenty-nine (4.0%) of the blood cultures were contaminated. Eight (2.0%) participants had malaria parasites present on blood film; all were identified as *Plasmodium falciparum* and the median density of asexual forms was 46860 parasites/μL (range, 174–113880 parasites/μL). Of patients with *P. falciparum* infection, 6 (75.0%) had >500 parasites/μL. One hundred fifty-seven (39.0%) of the participants were HIV seropositive, including 62 (39.5%) with no prior history of a positive HIV test, and 1 (0.2%) participant was HIV seronegative but had an HIV-1 RNA load of >100000 copies/mL, which is consistent with acute HIV infection. Of HIV-infected patients, the median CD4 cell count was 98 cells/μL (range, 1–1,105 cells/μL), 96 (61.1%) had CD4 cell counts of <200 cells/μL, 29 (18.5%) were taking trimethoprim-sulfamethoxazole (SXT) prophylaxis, and 20 (12.7%) were receiving ART. Two hundred fifty-four (63.0%) of 403 patients received provisional diagnoses of malaria.

**Relationship Between HIV and Invasive Infections**

The relationship between invasive disease and HIV infection is shown in Table 1, and the leading causes of invasive infection are shown in Table 2. Neither of the HIV-infected patients who had *Salmonella* Typhi bacteremia were receiving SXT prophylaxis and their CD4 cell counts were 182 and 94 cells/μL. Of 7 *S. pneumoniae* bloodstream isolates, 1 (14.3%) belonged to serotype 1, 2 (28.6%) to serotype 13, 2 (28.6%) to serotype 19A, 1 (14.3%) to serotype 19F, and 1 (14.3%) to serotype 46. Of 2 nontyphoidal *Salmonella* bloodstream isolates, both were serotype Typhimurium. Of 7 *S. pneumoniae* isolates, 7 (100%) were susceptible to chloramphenicol, 6 (85.7%) were susceptible to erythromycin, and the remainder were resistant; 5 (71.4%) were susceptible to penicillin and the remainder showed intermediate susceptibility; and 3 (42.9%) were susceptible to SXT and the remainder were resistant. Of 28 *Salmonella enterica* isolates, 3 (10.7%) were susceptible to ampicillin and the remainder were resistant; 28 (100%) were susceptible to ceftriaxone; 20 (71.4%) were susceptible to chloramphenicol and the remainder were resistant; and 28 (100%) were susceptible to ciprofloxacin, and none showed decreased ciprofloxacin susceptibility [26].

**Antimicrobial Use Prior to Admission**

Among those whose urine was tested, 87 (22.3%) demonstrated urine antimicrobial activity. Of 294 blood cultures drawn from patients without demonstrated urine antimicrobial activity, 51 (17.3%) were positive; whereas of 79 blood cultures drawn from patients with urine antimicrobial activity, 19 (24.1%) were positive (odds ratio, 1.51 [95% confidence interval, 0.83–2.74]; *P* = .175).

**Predictors of Invasive Disease**

Risk factors for predicting invasive disease were identified by multivariable logistic regression analysis and are displayed in Table 3.
Table 1. Invasive Infections Among HIV-Infected and HIV-Uninfected Participants, Kilimanjaro Christian Medical Centre and Mawenzi Regional Hospital, 2007–2008

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. (%) of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n=403)</td>
</tr>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7 (1.7)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Salmonella serotype Typhi</td>
<td>26 (6.4)</td>
</tr>
<tr>
<td>Nontyphoidal Salmonella</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td><strong>Other gram-negative organisms</strong></td>
<td></td>
</tr>
<tr>
<td>Legionella pneumophila serogroup</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Neisseria species</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td><strong>Gram-positive organisms</strong></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Streptococcus pneumonia&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24 (5.9)</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td><strong>Yeasts</strong></td>
<td></td>
</tr>
<tr>
<td>Cryptococcus neoformans&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17 (4.2)</td>
</tr>
<tr>
<td>Histoplasma capsulatum&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4 (1.0)</td>
</tr>
<tr>
<td><strong>Mycobacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Mycobacterium simiae</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>12 (3.0)</td>
</tr>
<tr>
<td><strong>Plasmodia</strong></td>
<td></td>
</tr>
<tr>
<td>Plasmodium falciparum</td>
<td>8 (2.0)</td>
</tr>
<tr>
<td>Other Plasmodium species</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total no. of participants with invasive infections</strong>&lt;sup&gt;f&lt;/sup&gt;</td>
<td>112 (27.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers are too small to calculate a test statistic.
<sup>b</sup> By urine antigen testing.
<sup>c</sup> Of invasive S. pneumoniae infection diagnoses, 7 were by blood culture and the remainder were by urine antigen detected with negative blood culture.
<sup>d</sup> Of invasive C. neoformans infection diagnoses, 6 were by blood culture and the remainder were by serum antigen detection with negative blood culture.
<sup>e</sup> All H. capsulatum infection diagnoses were by urine antigen detection.
<sup>f</sup> Three patients had 2 organisms isolated from their blood and 1 patient had 3 organisms isolated.

**DISCUSSION**

We demonstrate that invasive bacterial and fungal disease and HIV infection are common among febrile adult and adolescent inpatients in Moshi, Tanzania, and that the majority of these patients received a provisional diagnosis of malaria. Salmonella Typhi and S. pneumoniae are the leading causes of bloodstream infection in this setting. HIV-associated M. tuberculosis bacteremia and C. neoformans invasive disease are important causes of febrile illness and death, even 3 years after the availability of free ART.

While Salmonella enterica is known to be a leading cause of community-acquired bloodstream infection in Sub-Saharan Africa, the marked increase in C. neoformans invasive disease in this setting may reflect earlier availability of ART and a recent rise in cryptococcal disease.

**In-Hospital Case Fatality**

The hospital outcome was known for 398 (98.8%) participants of whom 41 (10.3%) died. Those who died in hospital included 2 (18.2%) with M. tuberculosis bloodstream infection, 5 (31.3%) with C. neoformans infection, 2 (7.4%) with S. pneumoniae infection, and 12 (10.9%) with any invasive infection. There were no deaths associated with histoplasmosis, typhoid fever, or malaria. Eleven (9.9%) participants with invasive infection died in hospital, whereas 30 (10.3%) without invasive infection died (P = .736). Thirty (19.7%) HIV-infected participants died in hospital, whereas 11 (4.8%) of the HIV-uninfected participants died (P < .001).
Table 2. Leading Organisms Identified by Blood Culture and Antigen Detection Among 112 Participants With Invasive Infections, Kilimanjaro Christian Medical Centre and Mawenzi Regional Hospital, 2007–2008

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. (%) identified by blood culture or antigen detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella serotype Typhi</td>
<td>26 (23.2)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>24 (21.4)</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>17 (15.2)</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>12 (10.7)</td>
</tr>
<tr>
<td>Plasmodium falciparum</td>
<td>8 (7.1)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7 (6.3)</td>
</tr>
<tr>
<td>Others</td>
<td>18 (16.1%)</td>
</tr>
</tbody>
</table>

*a* Of invasive *S. pneumoniae* diagnoses, 7 were by blood culture and the remainder were by urine antigen detected with negative blood culture.

*b* Of invasive *C. neoformans* diagnoses, 6 were by blood culture and the remainder were by serum antigen detection with negative blood culture.

Africa, nontyphoidal *Salmonella* serotypes usually predominate and *Salmonella*. Typhi has been uncommon [2, 27]. Malaria has been associated with risk for invasive nontyphoidal *Salmonella* infection in Africa; it is possible that the low prevalence of malaria in Moshi may be linked to the uncommon occurrence of invasive nontyphoidal *Salmonella* infection [28]. The high prevalence of both typhoid fever and HIV infection in this study allowed us to examine the relationship between HIV infection and typhoid fever. Our study showed an apparent protective effect of HIV against *Salmonella*. Typhi bacteremia—an effect that has also been observed in a meta-analysis of studies of community-acquired bloodstream infection in Sub-Saharan Africa [2]. It is possible that this finding is a result of a patient selection effect resulting from studying patients at the hospital rather than the community level [29]. Alternatively, HIV may modify the risk of the host for *Salmonella*. Typhi infection or disease directly through changes in the gut mucosa or through modification of the host immune response. HIV-infected persons may use SXT prophylaxis which in turn could protect against typhoid fever, although the low prevalence of use of SXT prophylaxis among HIV-infected persons enrolled in this study makes this explanation less likely.

HIV infection was common among study participants, who frequently presented with immunologically advanced HIV disease. HIV-associated *M. tuberculosis* bacteremia and *C. neoformans* invasive disease were common and were associated with high in-hospital case fatality rates. The predominance of *M. tuberculosis* and *C. neoformans* 3 years after free ART became available in the catchment area of the study hospitals is striking and is not dissimilar to the results of a study completed a decade earlier elsewhere in Tanzania, long before the advent of ART programs [12]. These findings underscore the importance of promoting HIV counseling and testing services [30, 31], improving access of persons with HIV to care and treatment [32], and, while such programs are expanded, continuing to provide adequate support to services for the management of opportunistic infections. Acute HIV infection was identified as the likely cause of febrile illness in <1% of participants in our study, which confirms that the diagnosis is rare but warrants consideration in this setting. However, the prevalence of acute HIV infection was much lower than that observed among febrile outpatients in Uganda [33]. The lower prevalence of acute HIV infection in our study could be due to geographic differences in HIV epidemiology or to the fact that patients with acute HIV infection may be more likely to seek outpatient care than to be hospitalized.

As anticipated for an area of low malaria transmission intensity [17], malaria was a relatively uncommon cause of fever in this study. Despite malaria being uncommon, the majority of study participants received a provisional diagnosis of malaria, which is consistent with other studies [34]. This finding illustrates the importance of improving clinician awareness of bacterial and fungal bloodstream infections and broadening empiric treatment strategies to include bacterial pathogens. Antimicrobial susceptibility testing of common bacterial pathogens in this study demonstrates a high prevalence of resistance to ampicillin and SXT and the presence of chloramphenicol resistance. Third-generation cephalosporins provide good coverage for common bacterial pathogens, and fluoroquinolones remain active for *S. enterica*. Pre-hospital use of antibiotic and antimarial drugs, confirmed by detection of urine antimicrobial activity, was common in this study. Exposure to antimicrobial agents prior to hospital admission was not associated with decreased or increased risk for bloodstream infection. However, it is likely that the frequent pre-hospital use of antimicrobials would lead to differences in the spectrum of pathogens observed at the hospital compared with the community level [29].

While distinguishing the various causes of febrile illness clinically is problematic, we did identify a number of clinical or simple laboratory tests that can aid in diagnosis in this setting. HIV antibody testing can be of great value in identifying a group at high risk for *C. neoformans* and *M. tuberculosis* infection. A history of headache and weight loss and a positive Kernig sign are useful for identifying those with cryptococcal disease; chronic cough and lymphadenopathy were associated with disseminated tuberculosi. A history of dyspnea, elevated white cell count, and hypotension were associated with pneumococcal disease, whereas patients with typhoid fever tended to be younger and have a higher magnitude of fever, a history of rigors, and diarrhea.

Among patients with invasive disease, most deaths were recorded in those with *M. tuberculosis*, *C. neoformans*, and *S. pneumoniae* disease. No in-hospital deaths occurred in those with histoplasmosis, typhoid fever, or malaria. While HIV-infected patients were more likely to die during hospitalization...
Table 3. Predictors of Invasive Infections Among All Study Participants, Kilimanjaro Christian Medical Centre and Mawenzi Regional Hospital, 2007–2008

<table>
<thead>
<tr>
<th>Infection</th>
<th>Finding</th>
<th>Odds Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcus neoformans</td>
<td>Kernig sign</td>
<td>6.96</td>
<td>.049</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td>4.81</td>
<td>.039</td>
</tr>
<tr>
<td></td>
<td>Weight loss</td>
<td>3.56</td>
<td>.040</td>
</tr>
<tr>
<td></td>
<td>Past HIV infection diagnosis</td>
<td>3.25</td>
<td>.069</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte count per 1 cell/μL</td>
<td>1.04</td>
<td>.007</td>
</tr>
<tr>
<td></td>
<td>Platelet count per 1 cell/μL</td>
<td>1.01</td>
<td>.004</td>
</tr>
<tr>
<td></td>
<td>Male sex</td>
<td>.21</td>
<td>.013</td>
</tr>
<tr>
<td></td>
<td>Infiltrate on chest radiograph</td>
<td>.18</td>
<td>.029</td>
</tr>
<tr>
<td></td>
<td>Fever per 1°C</td>
<td>.04</td>
<td>.015</td>
</tr>
<tr>
<td>Histoplasma capsulatum</td>
<td>Skin lesions</td>
<td>13.07</td>
<td>.054</td>
</tr>
<tr>
<td></td>
<td>Oxygen saturation per 1%</td>
<td>.81</td>
<td>.002</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Past HIV infection diagnosis</td>
<td>20.64</td>
<td>.008</td>
</tr>
<tr>
<td></td>
<td>Chronic cough</td>
<td>7.54</td>
<td>.013</td>
</tr>
<tr>
<td></td>
<td>Lymphadenopathy</td>
<td>5.88</td>
<td>.048</td>
</tr>
<tr>
<td></td>
<td>Heart rate per 1 beat/min</td>
<td>1.05</td>
<td>.017</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte count per 1 cell/μL</td>
<td>.87</td>
<td>.006</td>
</tr>
<tr>
<td></td>
<td>Hematocrit level per 1%</td>
<td>.49</td>
<td>.004</td>
</tr>
<tr>
<td></td>
<td>Dyspnea</td>
<td>.05</td>
<td>.007</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Dyspnea</td>
<td>4.53</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>White blood cell count per 1 cell/μL</td>
<td>1.16</td>
<td>.006</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte count per 1 cell/μL</td>
<td>1.04</td>
<td>.049</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure per 1 mm Hg</td>
<td>.96</td>
<td>.020</td>
</tr>
<tr>
<td></td>
<td>Chronic fever</td>
<td>.28</td>
<td>.444</td>
</tr>
<tr>
<td>Salmonella serotype Typhi</td>
<td>Rigors</td>
<td>3.66</td>
<td>.084</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>3.03</td>
<td>.026</td>
</tr>
<tr>
<td></td>
<td>Fever per 1°C</td>
<td>1.43</td>
<td>.011</td>
</tr>
<tr>
<td></td>
<td>Age per 1 year</td>
<td>.95</td>
<td>.011</td>
</tr>
<tr>
<td></td>
<td>Crepitations on chest auscultation</td>
<td>.20</td>
<td>.018</td>
</tr>
<tr>
<td>Any invasive infection</td>
<td>Normal breath sounds on chest auscultation</td>
<td>2.12</td>
<td>.004</td>
</tr>
<tr>
<td></td>
<td>Fever per 1°C</td>
<td>1.80</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Weight loss</td>
<td>1.69</td>
<td>.056</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte count per 1 cell/μL</td>
<td>1.22</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Neutrophil count per 1 cell/μL</td>
<td>1.16</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Diastolic blood pressure per 1 mm Hg</td>
<td>.98</td>
<td>.020</td>
</tr>
<tr>
<td></td>
<td>Oxygen saturation per 1%</td>
<td>.93</td>
<td>.007</td>
</tr>
<tr>
<td></td>
<td>Hematocrit level per 1%</td>
<td>.54</td>
<td>.10</td>
</tr>
</tbody>
</table>

than those without HIV, there was no difference in risk for in-hospital death between those with and those without bloodstream infection. It is likely that the diagnostic services provided to clinicians through this study facilitated the targeting of antimicrobial therapy and may have improved patient outcomes. However, it is notable that 10% of febrile patients without a diagnosed invasive infection died in hospital. This finding suggests that further work to explore additional etiologies of fever is warranted.

This study had a number of limitations. Bias may have been introduced due to failure to enroll all eligible patients. Furthermore, the study duration of only 1 year did not allow us to assess changes across longer periods.

In summary, we demonstrate that invasive bacterial and fungal diseases are common causes of febrile illness among hospitalized patients in northern Tanzania. Although free ART had been available in the hospital catchment area for 3 years at the time of the study, many febrile patients had HIV infection with immunologically advanced HIV disease. HIV-associated disseminated tuberculosis and cryptococcal disease were common and associated with high inpatient case fatality rates. The high prevalence of typhoid fever in the study area provided a rare opportunity to study its epidemiologic interaction with HIV. HIV infection appeared to provide a protective effect against Salmonella. Typhi bacteremia—a finding that warrants further study. Many patients received a provisional diagnosis of...
malaria, although malaria was uncommon. Improved clinician awareness of invasive bacterial and fungal disease, strengthened clinical microbiology services, and use of empiric treatment directed at causes of fever other than malaria may improve patient outcomes. Since antimicrobial resistance to ampicillin, SX(T, and, to a lesser extent, chloramphenicol is common, third-generation cephalosporins and fluoroquinolones may be useful agents for empiric management of bacterial sepsis. We identified a number of clinical features that may help to identify patients with any invasive infection and those with specific infections. The fact that 10% of patients with febrile illness and no etiologic diagnosis died in hospital suggests that etiologies of fever other than those examined in this study may contribute to patient outcomes and should be the subject of future research.

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Potential conflicts of interest. J.A.B. is on the speaker’s bureau for the American Society of Tropical Medicine and Hygiene Intensive Review Course and the Infectious Diseases Society of America. He is also on the Data and Safety Monitoring Board service for Harvard School of Public Health and Kendle. All other authors: No conflicts.

References

5.23. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected children and infants in northern Tanzania


CONTRIBUTION

I identified the research question, conceived the study, implemented and managed the research in Tanzania including establishment of a full service clinical microbiology laboratory, and wrote the manuscript. I mentored my junior Tanzanian colleague Dr. Habib Ramadhani. Dr. Ramadhani assisted with day­today operations of the research until he began his PhD studies. Crump, Morrissey and Morpeth led laboratory aspects of the research. Msuya, Reyburn, Njau, Shaw, Shao, Schimana, and Kinabo coordinated activities at the Kilimanjaro Christian Medical Centre Site. Yang and Chow led statistical aspects of the research. Cunningham provided technical input in the discipline of pediatric infectious diseases. Bartlett sought and obtained funding and participated in study design. Diefenthal did radiologic assessments. Kinabo ensured that research activities were harmonized with patient care, communicated findings to clinicians, and provided critical feedback on drafts of the manuscript. All authors participated in revision of the manuscript.

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Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected children and infants in northern Tanzania

John A. Crump1,2,3,4, Habib O. Ramadhani3,4, Anne B. Morrissey1, Levina J. Msuya3,4, Lan-Yan Yang5,6, Shien-Chung Chow6, Susan C. Morpeth1, Hugh Reyburn1, Boniface N. Njau1, Andrea V. Shaw1, Helmut C. Diefenthal3,4, John A. Bartlett1,2,3,4, John F. Shao3,4, Werner Schimana3, Coleen K. Cunningham8 and Grace D. Kinabo3,4

1 Division of Infectious Diseases and International Health, Department of Medicine, and Department of Pathology, Duke University Medical Center, Durham, NC, USA
2 Duke Global Health Institute, Duke University, Durham, NC, USA
3 Kilimanjaro Christian Medical Centre, Moshi, Tanzania
4 Kilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania
5 Department of Biostatistics and Bioinformatics, Duke University School of Medicine, Durham, NC, USA
6 National Cheng Kung University, Tainan, Taiwan
7 London School of Hygiene and Tropical Medicine, UK
8 Division of Pediatric Infections Diseases, Department of Pediatrics, Duke University Medical Center, Durham, NC, USA

Summary

OBJECTIVE To describe the contribution of paediatric HIV and of HIV co-infections to admissions to a hospital in Moshi, Tanzania, using contemporary laboratory methods.

METHODS During 1 year, we enrolled consecutively admitted patients aged ≥2 months and <13 years with current or recent fever. All patients underwent standardized clinical history taking, a physical examination and HIV antibody testing; standard aerobic blood cultures and malaria film were also done, and hospital outcome was recorded. Early infant HIV diagnosis by HIV-1 RNA PCR was performed on those aged <18 months. HIV-infected patients also received serum cryptococcal antigen testing and had their CD4-positive T-lymphocyte count and percent determined.

RESULTS A total of 467 patients were enrolled whose median age was 2 years (range 2 months–13 years); of those patients, 57.2% were female and 12.2% were HIV-infected. Admission clinical diagnosis of HIV disease was made in 10.7% and of malaria in 60.4%. Of blood cultures, 5.8% grew pathogens; of these 25.9% were Salmonella enterica (including 6 Salmonella Typhi) and 22.2% Streptococcus pneumoniae. Plasmodium falciparum was identified on blood film of 1.3%. HIV infection was associated with S. pneumoniae (odds ratio 25.7, 95% CI 2.8, 234.0) bloodstream infection (BSI), but there was no evidence of an association with Escherichia coli or P. falciparum; Salmonella Typhi BSI occurred only among HIV-uninfected participants. The sensitivity and specificity of an admission clinical diagnosis of malaria were 100% and 40.3%; and for an admission diagnosis of bloodstream infection, they were 9.1% and 86.4%, respectively.

CONCLUSION Streptococcus pneumoniae is a leading cause of bloodstream infection among paediatric admissions in Tanzania and is closely associated with HIV infection. Malaria was over-diagnosed clinically, whereas invasive bacterial disease was underestimated. HIV and HIV co-infections contribute to a substantial proportion of paediatric febrile admissions, underscoring the value of routine HIV testing.

keywords Africa, bacteremia, HIV, paediatrics, Salmonella enterica, Streptococcus pneumoniae

Introduction

Fever is a common presenting feature among children and infants admitted to hospital in sub-Saharan Africa (Petit & van Ginneken 1995). Malaria, bacteremia, or other infectious conditions presenting as a febrile illness account for a large proportion of deaths in sub-Saharan Africa (Bryce et al. 2005), yet the laboratory capacity to make aetiological diagnosis is often lacking (Archibald & Reller 2001; Petti et al. 2006). Consequently, clinicians often rely on syndromic approaches to patient management (WHO 2000, 2005) and while application of such
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algorithms improves patient care, a proportion of patients inevitably do not receive specific therapy for potentially life threatening infections (Reyburn et al. 2004). Although malaria is often considered to be the leading life threatening cause of febrile illness in sub-Saharan Africa, malaria transmission intensity varies considerably by location (Hay et al. 2009). Furthermore, recent efforts to expand coverage with insecticide-treated bed nets and switch to more effective malaria treatment regimens have been associated with substantial declines in malaria parasite rates in the community and in the proportion of outpatient visits and hospital admissions because of malaria in a number of countries (WHO 2009; O’Meara et al. 2010).

While routine use of blood cultures is uncommon in sub-Saharan Africa, expanded use of blood culture at a few sentinel sites has highlighted the importance of invasive bacterial disease as a cause of illness and death among children both within and outside the hospital (Campbell et al. 2004; Berkley et al. 2005; Brent et al. 2006; Gordon et al. 2008; Sigauque et al. 2009; Nadim et al. 2010; Reddy et al. 2010). Patients with invasive bacterial disease frequently are treated empirically with antimalarial drugs without antibacterial therapy, resulting in adverse outcomes (Reyburn et al. 2004). To investigate the aetiology, management and outcomes of febrile illness in an area of low malaria transmission intensity (Hay et al. 2009), we studied paediatric inpatients at a consultant referral hospital in northern Tanzania. The results of a similar study among adults and adolescents are described elsewhere (Crump et al. 2011).

**Methods and materials**

**Setting**

Moshi (population >144 000) is the administrative centre of Kilimanjaro Region (population >1.4 million) in northern Tanzania, situated at 890 m above sea level. The climate is characterized by a long rainy period (March–May) and a short rainy period (October–December; National Bureau of Statistics & ORC Macro 2005). Kilimanjaro Christian Medical Centre (KCMC), a consultant referral hospital with 458 inpatient beds serving several regions in northern Tanzania, is an important provider of hospital care to residents of Moshi. In 2008, KCMC admitted 22 099 patients. Neither *Haemophilus influenzae* type B nor pneumococcal conjugate vaccine was available through the Tanzania expanded programme on immunizations during the study period. The HIV seroprevalence in Tanzania from population-based surveys of adults in 2003–2004 was 7.0% (Mishra et al. 2006).

**Participants**

Participants were prospectively identified from among paediatric inpatients at KCMC in Moshi, Tanzania, from 17 September 2007 through 25 August 2008. Admitted patients aged from 22 months to <13 years, with a history of fever in the past 48 h or an axillary temperature ≥37.5 °C or a rectal temperature of ≥38.0 °C, were eligible to participate in the study. Patients admitted with known malignancy, renal failure, hepatic failure, bone marrow aplasia, trauma or surgery were excluded. A standardized clinical history and a physical examination were performed by a trained clinical officer who was a member of the study team. Provisional admission diagnoses of the usual hospital clinical team were recorded and coded using the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) codes. After cleansing of the skin with isopropyl alcohol and povidone iodine, blood was drawn for a single aerobic blood culture (4 ml) as well as for complete blood count, examination for blood parasites and HIV antibody testing. The case definition for attribution of febrile illness to malaria for children aged ≤5 years was ≥1000 asexual parasites/μl and ≥500 parasites/μl for children aged ≥5 years (Chandler et al. 2006). Acute serum, plasma and whole blood were archived on all participants. For patients found to be HIV seropositive, CD4-positive T-lymphocyte count and per cent and serum cryptococcal antigen were also measured. Early infant diagnosis for those aged <18 months was performed by HIV-1 RNA PCR. Urine was collected as soon as possible after admission for detection of urine antimicrobial activity and for antigen detection of *Histoplasma capsulatum* and *Legionella pneumophila* serogroup 1. When requested by the hospital clinical team, a chest radiograph was ordered and reported using a standardized form by a radiologist (HCD). A discharge form was completed at the time of discharge from hospital that captured in-hospital management, whether the patient died in hospital, and the discharge diagnoses coded using ICD-10 codes. The results of all study investigations were provided immediately to the hospital clinical team to inform patient management.

**Laboratory methods**

Complete blood count and differential were performed using the CellDyn 3500 automated haematology analyzer (Abbott Laboratories, Abbott Park, IL, USA) and interpreted using locally established reference ranges (Buchanan et al. 2010). Thick and thin blood films stained with Giemsa were examined for blood parasites by oil immersion microscopy. Parasite density was determined by standard methods (Greenwood & Armstrong 1991).
Blood culture bottles were assessed for volume adequacy by comparing the weight before and after inoculation with blood. Adequate volume was defined as recommended by the College of American Pathologists, the Viral Quality Assurance programme of the AIDS Clinical Trials Group, and the United Kingdom National External Quality Assessment Service.

During the study, the laboratory participated successfully in relevant external quality assurance programmes of the College of American Pathologists, the Viral Quality Assurance programme of the AIDS Clinical Trials Group, and the United Kingdom National External Quality Assessment Service.

Statistical analysis

Data were entered using the Cardiff Teleform system (Cardiff Inc., Vista, CA, USA) into an Access database (Microsoft Corp). For continuous responses, analysis of variance was used to assess treatment difference between groups. For categorical data and binary responses, the Cochran-Mantel-Haenszel test was performed. Descriptive statistics were used for comparisons of baseline demographics and patient characteristics. Weight for age z-scores (WAZ) were calculated using WHO (WHO) AnthroPlus software (WHO, Geneva, Switzerland) and WHO growth standards. The performance of clinical diagnosis at admission, the WHO manual ‘Management of the child with severe infection or severe malnutrition: guidelines for care at first-referral level in developing countries’ (WHO 2000), typhoid fever, and suspicion of HIV (WHO 2000), and WHO Integrated Management of Childhood Illness (IMCI) guidelines for malaria were assessed against laboratory findings. For the septicaemia analysis, the following signs were used: fever with no obvious focus of infection; blood film for malaria is negative; no stiff neck or other specific signs of meningitis, or a lumbar puncture for meningitis is negative; and signs of systemic upset (e.g., inability to drink or breastfeed, convulsions, lethargy or vomiting everything). For the typhoid fever analysis, the following signs were used: fever (particularly ≥7 days), plus any of the following: diarrhoea or constipation, vomiting, abdominal pain, headache or cough; fever with no obvious focus of infection; no stiff neck or other specific signs of meningitis, or a lumbar puncture for meningitis is negative; signs of systemic upset; and blood smear for malaria is negative. All statistical tests were performed at the 5% level of significance (two-sided) with SPSS 12.0 software (SPSS Inc, Chicago, IL, USA).

Research ethics

This study was approved by the KCMC Research Ethics Committee, the Tanzania National Institute for Medical Research National Research Ethics Coordinating Committee and an Institutional Review Board of Duke University Medical Center.
Results
Over the study period, 11,54 patients admitted to the pediatric services of KCMC were screened for eligibility. Of these, 644 (55.8%) met eligibility criteria. Ultimately, 467 (72.5%) were enrolled in the study. The median age (range) of study participants was 2 years (2 months, 13 years) and 267 (57.2%) were female. The median (range) volume of blood inoculated into PF bottles was 2.9 ml (0.15, 9.35 ml), and 139 (29.8%) were classified as adequately fitted. Of enrolled patients, 27 (5.8%) had a clinically important organism isolated from blood culture and 16 (3.4%) had an organism classified as a contaminant isolate. Fifty-seven (12.2%) participants were HIV-infected. Of HIV-infected patients, the median (range) CD4-positive T-lymphocyte value was 11% (6–21%) for those aged <12 months, 16% (3–34%) for those aged ≥12 months to <5 years and 203 cells/mm³ (2–1631 cells/mm³) for those aged ≥5 years of age. Of 425 participants aged ≥10 years, the median (range) WAZ was −0.93 (5.00–6.00); 106 (24.9%) had WAZ <−2 and 49 (11.5%) had WAZ <−3.

Relationship between HIV and invasive infections
The relationship between invasive disease and HIV infection is shown in Table 1. Of 26 invasive infections diagnosed, Plasmodium falciparum, Salmonella Typhi and Streptococcus pneumoniae accounted for 6 (22.2%) each; 2 (7.4%) were because of Cryptococcus neoformans. Of three children aged <5 years with positive malaria films, 2 (66.7%) had ≥1000 asexual parasites/µl. Of three children aged ≥5 years, none had ≥500 parasites/µl. Of six S. pneumoniae bloodstream isolates, one (16.7%) belonged to serotype 1; one (16.7%) to serotype 4; one (16.7%) to serotype 5; one (16.7%) to serotype 6B; and two (33.3%) to serotype 14. The non-typhoidal Salmonella bloodstream isolate was serotype Typhimurium. Of six S. pneumoniae isolates, six (100%) were susceptible to chloramphenicol; six (100%) were susceptible to erythromycin; four (66.7%) were susceptible to penicillin by meningitis breakpoints and two showed intermediate susceptibility; one (16.7%) was susceptible to trimethoprim-sulfamethoxazole (SXT) and the remaining were resistant. Of seven Salmonella enterica, two (28.6%) were susceptible to ampicillin and the remaining were resistant; seven (100%) were susceptible to ceftriaxone and none produced extended-spectrum β-lactamases; three (42.9%) were susceptible to chloramphenicol and the remaining were resistant; two (28.6%) were susceptible to SXT and the remaining were resistant; and seven (100%) were susceptible to ciprofloxacin and none showed decreased ciprofloxacin susceptibility (Crump et al. 2003).

Antimicrobial use prior to admission
A total of 220 (47.1%) patients reported taking antibacterial drugs during their illness prior to admission to hospital. Of these, 47 (21.4%) reported taking SXT, 53 (24.1%) reported taking amoxicillin and 120 (54.5%) reported taking another antibacterial drug. In addition, 143 (30.6%) patients reported taking antimarial drugs during their illness and prior to admission to hospital. However, among those whose urine was tested, 154 (33.0%) demonstrated urine antimicrobial activity. Of 105 blood cultures drawn from patients with urine antimicrobial activity, 4 (3.8%) were positive whereas 12 (5.1%) of 236 blood cultures drawn from patients without urine antimicrobial activity were positive (OR = 0.74; 95% CI, 0.23–2.35; P = 0.610).

In-hospital case fatality
Of 464 (99.4%) patients whose hospital outcome was known, 34 (7.3%) died, 2 (0.4%) with invasive infection and 32 (6.8%) without documented invasive infection (P = 0.840). One (0.2%) child with S. pneumoniae BSI died. However, there were no deaths among those with typhoid fever, cryptococcal disease, or malaria. Of HIV-infected participants, 5 (8.8%) died in hospital, against 18 (5.3%) of HIV-uninfected participants (P = 0.460).

Reliability of clinical diagnoses
The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of an admission clinical diagnosis of malaria for the presence of malaria parasites on blood smear was 100%, 40.3%, 3.9% and 100%. The sensitivity, specificity, PPV and NPV of an admission diagnosis of bloodstream infection were 91.1%, 85.4%, 3.2% and 95.0%, respectively. The sensitivity, specificity, PPV, and NPV of an admission diagnosis of HIV infection was 66.7%, 98.8%, 90.0% and 94.6%, respectively.

Diagnostic performance of first-referral level guideline
The sensitivity, specificity, PPV and NPV of the WHO manual for septicaemia (blood culture positive for bacterial pathogen) were 94.1%, 5.2%, 4.4% and 90.0%, respectively, and were 60%, 44.4%, 13.4% and 1.2% for typhoid fever (blood culture positive for Salmonella Typhi). For suspicion of HIV infection, the sensitivity, specificity, PPV and NPV of the guidelines were 86.4%, 44.1% 20.9% and 95%, respectively. The sensitivity, specificity, PPV and NPV of the IMCI guidelines for the
Table 1 Invasive infections among HIV-infected and HIV-uninfected paediatric participants, KCMC 2007–2008

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Participants n (%)</th>
<th></th>
<th></th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n = 398)†</td>
<td>HIV-infected (n = 57)</td>
<td>HIV-uninfected (n = 341)</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>3 (0.75)</td>
<td>1 (1.75)</td>
<td>2 (0.59)</td>
<td>3.03 (0.27, 33.94)</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em></td>
<td>6 (1.51)</td>
<td>0 (0.00)</td>
<td>6 (1.76)</td>
<td>Undefined</td>
</tr>
<tr>
<td><em>Salmonella non-typhoidal</em></td>
<td>1 (0.25)</td>
<td>1 (1.75)</td>
<td>0 (0.00)</td>
<td></td>
</tr>
<tr>
<td>Other gram-negative organisms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Legionella pneumophila serogroup 1†</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td></td>
</tr>
<tr>
<td>*Haemophilus influenzae type b</td>
<td>1 (0.25)</td>
<td>0 (0.00)</td>
<td>1 (0.29)</td>
<td></td>
</tr>
<tr>
<td>Gram-positive organisms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Streptococcus pneumoniae</td>
<td>5 (1.26)</td>
<td>4 (7.02)</td>
<td>1 (0.29)</td>
<td>25.66 (2.81, 234.00)</td>
</tr>
<tr>
<td>Yeasts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Cryptococcus neoformans§</td>
<td>2 (0.50)</td>
<td>2 (3.51)</td>
<td>0 (0.00)</td>
<td>Undefined</td>
</tr>
<tr>
<td><em>Histoplasma capsulatum</em></td>
<td>2 (0.50)</td>
<td>1 (1.75)</td>
<td>1 (0.29)</td>
<td>6.07 (0.37, 98.48)</td>
</tr>
<tr>
<td>Plasmodia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Plasmodium falciparum</td>
<td>6 (1.51)</td>
<td>0 (0.00)</td>
<td>6 (1.76)</td>
<td>Undefined</td>
</tr>
<tr>
<td>*Plasmodium non-falciparum</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td></td>
</tr>
<tr>
<td>Total number of participants with invasive infections**</td>
<td>26 (6.53)</td>
<td>9 (15.79)</td>
<td>17 (4.99)</td>
<td>3.57 (1.51, 8.47)</td>
</tr>
</tbody>
</table>

*Numbers too small to calculate a test statistic.
†HIV status was unknown for 69 participants.
‡By urine antigen testing.
§Of invasive Cryptococcus neoformans diagnoses, all were by serum antigen detection with negative blood culture.
¶All H. capsulatum diagnoses were by urine antigen detection.
**No patients had more than one invasive infection confirmed by blood culture and antigen detection.

Diagnosis of malaria (blood film positive for Plasmodium spp) were 70%, 16.1%, 1.9% and 95.8%, respectively.

Appropriateness of WHO manual for antimicrobial management of septicaemia

The WHO manual (WHO 2000) for antimicrobial management of septicaemia recommends the combination of benzylpenicillin and chloramphenicol switching to chloramphenicol and ampicillin if there has been a poor response after 48 h. In settings where antimicrobial resistance is common among Gram-negative bacteria, the use of a third generation cephalosporin is suggested to be appropriate (WHO 2000). Among all bacterial pathogens isolated from the bloodstream, 12 (48%) were resistant toampicillin, 5 (20%) were resistant to chloramphenicol and 4 (16%) were resistant to both. No resistance toceftriaxone or ciprofloxacin was identified.

Discussion

We demonstrate that febrile illness is a common presenting feature among paediatric patients admitted to a tertiary hospital in northern Tanzania in an area of low malaria transmission intensity. Evaluation for malaria, HIV, and invasive bacterial and fungal disease confirmed that malaria was uncommon and that a substantial minority of patients were HIV-infected. Pneumococcal disease, often HIV-associated, and Salmonella enterica accounted for a substantial proportion of invasive bacterial disease. The diagnostic tests used in this study failed to identify a laboratory diagnosis for a large majority of patients. Malaria was over-diagnosed and invasive bacterial disease was under-diagnosed clinically. Bacterial pathogens were often resistant to commonly used antimicrobial agents. Despite this, clinical outcomes for those with laboratory-confirmed infections were good. However, a larger proportion of those without a laboratory diagnosis using the tests available in this study died prior to hospital discharge. The high case-fatality rate in the group without a laboratory diagnosis underscores the need for further investigation for both infectious and non-infectious conditions.

As anticipated in a low malaria transmission area, malaria was relatively uncommon among febrile patients enrolled in this study (Hay et al. 2009) and no malaria-associated deaths occurred. Although most participants did not have laboratory evidence of malaria, malaria was a
common clinical diagnosis on admission presumably associated with initial over use of antimalarial medications and under treatment of other conditions. This finding supports the recent WHO recommendation to use malaria diagnostic testing in all cases of suspected malaria before treatment (WHO 2010).

Invasive bacterial disease was predominantly caused by *S. pneumoniae* and *Salmonella enterica*; cryptococcal disease and histoplasmosis were also identified. *Haemophilus influenzae* bloodstream infection was uncommon despite the use of laboratory techniques that would optimize its isolation. Non-malaria bloodstream infection was under-diagnosed clinically, suggesting that antibacterial therapy was under-utilized on admission. While malaria may be excluded using diagnostic tests that are available or could be made widely available in sub-Saharan Africa (WHO 2010), diagnostic tests for invasive bacterial and fungal disease are not widely available at healthcare facilities in sub-Saharan Africa (Archibald & Reller 2001; Petti et al. 2006). In a tertiary hospital setting such as KCMC, treatment of febrile patients without malaria following current guidelines (WHO 2000) would result in substantial over use of antibacterial therapy, illustrating the limitations of empiric treatment strategies. Consistent with many other studies from sub-Saharan Africa, bacterial bloodstream isolates were frequently resistant to the commonly used antimicrobial agents ampicillin, chloramphenicol and SXT (Mandomando et al. 2010; Nadim et al. 2010; Reddy et al. 2010). Taken together, this evidence suggests that consideration should be given to the re-evaluation of recommendations for first-line antimicrobial management of paediatric bacterial sepsis in sub-Saharan Africa (WHO 2000).

Of *Salmonella enterica* bloodstream isolates, serotype Typhi was much more common than non-typhoidal *Salmonella*. This pattern of invasive salmonellosis differs from that reported in most other studies in sub-Saharan Africa including studies performed elsewhere in Tanzania (Nadjm et al. 2010), where non-typhoidal *Salmonella* predominates (Crump et al. 2004; Mweu & English 2008; Morpeth et al. 2009; Reddy et al. 2010). The apparent protective effect of HIV against typhoid fever is consistent with the results of a recent meta-analysis and of a study among adults and adolescents in the same area of Tanzania (Reddy et al. 2010; Crump et al. 2011). This finding raises interesting questions about the epidemiology of invasive salmonellosis in sub-Saharan Africa, including the role of HIV and malaria, that warrant further research (Morpeth et al. 2009; MacKenzie et al. 2010; Levine & Farag 2011).

HIV infection, documented in 12.2% of febrile patients, was often immunologically advanced. HIV was not always identified based on clinical suspicion nor would HIV always have been detected by following clinical guidelines (WHO 2000). Pneumococcal disease and cryptococcal disease were associated with HIV infection, and HIV-infected patients were less likely to be discharged alive from the hospital than those without HIV infection. These findings underscore the benefit of routinely offering HIV testing to hospitalized paediatric patients through provider-initiated testing (WHO/UNAIDS 2007).

More than 90% of enrolled patients had no laboratory diagnosis confirmed by the expanded laboratory evaluations provided by the study. Furthermore, while deaths in hospital were uncommon among those patients with laboratory-confirmed malaria and invasive bacterial and fungal disease, almost 7% of those patients without a laboratory diagnosis died. Although the range and consistent application of laboratory tests performed through this study was greater than that available for routine care, we were not able to make other laboratory evaluations that could have explained some of the deaths. For example, we did not have access to cerebrospinal fluid samples; we did not study respiratory tract samples to support the aetiological diagnosis of pneumonia; nor did we examine stool in patients with diarrhoea. Furthermore, the sensitivity of a single blood culture is <100% and may be further diminished by the prior antimicrobial exposure common among patients at a tertiary hospital. However, it is also possible that infections beyond those identified by examination of such specimens could have played a role. Evaluation of this patient group for other infectious aetiologies is warranted.

This study had a number of limitations. Bias may have been introduced because of failure to enroll all eligible patients. Because the study was designed before the availability of the WHO pocket book of hospital care of children (WHO 2005), the performance of those guidelines could not be assessed. Furthermore, the study duration of only 1 year did not allow us to assess changes across longer time periods.

In summary, the hospital management of febrile illness in sub-Saharan Africa poses many challenges. Recent recommendations to use malaria diagnostic tests to guide the use of antimalarial medications, if adopted, should lead to reductions in the over-diagnosis of malaria particularly in low transmission intensity areas, such as Moshi. Full implementation of routine provider-initiated HIV testing should contribute to ensuring that infants and children with HIV and associated co-infections receive appropriate care. However, targeting antibacterial treatment to those with the highest risk of invasive bacterial disease is challenging. Whereas current practice in many settings underutilizes antibacterial therapy (Reyburn et al. 2004),
following existing guidelines may result in overuse of antibacterial therapy. Expansion of clinical microbiology services and focused research would provide useful evidence to guide policy on targeted and empiric management. Furthermore, a large proportion of paediatric patients without laboratory evidence of malaria or invasive bacterial disease died in hospital. Further research on this group using an expanded range of diagnostic evaluations may contribute to reducing mortality in these children.

Acknowledgements
This paper was presented in part at the 58th American Society of Tropical Medicine and Hygiene annual meeting, Washington, DC, 18–22 November 2009, abstract 472. Its content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, which largely funded the work. The authors thank Ahaz T. Kulanga, MBA, for providing administrative support to this study and Pili M. Chambo, Beata V. Kyara, Beatu A. Massawe, Anna D. Mtei, Godfrey S. Mushii, Lillian E. Ngowi, Flora M. Nyka and Winfrida H. Shirima for reviewing and enrolling study participants. We are grateful to the leadership, clinicians and patients of KCMC for their contributions to this research. We thank Angela Karani, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, for serotyping S. pneumoniae strains, and Gino R. Micalizzi and John R. Bates, Queensland Health Forensic and Scientific Services, Brisbane, Australia, for assistance with serotyping non-typhoidal Salmonella strains. We thank Inverness Medical for providing Binax® NOW Legionella Urinary Antigen Test kits for the study. We thank Miravista Diagnostics, Indianapolis, Indiana, USA, for performing Histoplasma capsulatum Quantitative Antigen EIA on patient samples. We acknowledge the Hubert-Yeargan Center for Global Health at Duke University for critical infrastructure support for the Kilimanjaro Christian Medical Centre-Duke University Collaboration.

References


5.24. Invasive disease caused by nontuberculous mycobacteria in Tanzania


CONTRIBUTION

I identified the research question, conceived the study, implemented and managed the research in Tanzania including establishment of a full service clinical microbiology laboratory, and wrote the manuscript. van Ingen, Boeree, and van Soolingen conducted mycobacterial reference laboratory testing. Morrissey oversaw the laboratory that did primary isolation of mycobacteria. Mavura and Grossman identified and managed one of the patients. Crump, Thielman, Bartlett, and Maro identified and managed all remaining patients. Swai did histopathologic studies and photography. All authors participated in revision of the manuscript.

CITATIONS

Thomson Reuters Web of Knowledge through 1 August 2012

14

JOURNAL IMPACT FACTOR

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6.169
Invasive Disease Caused by Nontuberculous Mycobacteria, Tanzania


Data on nontuberculous mycobacterial (NTM) disease in sub-Saharan Africa are limited. During 2006–2008, we identified 3 HIV-infected patients in northern Tanzania who had invasive NTM; 2 were infected with *Mycobacterium sherrisi* and 1 with *M. avium* complex seqeuvar MAC-D. Invasive NTM disease is present in HIV-infected patients in sub-Saharan Africa.

In sub-Saharan Africa, mycobacterial infections are predominantly caused by *Mycobacterium tuberculosis* (1). In more developed countries, *M. avium* and *M. simiae* are responsible for disseminated disease in HIV-infected persons (2). To better understand invasive nontuberculous mycobacterial (NTM) infections in HIV-infected persons in sub-Saharan Africa, we studied patients at 2 hospitals in northern Tanzania.

The Study

From July 2006 through August 2008, we collected blood from 723 patients ≥13 years of age who had auxiliary temperatures ≥38°C and who had been admitted to Kilimanjaro Christian Medical Centre and Mawenzi Regional Hospital in Moshi, Tanzania. Standardized clinical information was collected from all patients. For mycobacterial culture, 5 ml from each patient was inoculated into a BacT/ALERT MB bottle and monitored in a BacT/ALERT 3D (bioMérieux, Durham, NC, USA) automated liquid culture instrument. Other tissue samples (not blood) were obtained from patients with suspected invasive mycobacterial disease and incubated on Middlebrook 7H10 and Lowenstein-Jensen media at 36°C. We used AccuProbe MTB and MAC kits (GenProbe, San Diego, CA, USA) to identify members of *M. tuberculosis* complex and *M. avium* complex. NTM were further identified by INNO-LiPA Mycobacteria v2 reverse line blot (Innogenetics, Gent, Belgium). All assays were used according to the manufacturer’s instructions. All reverse line blot identifications were confirmed by performing additional sequencing of the complete 16S rDNA gene, the 16S–23S internal transcribed spacer (ITS), and the heat shock protein 65 (hsp65) gene (3,4).

Of the 723 patients, 30 (4.1%) had mycobacterial bloodstream infections, of which 2 (9%) were NTM. In 1 additional patient, NTM was identified in a tissue specimen. We describe the 3 patients with NTM infections.

The first patient was a 49-year-old man with cough and weight loss. His sputum contained acid-fast bacilli, and he simultaneously received a diagnosis of HIV infection with a CD4-positive T-lymphocyte count (CD4 count) of 9 cells/mm³. Tuberculosis therapy was begun and comprised isoniazid, rifampin, pyrazinamide, and ethambutol; he was also started on a fixed-dose combination of zidovudine, lamivudine, and abacavir. DNA was extracted from the initial sputum smear taken at the time of presumptive tuberculosis diagnosis according to previously published methods (5). The GenoType CM/AS reverse line blot assay (Hain Lifesciences, Nehren, Germany) was weakly positive for *M. tuberculosis* complex. The patient’s cough resolved, and he completed a 9-month course of tuberculosis therapy. When fever subsequently developed, he was admitted to the hospital; CD4 count was 13 cells/mm³. Mycobacterial blood culture grew acid-fast bacilli after 12 days of incubation; results of AccuProbe MTB and MAC tests were negative. Heat-killed cells from the positive blood culture were identified as *M. simiae* by the INNO-LiPA reverse-line blot. Sequencing of the full 16S rDNA gene, ITS, and hsp65 gene identified the isolate as “*M. sherrisi*.” The 16S rDNA and hsp65 sequences were identical to the *M. sherrisi* American Type Culture Collection (ATCC; Manassas, VA, USA) BAA-832 strain sequences deposited in the GenBank sequence database under accession nos. AY353699 (16S rDNA) and AY365190 (hsp65). The ITS sequence was identical to that of *M. sherrisi* strain FI-95229 (accession no. DQ185132), isolated from sputum of a patient in Italy (6). The Tanzania patient was treated with azithromycin, 500 mg/day, and ethambutol, 800 mg/day. His fever abated
and he remained well, with 169 CD4 cells/mm$^3$ as of last follow-up in 2008.

The second patient was a 36-year-old HIV-infected man with a 3-month history of fever and weight loss and 31 CD4 cells/mm$^3$. He had been taking fixed-dose combination stavudine, lamivudine, and nevirapine for 5 months, but his adherence to therapy was poor. A mycobacterial blood culture grew acid-fast bacilli after 15 days of incubation; AccuProbe MTB and MAC test results were negative. Heat-killed cells from the positive blood culture were identified as $M. simiae$ by the INNO-LiPA reverse-line blot and again as $M. sherrisii$ by sequencing of the full 16S rDNA gene, ITS, and the hsp65 gene. The 16S rDNA gene had a single base-pair difference when compared with the $M. sherrisii$ ATCC BAA-832 strain sequence in GenBank. We deposited the new 16S rDNA sequence in GenBank under accession no. EU883389. The hsp65 sequence was identical to the $M. sherrisii$ ATCC BAA-832 strain sequence (accession no. AY365190); the ITS sequence was identical to the $M. sherrisii$ strain H-95229 (accession no. DQ185132) sequence (6). The patient was treated with azithromycin, 500 mg/day, and ethambutol, 800 mg/day; fever abated. At follow-up in 2008, the patient was continuing treatment with azithromycin and ethambutol but had abdominal pain and hepatosplenomegaly. Abdominal ultrasonography showed retroperitoneal lymphadenopathy. Follow-up mycobacterial blood cultures have been negative.

The third patient was a 36-year-old HIV-infected woman with a 4-month history of bilateral skin lesions affecting the lower extremities (Figure) and 206 CD4 cells/mm$^3$. HIV infection had been diagnosed 18 months earlier; baseline CD4 count was 6 cells/mm$^3$. She began fixed-dose combination stavudine, lamivudine, and nevirapine soon after her HIV diagnosis. An incisional biopsy from the active margin of a leg lesion showed several foci of dermal necrosis with dense lymphocytic infiltrate and Langhans-type giant cells consistent with granulomatous inflammation of tuberculosis (Figure). Culture of biopsy material was positive for $M. avium$ complex. The isolate reacted only with the $M. avium$-intracellulare-scrofulaceum complex probe of the INNO-LiPA reverse-line blot. The 16S rDNA gene and ITS sequences were identical to the $M. avium$ complex ATCC 35770 (Melnick) strain sequences published by Böddinghaus et al. (7) and available in the Ribosomal Differentiation of Microorganisms database (http://rdna.ridom.de). The ITS sequence was also identical to the MAC ATCC 35770 strain sequence available in GenBank (ITS sequevar MAC-D, accession no. L07851). The hsp65 sequence was identical to the ATCC 35770 sequence (accession no. U85637). Because the full 16S rDNA gene sequence of this strain was not available in GenBank and only a small fragment of hsp65 was available, we deposited our sequences under accession nos. EU815938 (16S rDNA) and EU935586 (hsp65). This patient was treated with azithromycin, 500 mg/day, ethambutol, 800 mg/day, and rifampin, 600 mg/day. Her lesions abated over the subsequent weeks, and she remained well as of follow-up in 2008.

**Conclusions**

Improved laboratory techniques enabled us to demonstrate that invasive NTM infections occur in northern Tanzania and include $M. sherrisii$ and $M. avium$ complex. $M. sherrisii$ still awaits official recognition (8). Of $M. sherrisii$ infections reported to date (6,9–12), most have been in HIV-infected patients from Africa (9–11). Although recommendations for the antimicrobial drug management of these infections have not yet been established, our 2 patients with $M. sherrisii$ disseminated disease responded clinically to the optimization of their antiretrovi-
Invasive Nontuberculous Mycobacteria, Tanzania

Invasive therapy regimen and to the combination of ethambutol and azithromycin.

The *M. avium* complex isolated from our third patient is remarkable for its ITS sequavar type. MAC-D has not previously been associated with invasive disease in HIV-infected patients, in which *M. avium* sequvars, mainly Mav-A and -B, are most common (13). The *M. avium* complex ATCC 35770 reference strain was the first reported strain with a MAC-D ITS. The ATCC 35770 strain, however, was isolated from a sputum sample in a symptomatic patient in the United States (14). The isolate from our third patient and the ATCC 35770 strain are genetically divergent from other *M. avium* complex members and may represent a separate species within the *M. avium* complex.

Invasive NTM disease in HIV-infected populations in sub-Saharan Africa demands more attention in terms of identification of etiologic agents, clinical relevance, and management. Further insights would be gained if current and future studies on tuberculosis in the region included liquid culture and molecular identification to confirm *M. tuberculosis* infection and establish the epidemiology and clinical relevance of NTM.

This research was supported by an International Studies on AIDS Associated Co-infections (ISAAC) award, a United States National Institutes of Health (NIH)-funded program (U01 AI062563). Authors received support from NIH awards ISAAC (J.A.C., A.B.M., N.M.T., J.A.B., V.P.M.), AIDS International Training and Research Program D43 PA-03-018 (J.A.C., B.S., N.M.T., J.A.B., V.P.M.), and the Duke Clinical Trials Unit and Clinical Research Sites U01 AI069484-01 (J.A.C., N.M.T., J.A.B., V.P.M.).

Dr Crump is an associate professor of medicine with the Division of Infectious Diseases and International Health at Duke University Medical Center. He lives and works in Moshi, Tanzania, where he serves as director of Duke Tanzania Operations for the Duke Global Health Institute and director of the Kilimanjaro Christian Medical Centre Clinical Research Site. His work focuses on HIV prevention, treatment, and care and on infectious diseases in Tanzania.

References


5.25. Controlled comparison of BacT/ALERT MB system, manual MYCO/F LYTIC procedure, and ISOLATOR 10 system for detection of *Mycobacterium tuberculosis* bacteremia


**CONTRIBUTION**

I conceived the research idea, designed the study, wrote the protocol, implemented the research, participated in laboratory evaluations, entered and analyzed the data, and wrote the manuscript. Morrissey supervised the laboratory that I directed and oversaw day-to-day operations on the bench. Ramadhani and Njau managed participant enrollment on the hospital wards. Maro coordinated interactions between the study team and the Department of Medicine and was responsible for acting on laboratory results. Reller provided mentorship for study design, analysis, and interpretation. All authors contributed to revisions of the manuscript.

**CITATIONS**

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4.153
Controlled Comparison of BacT/Alert MB System, Manual Myco/F Lytic Procedure, and Isolator 10 System for Diagnosis of Mycobacterium tuberculosis Bacteremia

John A. Crump,1,2,3,4,5,6 Anne B. Morrissey,1 Habib O. Ramadhani,4,5 Boniface N. Njau,4,5 Venance P. Maro,1,4,5 and L. Barth Reller1,2

Division of Infectious Diseases and International Health, Department of Medicine,1 and Department of Pathology,2 Duke University School of Medicine, and Duke Global Health Institute,3 Duke University, Durham, North Carolina, and Kilimanjaro Christian Medical Centre4 and Kilimanjaro Christian Medical College,5 Tumaini University,6 Moshi, Tanzania

Received 20 May 2011/Accepted 27 May 2011

We compared the performance of the BacT/Alert MB system, that of the manual Bactec Myco/F Lytic procedure, and that of the Isolator 10 lysis-centrifugation system in the detection of Mycobacterium tuberculosis bacteremia. Mean times to detection were 16.4 days for BacT/Alert MB versus 20.0 days for Myco/F Lytic, 16.5 days for BacT/Alert MB versus 23.8 days for Isolator 10, and 21.1 days for Bactec Myco/F Lytic versus 22.7 days for Isolator 10. There were no significant differences in yields. The mean (range) magnitude of mycobacteremia was 30.0 (0.4, 90.0) CFU/ml and was correlated with the time to positivity in the BacT/Alert MB system (r = −0.4920). M. tuberculosis bacteremia was detected more rapidly in a continuously monitored liquid blood culture system, but the mean time to positivity exceeded 3 weeks.

Mycobacterium tuberculosis bloodstream infection was described within a few decades of the discovery of the tubercle bacillus (7, 14). Disseminated tuberculosis remains a major health problem in countries where generalized HIV epidemics coincide with high tuberculosis incidence rates, often causing fatal illness in patients with immunologically advanced HIV disease (1, 4-6, 15, 17, 21). In-hospital case fatality rates for bacercemic disseminated tuberculosis in the era before the widespread availability of antiretroviral therapy approached 50% (1). Early recognition and treatment are likely to be important to avoid death (13).

Although the incremental value of mycobacterial blood culture for the diagnosis of disseminated tuberculosis has been both recognized and debated (10, 16, 19), there have been few evaluations of blood culture systems for the detection of M. tuberculosis (2, 3). Mycobacterial blood culture methods in common use include visual inspection of processed blood inoculated on a solid medium (e.g., the Isolator 10 system) and continuous detection in liquid medium inoculated with blood (e.g., the BacT/Alert MB system or the Bactec Myco/F Lytic system). In a mycobacterial blood culture study in the United States, we noted that bottles with M. tuberculosis were positive after a mean of 22.8 to 28.0 days of incubation, compared with 9.9 to 20.4 days for bottles with Mycobacterium avium complex (MAC) (11). In order to investigate the performance of mycobacterial blood culture methods for detecting M. tuberculosis bacteremia, we conducted a controlled study to compare the performances of the BacT/Alert MB system, the manual Bactec Myco/F Lytic procedure, and the Isolator 10 system for the detection of mycobacteremia among febrile patients admitted to two hospitals in northern Tanzania, a country experiencing a generalized HIV epidemic and a high incidence of tuberculosis.

Samples for blood cultures were collected from patients aged ≥13 years hospitalized at the Kilimanjaro Christian Medical Centre (KCMC) and Mawenzi Regional Hospital (MRH) in Moshi, Tanzania, from July 2006 through October 2009 (9). Patients with oral temperatures of ≥38.0°C were invited to participate in the study. Blood from all qualifying study participants was inoculated into a bioMérieux BacT/Alert mycobacterial (MB) bottle. In addition, blood from study participants with oral temperatures of ≥38.0°C, HIV infection, a fever duration of >1 month, and a presumed or measured weight loss of >10% was also inoculated into a Bactec Myco/F-Lytic bottle and a Wampole Isolator 10 lysis-centrifugation tube. Blood cultures were processed at KCMC.

Blood culture bottles were assessed for volume adequacy by comparing the weights before and after inoculation with blood. A bottle was included in the primary data analysis only if it contained 4 to 6 ml of blood. A secondary analysis was performed without reference to volume adequacy.

After assessment of the adequacy of the blood volume, MB bottles were loaded into the bioMérieux BacT/Alert 3D automated microbial detection system (bioMérieux Inc., Durham, NC), where they were incubated for 42 days. Bactec Myco/F-Lytic bottles (Becton Dickinson, Franklin Lakes, NJ) were incubated at 35°C for 42 days; bottle bottoms were examined for fluorescence daily using Wood's lamp. Isolator 10 lysis-centrifugation tubes were centrifuged and processed using the Wampole Isostat/Isolator Microbial System (Inverness Medical, Princeton, NJ), plated to Middlebrook 7H10 agar, and incubated at 35°C in 5% CO2 for 42 days. Bottles and plates with growth were processed according to standard techniques (20). AccuProbe Culture Identification
TABLE 1. Clinically important organisms recovered from BacT/Alert MB bottles

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of sets in which organism was detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis complex</td>
<td>26</td>
</tr>
<tr>
<td>M. sherrisi</td>
<td>4</td>
</tr>
<tr>
<td>Salmonella enterica serovar Typhi</td>
<td>31</td>
</tr>
<tr>
<td>Esherichia coli</td>
<td>14</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>12</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>7</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>4</td>
</tr>
<tr>
<td>Kibebia sp.</td>
<td>2</td>
</tr>
<tr>
<td>Brucella sp.</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
</tr>
</tbody>
</table>

Test MTB and MAC kits (Gen-Probe Inc., San Diego, CA) were used to identify M. tuberculosis complex and MAC members. Mycobacteria other than M. tuberculosis and MAC were identified at the National Institute for Public Health and the Environment, Bilthoven, the Netherlands (12, 23). Colonies growing on Middlebrook 7H10 plates from lysis-centrifugation specimens were counted, and numbers of CFU/ml of blood were calculated. Negative companion bottles from positive sets were subcultured at the end of the 42-day protocol.

Comparison of recovery rates was analyzed by the McNemar chi-square test with Yates’ correction for small numbers when necessary (18). Times to detection were compared by the paired two-sample t test for means after log transformation was performed to correct for the observed positively skewed (non-parametric) distributions. All analyses were done with the SAS system for Windows (release 9.1; SAS Institute, Cary, NC).

This study was approved by the KCME Research Ethics Committee, the Tanzania National Institutes for Medical Research, the National Research Ethics Coordinating Committee, and the Institutional Review Board of the Duke University Medical Center.

A total of 759 BacT/Alert MB blood cultures were processed; without restriction for volume adequacy, 26 (3.4%) yielded M. tuberculosis, 3 (0.4%) yielded M. sherrisi, and 77 (10.1%) yielded other pathogens, as shown in Table 1. Sixteen (2.1%) yielded organisms classified as contaminants. Of 20 complete (two bottles and one tube), adequately filled (5 ± 1 ml) sets from which M. tuberculosis was recovered from at least one bottle, the BacT/Alert MB system was positive for 12 (60.0%), the Bactec Myco/F Lytic procedure was positive for 20 (100.0%), and the Isolator 10 system was positive for 9 (45.0%).

Table 2 shows the yields of M. tuberculosis for all three two-way comparisons of adequately filled pairs of bottles and for all bottles. For adequately filled pairs, although there were trends toward the manually read Bactec Myco/F Lytic bottle detecting M. tuberculosis more frequently than the BacT/Alert MB system and the Isolator 10 system, there were no significant differences between any two bottles compared. For all bottle pairs, there was also a trend toward the manually read Bactec Myco/F Lytic bottles detecting M. tuberculosis more frequently than the BacT/Alert MB system and the yield was significantly greater in the manually read Bactec Myco/F Lytic bottles than in the Isolator 10 system. Of 17 adequately filled MB bottles yielding M. tuberculosis, the mean (range) time to positivity was 22.6 (9.4 to 37.5) days. Of the three possible two-way comparisons of times to detection for adequate pairs of positive blood cultures, the mean time to detection was the shortest for the continuously monitored BacT/Alert MB system, followed by the manually read Bactec Myco/F Lytic bottle and the Isolator 10 system (Table 3). When all of the bottles were considered, the time to detection was significantly shorter for the BacT/Alert MB system than for both the manually read Bactec Myco/F Lytic bottle and the Isolator 10 system (Table 3).

We have demonstrated that M. tuberculosis is a leading cause of bloodstream infection among febrile inpatients in northern Tanzania (9). However, even with the continuously monitored BacT/Alert MB system, the mean (range) time to positivity exceeded 3 weeks. The mean time to positivity for the continuously monitored BacT/Alert MB system was significantly shorter than for the Isolator 10 plated to Middlebrook 7H10 solid medium considering either adequately filled bottles or all bottles. There was no nonsignificant trend toward the continuously monitored BacT/Alert MB system having shorter times to detection than the manually read Bactec Myco/F Lytic bottle for adequately filled bottles; this difference was significant when all of the bottles were considered. While there were no statistically significant differences in sensitivity between the systems when adequately filled bottles were considered, M. tuberculosis was detected by only one member of a bottle pair on 42% of the occasions. Furthermore, consistent with other studies (3), a comparison without respect to volume adequacy showed that the Bactec Myco/F Lytic bottle was more sensitive than the Isolator 10 plated to Middlebrook 7H10 solid medium.

<table>
<thead>
<tr>
<th>Bottle pair (bottle 1, bottle 2)</th>
<th>No. of bottles in which MTB was detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Both bottles</td>
</tr>
<tr>
<td>Adequately filled bottles</td>
<td></td>
</tr>
<tr>
<td>BacT/Alert MB, Myco/F Lytic</td>
<td>12</td>
</tr>
<tr>
<td>BacT/Alert MB, Isolator 10</td>
<td>8</td>
</tr>
<tr>
<td>Myco/F Lytic, Isolator 10</td>
<td>8</td>
</tr>
<tr>
<td>Adequately filled bottles</td>
<td></td>
</tr>
<tr>
<td>BacT/Alert MB, Myco/F Lytic</td>
<td>7</td>
</tr>
<tr>
<td>BacT/Alert MB, Isolator 10</td>
<td>5</td>
</tr>
<tr>
<td>Myco/F Lytic, Isolator 10</td>
<td>2</td>
</tr>
</tbody>
</table>

(Continued)
Whereas *M. tuberculosis* was detected after a mean of 22.6 days by the continuously monitored BacT/Alert MB system, *M. sherrisi*, the second most frequently isolated *Mycobacterium* species in this study (12, 23), was detected after a mean of 15.2 days by the same system. The observed differences in the time to detection between *M. tuberculosis* and nontuberculous mycobacteria in liquid systems may be due to constitutional differences in the rate of replication by species, differences in the optimization of the medium for the growth of different mycobacterial species, exposure to drugs with antimycobacterial activity at the time of sample collection, and the sizes of the initial inoculums of mycobacteria placed into the blood culture bottle.

The quantitative study demonstrated a wide variation in the magnitude of *M. tuberculosis* bacteremia in this predominantly HIV-infected population (9) of <1 CFU/ml to 90 CFU/ml. We also demonstrated a trend toward longer times to detection in the continuously monitored BacT/Alert MB system among patients with a lower magnitude of mycobacteremia. The occurrence of *M. tuberculosis* bacteremia at magnitudes of <1 CFU/ml may go some way toward identifying why *M. tuberculosis* was detected in only one member of a large proportion of many bottle pairs.

Our study had a number of limitations. In order to target the use of diagnostics to a population with higher pretest probability for disseminated tuberculosis, blood was collected for mycobacterial culture in all three blood culture systems only from patients meeting the more rigorous inclusion criteria of an oral temperature of ≥38.0°C, the presence of HIV infection, a fever duration of >1 month, and a presumed or measured weight loss of >10%. However, 10 (38.5%) of 26 participants with *M. tuberculosis* bloodstream infection did not meet these criteria, which resulted in some participants with *M. tuberculosis* bacteremia having blood inoculated into a BacT/Alert MB bottle only, thus limiting our power to compare the systems. We recommend that future studies use broader inclusion criteria, for example, focused on the presence of fever and HIV infection. The lack of availability of a Bactec continuously monitored blood culture instrument at the study site meant that Bactec Myco/F Lytic bottles had to be read manually on a daily basis, placing the Bactec Myco/F Lytic procedure at a disadvantage for comparisons of times to detection relative to the BacT/Alert MB system. To standardize volumes, we inoculated 5 ml of blood into each bottle or tube, including the Isolator 10 system, which is designed for 10 ml of blood. The lower ratio of blood to lytic agent and anticoagulant in the Isolator 10 system may have inhibited mycobacterial growth (25). However, Isolator 10 tubes were processed within 8 h of collection and the sediment was plated to solid medium rather than into broth (24).

In summary, *M. tuberculosis* is a common cause of bloodstream infection in northern Tanzania. Although it is detected more rapidly in a continuously monitored liquid blood culture system than by lysis-centrifugation with plating to solid medium, a mean time to positivity exceeding 3 weeks may be too long to lead to potentially lifesaving early initiation of tuberculosis treatment. The occurrence of *M. tuberculosis* bacteremia at magnitudes of <1 CFU/ml may contribute to long times to detection in some cases and could explain, in part, the large proportion of blood culture pairs where *M. tuberculosis* was isolated from only one bottle. Future work on the detection of *M. tuberculosis* bloodstream infection should focus on optimizing both sensitivity and time to detection. Possibly strategies could include further development of *M. tuberculosis* nucleic amplification techniques on large-volume peripheral blood specimens (8, 22).

This research was supported by an International Studies of AIDS-Associated Co-Infections (ISAAAC) award, a United States National Institutes of Health (NIH)-funded program (U01 AI062563). We received support from NIH ISAAC awards (J.A.C., A.B.M., H.O.R., B.N.N., V.P.M.), the AIDS International Training and Research Program (D43 PA-03-018) (J.A.C., H.O.R., B.N.N., V.P.M.), the Duke Clinical Trials Unit and Clinical Research Sites (U01 AI069484).
(J.A.C., V.P.M.), and the Center for HIV/AIDS Vaccine Immunology (U01 AI067854) (J.A.C.).

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REFERENCES


5.26. Performance of nucleic acid amplification following extraction of 5mL of whole blood for the diagnosis of *Mycobacterium tuberculosis* bacteremia


CONTRIBUTION

I conceived the research idea, designed the study, wrote the protocol, implemented the research, participated in laboratory evaluations, entered and analyzed the data, and wrote the manuscript. Morrissey supervised the Tanzania laboratory that I directed and oversaw day-to-day operations on the bench. Tuohy and Procop oversaw nucleic acid amplification testing in Cleveland. Ramadhani and Njau managed participant enrollment on the hospital wards. Maro coordinated interactions between the study team and the Department of Medicine. Reller and Procop provided mentorship for study design, analysis, and interpretation. All authors contributed to revisions of the manuscript.

CITATIONS

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JOURNAL IMPACT FACTOR

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4.153
Performance of Nucleic Acid Amplification following Extraction of 5 Milliliters of Whole Blood for Diagnosis of Mycobacterium tuberculosis Bacteremia

John A. Crump,a,b,c,d,e Marion J. Tuohy,† Anne B. Morrissey,§ Habib O. Ranadhani,d,e Boniface N. Njau,d,e Venance P. Maro,d,e L. Barth Reller,a,b and Gary W. Procop†

Division of Infectious Diseases and International Health, Department of Medicine,a,b and Department of Pathology,c Duke University School of Medicine, and Duke Global Health Institute,d Duke University, Durham, North Carolina, USA; Kilimanjaro Christian Medical Centrea,d and Kilimanjaro Christian Medical College,e Tumaini University,c Moshi, Tanzania; and Department of Pathology, Cleveland Clinic, Cleveland, Ohio, USA

To investigate the performance of a nucleic acid amplification test (NAAT) for the diagnosis of Mycobacterium tuberculosis bacteremia, 5-ml aliquots of blood were inoculated into bioMérieux mycobacterial (MB) bottles and incubated, and 5-ml aliquots of blood were extracted and tested by real-time PCR. Of 25 samples from patients with M. tuberculosis bacteremia, 9 (36.0%) were positive and 1 (1.5%) of 66 control samples was positive by NAAT. The NAAT shows promise, but modifications should focus on improving sensitivity.

Disseminated tuberculosis is a major health problem in countries where generalized HIV epidemics coincide with high tuberculosis incidence rates, often causing fatal illness in patients with immunologically advanced HIV disease (14). Long recognized (5, 13), Mycobacterium tuberculosis bacteremia is common in sub-Saharan Africa (1, 2, 4, 16, 20) and Asia (3, 17). In-hospital case fatality rates are high (1), and median survival is short (1, 17, 19). Early recognition and treatment are likely to be important for averting mortality (12). Even when using mycobacterial blood culture systems with continuous detection, the time to positive may be too long to influence clinical management (7, 9). Nucleic acid amplification tests (NAAT) on whole-blood specimens have shown promise for the diagnosis of pulmonary tuberculosis (6, 21). We hypothesized that NAAT on whole blood may be useful for the early diagnosis of the disseminated form of tuberculosis.

Samples for blood cultures, NAAT, and other diagnostic tests were collected from patients aged ≥13 years hospitalized at the Kilimanjaro Christian Medical Centre (KCMC) and Mawenzi Regional Hospital (MRH) in Moshi, Tanzania, from July 2006 through October 2009 (8). Patients with oral temperatures of ≥38.0°C were invited to participate; 5 ml of blood was inoculated into a bioMérieux BacT/Alert MB bottle and 5 ml was inoculated into an EDTA tube for subsequent NAAT. Other study procedures are described elsewhere (7, 8, 10, 15, 23). Only the results of the MB bottle were considered in the classification of cases; a patient with a companion bottle positive for M. tuberculosis was not included in the control group even if the MB bottle was negative.

Blood culture bottles and tubes were assessed for volume adequacy by comparing the weights before and after inoculation. A bottle or tube was considered adequately filled if it contained 4 to 6 ml of blood. Only samples from patients with adequately filled bottles and tubes were included in the study. BacT/Alert MB bottle were loaded into the BacT/Alert 3D automated microbial detection system (bioMérieux Inc., Durham, NC) where they were incubated for up to 42 days.

Specimens were classified as being from a case patient with M. tuberculosis bacteremia if the MB blood culture bottle was positive for M. tuberculosis. Those with mycobacterial blood cultures negative for M. tuberculosis were classified as controls. The results of clinical evaluations and examination of nonblood specimens for mycobacteria were available for evaluation following completion of nucleic acid amplification testing (8).

EDTA-blood was transferred to cryovials and stored at −80°C for ≥2 to 5 years. Cryovials were shipped on dry ice to the Cleveland Clinic for nucleic acid amplification testing. Each 5-ml sample was thawed, mixed thoroughly, and transferred into an adult Warpole isolator tube (Inverness Medical Innovations, Inc., Princeton, NJ). Each isolator tube was gently vortexed for 3 to 10 s and held at room temperature for at least 1 h to inactivate HIV, if present (11). Following centrifugation at 3,000 × g for 30 min, a pellet was obtained using the manufacturer’s instructions. The 1.5-ml pellet was transferred into a 2-ml Sarstedt microcentrifuge tube and centrifuged at 10,000 × g for 10 min. Approximately 1.2 ml of supernatant was removed, and 500 µl of phosphate-buffered saline (PBS) was added. The suspension was vortexed and centrifuged at 10,000 × g for 10 min, and most of the supernatant was removed. A 180-µl volume of MagNA Pure bacteria lysis buffer (Roche, Indianapolis, IN) and 20 µl of protease K (Roche) were added to each pellet, and the mixture was incubated at 65°C for at least 2 h to overnight. The suspension was heated at 100°C for 10 min. Processing of the pellet was performed using a class II biosafety cabinet and a microcentrifuge with a removable rotor. The entire sample was added to 2 ml of NucliSENS EasyMag lysis buffer (bioMérieux, Durham, NC) and extracted on the EasyMag instrument. A final extraction volume of 50 µl was obtained.

PCR was performed using the LightCycler system (Roche) based on a previously described assay (22), with the following modifications. Asymmetric PCR was used by increasing the re-
TABLE 1 Patient samples with and without M. tuberculosis bacteremia selected for evaluation of the nucleic acid amplification test

<table>
<thead>
<tr>
<th>Designation</th>
<th>HIV serostatus</th>
<th>Invasive infection category</th>
<th>Bloodstream isolate</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>Infected</td>
<td>Met study eligibility, M. tuberculosis bloodstream infection</td>
<td>M. tuberculosis</td>
<td>25 (27.5)</td>
</tr>
<tr>
<td>Control</td>
<td>Infected</td>
<td>Non tuberculous mycobacterial bloodstream infection</td>
<td>Mycobacterium sherrisii (1), Mycobacterium simiae (1)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td>Met study eligibility, blood culture negative</td>
<td>Negative</td>
<td>13 (14.3)</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td>Nonmycobacterial bloodstream infection</td>
<td>Cryptococcus neoformans (3), Escherichia coli (3), Streptococcus pneumoniae (1)</td>
<td>9 (9.9)</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td>Nonmycobacterial bloodstream infection</td>
<td>E. coli (4), S. pneumoniae (2), Salmonella enterica serovar Typhi (16)</td>
<td>22 (24.2)</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td>Blood culture negative</td>
<td>Negative</td>
<td>20 (22.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>91 (100)</td>
</tr>
</tbody>
</table>

verse primer concentration from 0.25 μM to 0.5 μM. Additionally, PCR cycles were increased from 45 to 55, and step mode was selected for melting curve analysis. Positive and negative controls consisted of M. tuberculosis ATCC 27294 and PCR-grade water, respectively. If amplification occurred, then the identity of the Mycobacterium species as M. tuberculosis was confirmed using postamplification melt curve analysis by comparison to the positive control (≥2°C). Five 1-ml replicates of each sample were tested. All PCR-negative samples were further tested using the LightCycler control kit DNA (Roche), which is a PCR assay for a 110-bp fragment of the human β-globin gene.

Means and ranges were calculated for continuous data and compared by the paired two-sample t test for means after log transformation was performed to correct for the observed positively skewed (nonparametric) distributions. Proportions were compared using the chi-square test with Yates’ correction for small numbers when necessary. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for the NAAT compared with blood culture. All analyses were done with the SAS system for Windows (release 9.1; SAS Institute, Cary, NC). This study was approved by the KCMC Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an Institutional Review Board of Duke University Medical Center.

Of 91 participants included in the study, 25 (27.5%) had M. tuberculosis bacteremia and were classified as cases. All were HIV infected. The remaining 66 (72.5%) had mycobacterial blood cultures negative for M. tuberculosis and were classified as controls. Characteristics of control participants and samples are summarized in Table 1.

Of 25 samples with M. tuberculosis bacteremia, 9 (36.0%) were positive by NAAT. Of those positive by NAAT, the mean number of replicates that were positive was 3 (range, 1 to 5). For those with results available, the mean magnitude of mycobacteria was 58.1 CFU/ml (range, 17.0 to 90.0) among NAAT-positive samples, compared with 0.5 CFU/ml (mean and range) for NAAT-negative samples (P = 0.157). The mean time to positive in the continuously monitored BacT/Alert MB system was 16.8 days (range, 9.4 to 27.5) for NAAT-positive samples and 22.0 days (range, 11.3 to 30.9) for NAAT-negative samples (P = 0.062) (Table 2).

Of 66 control samples, 1 (1.5%) was positive for M. tuberculosis by NAAT. The sample was positive in 1 of 5 replicates. Evaluation of case report forms showed that this HIV-uninfected participant had clinical features consistent with pulmonary tuberculosis. All 81 PCR-negative samples were β-globin PCR positive, confirming successful specimen DNA extraction and absence of PCR inhibitors.

The sensitivity (95% confidence interval [CI]) of the NAAT for the diagnosis of M. tuberculosis bacteremia was 0.360 (CI, 0.187 to 0.573), and the specificity was 0.985 (CI, 0.907 to 0.999). The positive predictive value (95% CI) of the NAAT for the diagnosis of M. tuberculosis bacteremia was 0.900 (CI, 0.541 to 0.995), and the negative predictive value was 0.802 (CI, 0.696 to 0.879).

We found that extraction of 5 ml of whole blood followed by real-time PCR targeting of the mycobacterial 16S rRNA gene (22) detected approximately one-third of patients with M. tuberculosis bacteremia diagnosed by culture of an equivalent volume of blood. Specificity exceeded 98% in a control population that included HIV-infected persons enrolled in a country with a high incidence of tuberculosis. There was a trend toward patients with a higher magnitude of mycobacteremia being more likely to have a positive M. tuberculosis NAAT result.

Our NAAT was less sensitive in patients with confirmed M. tuberculosis bacteremia than an IS6110-based assay with patients with suspected pulmonary tuberculosis (6, 21). Possible explanations include that extracting whole blood rather than buffy coat may have increased the effect of blood-associated PCR inhibitors; this may have been compounded by the use of frozen rather than fresh whole blood. Differences in sensitivity may also relate to the nested design of the IS6110-based assay (6, 21) combined with a higher copy number of the IS6110 target compared with the mycobacterial 16S rRNA gene (22). Our assay may perform better in patients with higher magnitudes of mycobacteremia (7).

Despite studying a population with a high seroprevalence of HIV (8) and risk for pulmonary tuberculosis (18), the specificity of our assay was relatively high (6, 21). Efforts to increase the sensitivity of our NAAT may result in the detection of more patients with pulmonary tuberculosis and low-magnitude M. tuberculosis bacteremia not detected by blood culture. Specimens from control patients with a range of bacterial and fungal bloodstream infections were negative in the M. tuberculosis NAAT, confirming the specificity of the assay in the presence of a range of epidemiologically important conditions (20, 22).

In conclusion, we have demonstrated that a NAAT approach could provide a solution to the rapid diagnosis of bacteremic disseminated tuberculosis. Although our assay lacked sensitivity, the potential to detect more than a third of patients with M. tuberculosis bacteremia may be important step forward in laboratory diagnosis of a condition that is rapidly fatal in a large propor-
tion of patients. We recommend that future work be focused on improving the lower limit of detection of the assay.

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5.27. Bacteremic disseminated tuberculosis in sub-Saharan Africa: a prospective cohort study


CONTRIBUTION

I conceived the research idea, developed the protocol, collected, entered, and analyzed the data, and wrote the manuscript. Ramadhani, Njau, and Mushi oversaw day-to-day operations of participant enrollment. Morrissey supervised the laboratory that I directed in which diagnostic testing was done. Saganda and Mwako ensured that research activities were coordinated with patient care at the Mawenzi Regional Hospital site and were responsible for patient management there. Maro ensured that research activities were coordinated with patient care at the Kilimanjaro Christian Medical Centre site and was responsible for patient management there. Yang and Chow led statistical analyses. Reller provided technical input and mentorship on study design and laboratory testing. Bartlett sought and obtained funding and managed the parent grant. All authors contributed to revisions of the manuscript.

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Bacteremic Disseminated Tuberculosis in Sub-Saharan Africa: A Prospective Cohort Study

John A. Crump,1,2,3,5 Habib O. Ramadhani,1,6 Anne B. Morrissey,1 Wilbrod Saganda,6 Mumwwa S. Mwako,6 Lan-Yan Yang,7,8 Shein-Chung Chow,7 Boniface N. Njau,4 Godfrey S. Mushi,4 Venance P. Maro,4,5 Lisa A. Bartlett,1,3,4 and John A. Bartlett1,3,4

1Division of Infectious Diseases and International Health, Department of Medicine, and 2Department of Pathology, Duke University Medical Center, and 3Duke Global Health Institute, Duke University, Durham, North Carolina; 4Kilimanjaro Christian Medical Centre, 5Kilimanjaro Christian Medical College, Tumaini University, and 6Mawenzi Regional Hospital, Moshi, Tanzania; 7Department of Biostatistics and Bioinformatics, Duke University School of Medicine, Durham, North Carolina; and 8National Cheng Kung University, Tainan, Taiwan

Background. Disseminated tuberculosis is a major health problem in countries where generalized human immunodeficiency virus (HIV) infection epidemics coincide with high tuberculosis incidence rates; data are limited on patient outcomes beyond the inpatient period.

Methods. We enrolled consecutive eligible febrile inpatients in Moshi, Tanzania, from 10 March 2006 through 28 August 2010; those with Mycobacterium tuberculosis bacteremia were followed up monthly for 12 months. Survivors, predictors of bacteremic disseminated tuberculosis, and predictors of death were assessed. Anti-retroviral therapy (ART) treatment and bacteremia treatment were provided.

Results. A total of 508 participants were enrolled; 29 (5.7%) had M. tuberculosis isolated by blood culture. The median age of all study participants was 37.4 years (range, 13.6-104.8 years). Cough lasting >1 month (odds ratio [OR], 13.5; P < .001), fever lasting >1 month (OR, 7.8; P = .001), weight loss of >10% (OR, 10.0; P = .001), lymphadenopathy (OR 6.8; P = .002), HIV infection (OR, undefined; P < .001), and lower CD4 cell count and total lymphocyte count were associated with bacteremic disseminated tuberculosis. Fifty percent of participants with M. tuberculosis bacteremia died within 36 days of enrollment. Lower CD4 cell count (OR, 0.88; P = .049) and lower total lymphocyte count (OR, 0.76; P = .050) were associated with death. Magnitude of mycobacteremia tended to be higher among those with lower CD4 cell counts, but did not predict death.

Conclusions. In the era of free ART and access to tuberculosis treatment, almost one half of patients with M. tuberculosis bacteremia may die within a month of hospitalization. Simple clinical assessments can help to identify those with the condition. Advanced immunosuppression predicts death. Efforts should focus on early diagnosis and treatment of HIV infection, tuberculosis, and disseminated disease.

Disseminated tuberculosis is a major health problem in countries where generalized human immunodeficiency virus (HIV) infection epidemics coincide with high tuberculosis incidence rates [1]. Disseminated tuberculosis often causes rapidly fatal illness in patients with immunologically advanced HIV disease and has persisted as a public health problem even since the expansion of HIV care and treatment programs [2, 3]. Because the median survival of patients with bacteremic disseminated tuberculosis following admission to hospital may be very short [3-5], early recognition and treatment are likely to be important to avert mortality [6]. However, the clinical diagnosis of disseminated tuberculosis may be challenging in areas with limited laboratory services [7, 8], as patients may present with nonspecific symptoms and signs, and classic radiographic features of pulmonary or miliary tuberculosis may be absent [4, 5, 9]. Consequently, the diagnosis is often overlooked and is a frequent post-mortem finding among HIV-infected patients in areas

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Correspondence: John A. Crump, MB, ChB, DTM&H, Adjunct Professor of Medicine, Pathology, and Global Health, Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Box 102359, Durham, NC 27710 (john.crump@duke.edu).

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with high rates of tuberculosis [1]. Where laboratory services are available, disseminated tuberculosis may be strictly defined as isolation of Mycobacterium tuberculosis from blood or bone marrow, from a liver biopsy specimen, or from specimens from ≥2 noncontiguous organs in a single patient [10, 11].

*M. tuberculosis* was first recognized as a cause of bloodstream infection almost a century ago [12, 13]. Today, when sought using mycobacterial blood culture techniques, *M. tuberculosis* is a leading cause of community-acquired bloodstream infection among febrile hospitalized patients in sub-Saharan Africa [4, 14-18] and Asia [5, 9]. In-hospital case fatality rates for bacteremic disseminated tuberculosis in the era before the widespread availability of antiretroviral therapy (ART) approached 50% [4]. Unfortunately, the median time to positivity for *M. tuberculosis*, even in continuously monitored blood culture systems, exceeds 3 weeks, limiting its value for clinical management [19, 20]. *M. tuberculosis* nucleic acid amplification tests (NAATs) following extraction of large volumes of whole blood show promise for more rapid diagnosis, but they need refinement to improve sensitivity and adaptation for low-resource settings [21]. Although patient survival after *Mycobacterium avium* complex bacteremia was established for HIV-infected persons during the 1990s [22], little is understood about the natural history of *M. tuberculosis* bacteremia beyond the inpatient period or during the era of widespread availability of ART in endemic countries.

In order to improve approaches to the clinical recognition of disseminated tuberculosis, to enhance our understanding of the natural history of the disease in the context of timely use of tuberculosis treatment and ART, and to improve identification of patients at greatest risk for death, we enrolled and followed up consecutive patients with *M. tuberculosis* bacteremia identified at 2 hospitals in Tanzania, a country experiencing a generalized HIV epidemic and high incidence of tuberculosis.

**METHODS AND MATERIALS**

**Setting**

Moshi (population, >144 000) is the administrative center of the Kilimanjaro Region (population, >1.4 million) in northern Tanzania and is situated at an elevation of approximately 890 meters above mean sea level. Malaria transmission intensity is low [23]. National adult HIV seroprevalence was estimated at 7.0% in 2003-2004 [24] and has since declined; national tuberculosis incidence was estimated at 297 cases per 100 000 persons in 2007 [25]. Kilimanjaro Christian Medical Centre (KCMC) is a consultant referral hospital with 458 inpatient beds serving several regions in northern Tanzania, and Mawenzi Regional Hospital (MRH), with 300 beds, is the regional hospital for Kilimanjaro. Together KCMC and MRH serve as the main providers of hospital care in the Moshi area. In 2008, KCMC admitted 22 099 patients and MRH admitted 21 763 patients.

**Participants**

Participants were prospectively identified from among adult and adolescent inpatients at KCMC and MRH from 10 March 2006 through 28 August 2010. As described elsewhere, all admitted patients aged ≥13 years and with oral temperatures of ≥38°C were invited to participate in the study [2]. From 31 August 2008, enrollment was restricted to those who also had HIV infection, subjective fever for >1 month, and weight loss of >10%. A standardized clinical history was taken and physical examination was performed on consenting patients by a trained clinical officer who was a member of the study team. Following cleansing of the skin with povidone iodine and isopropanol alcohol, blood was drawn for aerobic blood culture (10 mL) and for up to 3 simultaneous mycobacterial blood cultures (5 mL each) as well as for complete blood count, examination for blood parasites, and HIV antibody testing. For patients found to be HIV seropositive, CD4-positive T-lymphocyte count (CD4 cell count) was also measured. A chest radiograph was ordered for all patients and was reported using a standardized form by a radiologist. The results of all study investigations were provided immediately to the hospital clinical team to inform patient management. A discharge form was completed at the time of discharge from hospital that captured whether the patient died in hospital. Participants with *M. tuberculosis* bloodstream infection were referred for immediate initiation to tuberculosis chemotherapy and asked to return for follow-up by the study team monthly for 12 months. Those who did not return for follow-up visits were contacted by telephone and, if necessary, sought at home by a field worker. Deaths were recorded and standardized verbal autopsies were performed for outpatient deaths. Those with HIV infection were referred to an HIV care and treatment center. The timing of initiation of ART was at the discretion of the clinical team, although national guidelines recommended that patients with immunologically advanced HIV disease and tuberculosis start ART with stavudine, lamivudine, and efavirenz as early as 2 weeks after the initiation of the intensive phase of tuberculosis treatment [26].

**Laboratory Methods**

Complete blood count and differential were performed using the CellDyn 3500 automated hematology analyzer (Abbott Laboratories, Abbott Park, Illinois). Manual differentials were performed when necessary on blood films stained with Giemsa.

Blood culture bottles were assessed for volume adequacy by comparing the weight before and after inoculation with blood. BacT/ALERT standard aerobic (SA) and mycobacterial (MB)
bottles were loaded into the BacT/ALERT 3D automated microbiological detection system (bioMérieux, Durham, North Carolina) where they were incubated for 5 and 42 days, respectively. BACTEC Myco/F Lytic bottles (Becton Dickinson, Franklin Lakes, New Jersey) were incubated at 35°C for 42 days; bottle bottoms were examined for fluorescence daily using a Wood lamp. ISOLATOR10 lysis-centrifugation tubes were centrifuged and processed using the Wampole ISOSTAT/ISOLATOR Microbial System (Inverness Medical, Princeton, New Jersey), plated to Middlebrook 7H10 agar, and incubated at 35°C in 5% carbon dioxide for 42 days. An aliquot of the blood-broth mixture was removed from bottles flagged positive by the instrument or by inspection of the bottom, using a 1-mL tuberculin syringe for SA and 5-mL syringe with 19-gauge needle for MB and Myco/F Lytic. A portion was examined by Gram stain, Kinyoun stain (MB and Myco/F Lytic), and India ink stain when yeast-like morphology was observed. Aliquots were plated to solid medium according to stain results. Nonmycobacterial plates were examined daily for growth and subsequent isolation and identification according to standard techniques. Mycobacterial plates were examined weekly for growth. AccuProbe Culture Identification Test MTB and MAC kits (Gen-Probe, San Diego, California) were used to identify members of M. tuberculosis complex and M. avium complex; other Mycobacterium species were identified by a reference laboratory [27, 28]. Colonies growing on lysis-centrifugation plates were counted and colony-forming units per milliliter of blood were calculated.

HIV type 1 (HIV-1) antibody testing was performed on whole blood using both the Capillus HIV-1/HIV-2 (Trinity Biotech, Bray, Ireland) and Determine HIV-1/2 (Abbott Laboratories) rapid HIV antibody tests. The Capillus test was replaced with the SD Bioline HIV-1/2 3.0 (Standard Diagnostics, Kyonggi-do, Korea) on 4 March 2008 after a change in Tanzania Ministry of Health HIV testing guidelines. If rapid tests were discordant, the sample was tested with an enzyme-linked immunosorbent assay (ELISA; Vironostika Uni-Form II plus O Ab, bioMérieux). If the ELISA was negative, no further testing was done. If the ELISA was positive, a Western blot (Genetic Systems HIV-1 Western Blot kit, BioRad, Hercules, California) was done to confirm [29]. The CD4 cell count was measured using the FACScalibur system (Becton Dickinson).

During the study, the laboratory participated successfully in external quality assurance programs of the College of American Pathologists for serology, bacteriology, mycology, mycobacteriology, blood parasites, rapid HIV, and India ink; the Viral Quality Assurance program of the AIDS Clinical Trials Group for HIV-1 RNA polymerase chain reaction; and the United Kingdom National External Quality Assessment Service for flow cytometry.

Statistics

Data were entered using the Cardiff Teleform system (Cardiff, Vista, California) into an Access database (Microsoft Corp, Redmond, Washington). For continuous responses, analysis of variance was used to assess treatment difference between groups. For categorical data and binary responses, Cochran-Mantel-Haenszel test was performed to compare groups. Descriptive statistics for demographics and patient characteristic baseline were obtained for baseline comparability. A logistic regression analysis was performed to identify risk factors associated with death with bacteremic disseminated tuberculosis. An estimate of survival curve using the Kaplan-Meier method was computed. The log-rank test was performed to compare difference between 2 survival distributions. The Kendall τ correlation was used to measure the association between 2 groups. All statistical tests performed were 2-sided at the 5% level of significance. Statistical analyses were performed with SPSS software, version 12.0 (IBM SPSS, Chicago, Illinois).

Research Ethics

This study was approved by the KCMC Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an institutional review board of Duke University Medical Center.

RESULTS

Of 508 participants enrolled during the total study period, 29 (5.7%) had M. tuberculosis bacteremia and 25 (4.9%) had both M. tuberculosis bacteremia and complete clinical data available. Of the 29 with M. tuberculosis bacteremia, 9 (31.0%) had both a positive lysis-centrifugation blood culture and CD4 cell count available. Of the 25 with complete clinical data, 12 (48.0%) were identified during the period of unrestricted enrollment. The median age of all study participants was 37.4 years (range, 13.6–104.3 years) and 281 (56.2%) were female; 236 (46.5%) of the participants were HIV seropositive. Of HIV-infected patients, the median CD4 cell count was 111 cells/μL (range, 1–1105 cells/μL), 69 (29.2%) were taking trimethoprim-sulfamethoxazole prophylaxis, and 84 (35.6%) were receiving ART. Of all participants, 403 (79.3%) were enrolled during the period of unrestricted enrollment and 12 (2.9%) of these had M. tuberculosis bloodstream infection. The characteristics of these patients have been described elsewhere [2]. An additional 105 participants (20.7%) were enrolled during the period of restricted enrollment after 31 August 2008.

Characteristics of Participants With and Without Bacteremic Disseminated Tuberculosis

The characteristics of participants enrolled from 17 September 2007 through 31 August 2008 during the period of unrestricted
Table 1. Characteristics of Participants With and Without Bacteremic Disseminated Tuberculosis, Kiliimanjaro Christian Medical Centre and Mawenzi Regional Hospital, 2007–2008

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n = 403)</th>
<th>Disseminated Tuberculosis (n = 12)</th>
<th>No Disseminated Tuberculosis (n = 391)</th>
<th>OR (95% CI)</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, median (range)</td>
<td>37.0 (13.6–95.5)</td>
<td>39.0 (21.2–55.7)</td>
<td>36.1 (13.6–95.5)</td>
<td>...</td>
<td>.935</td>
</tr>
<tr>
<td>Female sex</td>
<td>217 (53.8)</td>
<td>9 (75.0)</td>
<td>208 (53.2)</td>
<td>2.6</td>
<td>(.70–9.9)</td>
</tr>
<tr>
<td>Ever treated for tuberculosis</td>
<td>39 (9.7)</td>
<td>2 (16.7)</td>
<td>37 (9.5)</td>
<td>1.9</td>
<td>(.40–9.1)</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>261 (64.8)</td>
<td>10 (83.3)</td>
<td>251 (64.2)</td>
<td>2.8</td>
<td>(.60–12.9)</td>
</tr>
<tr>
<td>Cough &gt;1 month</td>
<td>80 (19.9)</td>
<td>9 (75.0)</td>
<td>71 (18.2)</td>
<td>13.5</td>
<td>(3.6–51.2)</td>
</tr>
<tr>
<td>Fever &gt;1 month</td>
<td>88 (21.8)</td>
<td>8 (66.7)</td>
<td>80 (20.5)</td>
<td>7.8</td>
<td>(2.3–26.5)</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>22 (5.5)</td>
<td>2 (16.7)</td>
<td>20 (5.1)</td>
<td>3.7</td>
<td>(1.7–18.1)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>45 (11.2)</td>
<td>3 (25.0)</td>
<td>42 (10.7)</td>
<td>2.8</td>
<td>(1.2–10.6)</td>
</tr>
<tr>
<td>Weight loss &gt;10%</td>
<td>99 (24.6)</td>
<td>9 (75.0)</td>
<td>90 (23.0)</td>
<td>10.0</td>
<td>(2.7–37.9)</td>
</tr>
<tr>
<td>Signs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, median (range)</td>
<td>21.0 (13.5–41.8)</td>
<td>19.1 (15.5–33.3)</td>
<td>21.1 (13.5–41.8)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>42 (10.4)</td>
<td>5 (41.7)</td>
<td>37 (9.5)</td>
<td>6.8</td>
<td>(2.1–22.6)</td>
</tr>
<tr>
<td>Systolic blood pressure, median (range), mm Hg</td>
<td>112 (60–200)</td>
<td>110 (84–146)</td>
<td>112 (60–200)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Diastolic blood pressure, median (range), mm Hg</td>
<td>70 (20–128)</td>
<td>70.0 (51–103)</td>
<td>70.0 (20–128)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Respiratory rate, breaths/minute, median (range)</td>
<td>25 (14–137)</td>
<td>28 (18–40)</td>
<td>25 (14–137)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Oxygen saturation, %, median (range)</td>
<td>96 (71–100)</td>
<td>95 (88–99)</td>
<td>96 (71.100)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Abnormal breath sounds</td>
<td>166 (40.5)</td>
<td>7 (58.3)</td>
<td>159 (40.4)</td>
<td>2.1</td>
<td>(.64–6.6)</td>
</tr>
<tr>
<td>Crepitations</td>
<td>178 (44.2)</td>
<td>8 (66.7)</td>
<td>170 (43.5)</td>
<td>2.6</td>
<td>(1.7–8.8)</td>
</tr>
<tr>
<td>Absent breath sounds</td>
<td>6 (1.5)</td>
<td>0 (0.0)</td>
<td>6 (1.5)</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>Bronchial breathing</td>
<td>57 (14.1)</td>
<td>4 (33.3)</td>
<td>53 (13.6)</td>
<td>3.2</td>
<td>(.93–11.0)</td>
</tr>
<tr>
<td>Pleural rub</td>
<td>15 (3.7)</td>
<td>1 (8.3)</td>
<td>14 (3.6)</td>
<td>2.5</td>
<td>(.30–20.3)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>15 (3.7)</td>
<td>0 (0.0)</td>
<td>15 (3.8)</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>32 (7.9)</td>
<td>1 (6.3)</td>
<td>31 (7.9)</td>
<td>1.1</td>
<td>(.13–8.5)</td>
</tr>
<tr>
<td>Abdominal mass</td>
<td>9 (2.2)</td>
<td>0 (0.0)</td>
<td>9 (2.3)</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>Glasgow coma score, median (range)</td>
<td>15.0 (3.0–15.0)</td>
<td>15.0 (14.0–15.0)</td>
<td>15.0 (3.0–15.0)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Neck stiffness</td>
<td>20 (5.0)</td>
<td>1 (6.3)</td>
<td>19 (4.9)</td>
<td>1.8</td>
<td>(22–44.5)</td>
</tr>
<tr>
<td>Kernig sign positive</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>Skin lesion</td>
<td>17 (4.2)</td>
<td>0 (0.0)</td>
<td>17 (4.3)</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>BCG scar</td>
<td>368 (91.3)</td>
<td>12 (100.0)</td>
<td>356 (91.0)</td>
<td>Und</td>
<td>Und</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infection</td>
<td>157 (39.0)</td>
<td>12 (100.0)</td>
<td>147 (37.6)</td>
<td>Und</td>
<td>Und</td>
</tr>
<tr>
<td>CD4 cell count, cells/µL, median (range)</td>
<td>105 (1–1400)</td>
<td>41 (3–179)</td>
<td>112 (1–1400)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Hemoglobin level, g/dL, median (range)</td>
<td>8.3 (6.0–16.0)</td>
<td>8.3 (6.0–11.0)</td>
<td>8.0 (5.0–16.0)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Lab test results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit level, %, median (range)</td>
<td>25 (14–45)</td>
<td>25 (18–33)</td>
<td>27.40 (14–45)</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Notes: All values are percentages unless otherwise indicated. Median and range are given for continuous variables. OR = odds ratio; 95% CI = 95% confidence interval; P Value = statistical significance. All comparisons were made using the Student’s t-test or the Mann-Whitney U test for continuous variables and the Pearson chi-square test for categorical variables. *P < .05.
Acknowledged
Table 2. Predictors of Death Among Participants With Bacteremic Disseminated Tuberculosis, Kilimanjaro Christian Medical Centre and Mawenzi Regional Hospital, 2006-2010 (n = 20)

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.0</td>
<td>.578</td>
</tr>
<tr>
<td>Sex</td>
<td>0.60</td>
<td>.583</td>
</tr>
<tr>
<td>Fever &gt;1 month</td>
<td>0.00</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>Cough &gt;1 month</td>
<td>0.00</td>
<td>.999</td>
</tr>
<tr>
<td>Weight loss &gt;1 month</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Receipt of antituberculosis drugs since current illness started</td>
<td>0.00</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>Receipt of antiretroviral therapy since current illness started</td>
<td>1.3</td>
<td>.796</td>
</tr>
<tr>
<td>Years since first positive HIV test</td>
<td>0.59</td>
<td>.538</td>
</tr>
<tr>
<td>Past treatment for tuberculosis</td>
<td>0.00</td>
<td>.999</td>
</tr>
<tr>
<td>Years since first treated for tuberculosis</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.91</td>
<td>.898</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.87</td>
<td>.318</td>
</tr>
<tr>
<td>Conjunctival pallor</td>
<td>0.45</td>
<td>.427</td>
</tr>
<tr>
<td>Oral candidiasis</td>
<td>6.7</td>
<td>.120</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>2.9</td>
<td>.287</td>
</tr>
<tr>
<td>Blood pressure, systolic</td>
<td>0.99</td>
<td>.681</td>
</tr>
<tr>
<td>Blood pressure, diastolic</td>
<td>0.99</td>
<td>.524</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>1.0</td>
<td>.812</td>
</tr>
<tr>
<td>Reduced oxygen saturation</td>
<td>1.4</td>
<td>.123</td>
</tr>
<tr>
<td>Abnormal breath sounds</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Presence of ascites</td>
<td>0.80</td>
<td>.881</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>1.3</td>
<td>.796</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Glasgow coma score</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Neck stiffness</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Kernig sign positive</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Presence of herpes zoster scar</td>
<td>0.70</td>
<td>.812</td>
</tr>
<tr>
<td>Presence of Kaposi sarcoma</td>
<td>0.00</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>Presence of BCG scar</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Abnormal chest radiograph</td>
<td>0.42</td>
<td>.512</td>
</tr>
<tr>
<td>Presence of infiltrates on chest radiograph</td>
<td>1.0</td>
<td>1.000</td>
</tr>
<tr>
<td>Presence of nodules on chest radiograph</td>
<td>0.00</td>
<td>.999</td>
</tr>
<tr>
<td>Presence of cavities on chest radiograph</td>
<td>2.0</td>
<td>6.338</td>
</tr>
<tr>
<td>Presence of pleural effusion on chest radiograph</td>
<td>0.80</td>
<td>.887</td>
</tr>
<tr>
<td>Presence of hilar lymphadenopathy on chest radiograph</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Presence of pericardial effusion on chest radiograph</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>HIV infection laboratory confirmed</td>
<td>1.3</td>
<td>.891</td>
</tr>
<tr>
<td>CD4 cell count</td>
<td>0.99</td>
<td>.049</td>
</tr>
<tr>
<td>Positive cryptococcal antigen test</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Hemoglobin level</td>
<td>1.2</td>
<td>5.84</td>
</tr>
<tr>
<td>Hematocrit level</td>
<td>1.1</td>
<td>.499</td>
</tr>
<tr>
<td>Total lymphocyte count</td>
<td>0.76</td>
<td>.050</td>
</tr>
<tr>
<td>Platelet count</td>
<td>1.0</td>
<td>.226</td>
</tr>
</tbody>
</table>

For continuous variables, odds ratios are per unit increase in value.

Abbreviations: HIV, human immunodeficiency virus; OR, odds ratio.

* Unavailable due to missing values, or the variable is constant for all data.

DISCUSSION

We describe the survival of patients with bacteremic disseminated tuberculosis over 12 months of follow-up in a setting where ART and tuberculosis treatment are readily available. In this context, we demonstrate that bacteremic disseminated tuberculosis is often rapidly fatal, with almost one-half of affected patients dying within a month of enrollment. We find that a range of clinical and laboratory features may assist with the identification of patients with bacteremic disseminated tuberculosis. Those patients with immunologically advanced HIV disease are at greatest risk of death and also experience the highest magnitudes of mycobacteremia.

Although almost one-half of participants with bacteremic disseminated tuberculosis had died by 1 month of follow-up, the fact that more than one-third of participants were still alive after 1 year is cause for some optimism. It is likely that earlier identification of patients with disseminated tuberculosis may increase the proportion of long-term survivors. We have previously demonstrated that mycobacterial blood culture plays an
increasingly important role in the diagnosis of disseminated tuberculosis in the United States [11]. However, the median time to positivity for *M. tuberculosis* in continuously monitored blood culture systems exceeds 3 weeks [19, 20]. Because bacteremic disseminated tuberculosis is rapidly fatal in a substantial proportion of patients before a mycobacterial blood culture instrument signals positivity, alternative approaches to facilitate early diagnosis are needed. *M. tuberculosis* NAATs on whole blood can yield results within a day, but improvements to sensitivity are needed to detect low-magnitude mycobacteria [21].

Previous research has shown that a substantial group of patients with bacteremic disseminated tuberculosis may be identified by consistently applying a routine approach to tuberculosis clinical and laboratory diagnosis [6, 30]. However, a proportion of patients with disseminated disease will not be detected with a standard approach, and the size of this group appears to vary according to local clinical practices, laboratory services, and epidemiologic conditions [5, 6, 31, 32]. It is notable that a substantial minority of patients have a normal chest radiograph. We confirm that chronic cough and fever lasting >1 month, a history of weight loss of >10%, lymphadenopathy, and HIV infection [4, 17, 33] assist with the identification of patients with disseminated tuberculosis. Furthermore, among those patients with HIV infection, we demonstrate that lower CD4 cell count is a useful predictor of disseminated disease. As far as we are aware, risk factors for death among patients with bacteremic disseminated tuberculosis have not previously been studied prospectively. Although the sample size was small, lower CD4 cell count and the related measure of lower total lymphocyte count were the only predictors of death identified among many evaluated by our study. Of interest, there was a trend of patients with bacteremic disseminated tuberculosis with immunologically advanced HIV disease having higher magnitudes of mycobacteria. However, magnitude of mycobacteria was not itself associated with increased risk for death. It is possible that host, organism, and clinical management factors not measured by our study could contribute to the outcome of survival. Although we did not study the role of *M. tuberculosis* genotype or antimicrobial resistance, the epidemiology of both has been described elsewhere among tuberculosis patients in northern Tanzania [34]. The most appropriate timing for initiation of ART among the subset of patients with bacteremic disseminated tuberculosis has not been specifically studied. However, randomized trials confirm that early initiation of ART is associated with improved outcomes in HIV and tuberculosis coinfected patients, with and without immunologically advanced disease [35–39]. Participants with tuberculosis identified in this study were recommended to have ART initiated 2 weeks after the start of treatment for tuberculosis [26], although clinician practices may have varied. It is not known whether *M. tuberculosis* bacteremia independently influences risk for the immune reconstitution inflammatory syndrome, and our study was not designed to answer this potentially important question.

The high case fatality rate of bacteremic disseminated tuberculosis underscores the importance of intervention earlier in the course of HIV infection and prior to the loss of immunologic control of tuberculosis among latently infected individuals or prior to dissemination in those with pulmonary infection. Provision and promotion of HIV counseling and testing services facilitates the early detection of HIV infection. Referral of HIV-infected individuals to healthcare services that identify and effectively treat active tuberculosis [40], treat latent tuberculosis infection [41], and initiate trimethoprim-sulfamethoxazole prophylaxis [42] and ART in a timely manner [43] may all contribute to a reduction in the number of persons presenting late with fatal disseminated tuberculosis. The use of empiric tuberculosis treatment in patients suspected to have disseminated tuberculosis can be considered and is an area where further research is needed [44].

Our study had a number of limitations. The number of patients with bacteremic disseminated tuberculosis identified was quite small, limiting our ability to identify minor risk factors. Nonetheless, our study was conducted over 4 years and likely represents one of the largest prospective studies on bacteremic disseminated tuberculosis yet reported. Although it was a component of the routine clinical care of study participants, the systematic collection of samples for routine diagnosis of pulmonary tuberculosis was not part of the study design, preventing assessment of sputum mycobacteriology as a predictor of disseminated tuberculosis. Our study was not designed to comprehensively evaluate host and organism factors associated with bacteremic disseminated tuberculosis. Future studies might build on our work by incorporating a pulmonary tuberculosis comparison arm, evaluating host genomic risk factors for extrapulmonary disease, and studying the role of *M. tuberculosis* genotype and antimicrobial resistance.

In conclusion, bacteremic disseminated tuberculosis is rapidly fatal in a large proportion of patients, especially those with immunologically advanced HIV disease. However, early recognition and consequently early initiation of potentially lifesaving treatment may be facilitated by careful clinical history, physical examination, and use of laboratory tests that are widely available at district hospitals in endemic areas [45]. Specific laboratory diagnosis can be augmented with mycobacterial blood culture, but assays yielding a more rapid result such as NAATs are needed to influence management in the first few days after presentation to a healthcare facility.

**Notes**

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References


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5.28. Capacity of health care facilities to deliver HIV treatment and care services, northern Tanzania, 2004


CONTRIBUTION

My position as last author reflects my role as senior, supervising author on the paper. I conceived the research idea, assisted with seeking and obtaining funding, and provided on-site, day-to-day leadership of all aspects of the research including data collection, design of the analysis, and write up. I mentored the medical student, Ms. Landman, as we designed the study, implemented the research, conducted the analyses, and wrote the paper together. Kinabo, Schimana, Dolmans, Swai, and Shao provided critical input for study design and insights into the Tanzania health service. All authors contributed to revisions of the manuscript.

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Capacity of health-care facilities to deliver HIV treatment and care services, Northern Tanzania, 2004

Keren Z Landman1, Grace D Kinabo MD2,3, Werner Schimana MD2,3, Wil M Dolmans MD3,4, Mark E Swai MD2,3, John F Shao MD PhD2,3 and John A Crump MB ChB DTM&H1,3,4

Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Box 3867, Durham, NC 27710, USA; Department of Pediatrics, Kilimanjaro Christian Medical Centre, PO Box 3010, Moshi;

Kilimanjaro Christian Medical College, Tumaini University, PO Box 2240, Moshi;

Department of Medicine, Department of Pathology, Kilimanjaro Christian Medical Centre, PO Box 3010, Moshi, Tanzania

Summary: Few data exist on the current capacity of Tanzanian health-care facilities to deliver antiretroviral therapy (ART). We evaluated this capacity among Northern Zone facilities in 2004 using a questionnaire that addressed human resources, clinical facilities and services, and laboratory capacity. Of 19 facilities surveyed, nine (47%) had staff trained to manage ART and three (16%) prescribed ART. Two (11%) offered CD4 counts, five (26%) offered liver function tests, 16 (84%) offered chest radiography, and 18 (95%) offered acid-fast sputum staining. Of 12 (67%) facilities offering outpatient HIV/AIDS services, 12 (100%) provided co-trimoxazole to outpatients and six (50%) provided isoniazid (INH). All 19 (100%) facilities offered rapid HIV tests and full blood pictures. Overall in 2004, facilities needed strengthening to increase staff training in ART management and to implement INH for treatment of latent tuberculosis. Laboratory facilities for ART monitoring were inadequate, and outpatient ART was limited.

Keywords: HIV, Tanzania, highly active antiretroviral therapy, health-care facilities, manpower, services

Introduction

Of the 40 million persons living with HIV/AIDS worldwide, approximately 1.9 million live in Tanzania. 1 In 2003, only 2% of adults in need of antiretroviral therapy (ART) in Africa were receiving it. 2 Several programmes have been established to improve access to ART, including the Global Fund to Fight AIDS, Tuberculosis, and Malaria and the United States’ President’s Emergency Plan for AIDS Relief. The World Health Organization (WHO) and its partners have promoted a target of treating three million people with ART by the end of 2005.

Kilimanjaro Christian Medical Centre (KCMC) is the referral hospital for the Northern Zone of Tanzania. With its community partner organization Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI; Women Against AIDS in Kilimanjaro), KCMC plays a central role in provision and capacity building for scale-up of HIV/AIDS treatment and care services for North-ern Tanzania. In an effort to understand and address the capacity and capability for delivery of HIV/AIDS treatment and care in Northern Tanzania, we conducted a survey of health-care facilities in the Northern Zone.

Methods

A standardized questionnaire was developed to gather data about human capacity and capability, clinical facilities and services, and laboratory capacity with respect to the delivery of ART and HIV treatment and care services. A sample of 20 health-care facilities was selected from a government list of over 50 facilities in the Northern Zone to provide geographic representation of the KCMC catchment area. The 20 selected facilities were grouped into four geographical areas, each visited by a survey team of 2-4 people. Each team included either a medical doctor or nurse and either a laboratory staff member or a pharmacy staff member. Teams were instructed to complete one questionnaire at each facility by interviewing the facility director or most senior medical staff person available and the most senior laboratory staff.
person available. Completed questionnaires were entered and analysed using EpiInfo 2002 software (Stone Mountain, GA, USA).

There are five levels of health-care facilities in the Tanzanian public health-care system. From the highest to the lowest levels of service, they are referral hospitals, regional hospitals, district hospitals, health centres, and dispensaries. Non-government facility types include mission hospitals and factory hospitals. Data were stratified and analysed by regional and non-regional health-care facilities.

Results

Visits were completed at 19 Northern Zone health-care facilities by the survey teams between 15 July and 31 August, 2004. Of the 19 facilities, five (26%) were regional hospitals, five (26%) were mission hospitals, seven (37%) were district hospitals, one (5%) was a national tuberculosis referral hospital, and one (5%) was a factory hospital. Facilities surveyed had a median (range) of 150 (54-450) inpatient beds with a median occupancy of 75% (50-100%) in 2003. A median of 600 (20-40,000) outpatients were reported to be seen at the facilities each week.

Human capacity and capability

Among 19 responding facilities, 15 (79%) reported having staff trained in the clinical management of people living with HIV/AIDS. Nine (47%) facilities reported that their staff had received training on the use of ART in the clinical management of HIV/AIDS.

Fourteen (74%) facilities reported having at least one medical doctor on staff, while 18 (95%) facilities reported having at least one assistant medical officer on staff. Other patterns of facility clinical staffing are detailed in Table 1.

Clinical facilities and services

Of 19 responding facilities, 16 (84%) offered chest radiography services. Of 18 responding facilities, 12 (67%) reported offering outpatient services, especially for people living with HIV/AIDS. Among the 12 facilities offering HIV/AIDS outpatient services, all had co-trimoxazole available for outpatient use by HIV patients and six (50%) had isoniazid (INH) available for the treatment of latent tuberculosis infection. Nine (75%) had fluconazole available. Of the 12 facilities offering HIV/AIDS outpatient services, four (33%) reported offering ART on an outpatient basis.

Of the 19 facilities, three (16%) reported offering services for the prevention of mother-to-child transmission of HIV (PMTCT) with a median of 20 (range 10-20) mothers being tested within PMTCT programmes each week. Of the three facilities offering PMTCT services, two used nevirapine and one used zidovudine as the most common drug in the programme.

All facilities reported having a pharmacy. Of these four (21%) reported that their pharmacies stocked ART.

Among 18 responding facilities, 12 (67%) reported that they partner with organizations providing HIV home-based care.

Laboratory capacity

All 19 (100%) facilities reported having a clinical laboratory performing HIV serology by simple rapid tests on-site. One (5%) facility reported having the capacity to perform serology by enzyme-linked immunosorbent assay, and none had the capacity to perform HIV Western blot. All 19 (100%) facilities had laboratories capable of performing full blood pictures, malaria smears, and stool examinations for ova, cysts, and parasites. Only two (11%) facilities could perform CD4-positive T-lymphocyte counts (CD4 count). Eighteen (95%) facilities could perform acid-fast staining for mycobacteria. Although 18 (95%) facilities could perform cerebrospinal fluid (CSF) Gram stains, only four (21%) facilities could perform CSF India ink preparations. Also, five (26%) facilities could perform liver function tests (LFT) and blood-urea nitrogen tests (BUN), four (21%) could perform CSF cultures, and one (5%) could perform blood cultures.
Key markers of capacity for delivering ART were not significantly different between regional hospitals and health facilities at lower levels of the Tanzanian public health-care system.

Discussion

We found that in 2004, health-care facilities in the Northern Zone of Tanzania required strengthening in the areas of human capacity and capability, clinical facilities and services, and laboratory capacity in order to provide universal access to quality HIV treatment and care services for Tanzanians.

Recent public health approaches to the delivery of ART have shifted responsibility for managing treatment and care from highly trained physicians to other health-care workers and community and family members. Uganda’s national ART programme has adopted a model wherein assessment, therapy initiation and changes, management of serious conditions, and staff supervision are the responsibilities of physicians, while follow-up of ART – including counselling and the initial diagnosis and treatment of common opportunistic infections – is the responsibility of clinical officers, nurses, and counsellors. Within this model, human capacity to deliver ART is largely demonstrated by the availability of clinical officers, nurses, and counsellors in individual facilities. Nurses were the best-represented staff among facilities we surveyed, followed closely by assistant medical officers. Even in the quarter of facilities surveyed that were not staffed by medical doctors, such staff could be trained to initiate and deliver follow-up care to patients taking ART.

Studies in the context of community-based directly administered ART have demonstrated significant association between staff training and patient adherence to ART. Less than half of the facilities we surveyed reported employing staff who had received training on the management of ART. Successful ART delivery programmes elsewhere in sub-Saharan Africa have prioritized the creation of teams of doctors and nurses intensively trained to specialize in HIV care, as well as construction of spaces for HIV outpatient care; setting similar priorities and devising strategies to achieve them will be an important step toward increasing capacity for ART delivery in the health facilities we visited.

Although our study was not designed to evaluate the relationship between staffing levels and clinical workload, studies from elsewhere in East Africa have demonstrated limitations of human resources, infrastructure, and consumable resources as barriers to the delivery of high-quality clinical services. In particular, inadequate numbers of nurses due to workforce migration and depletion by HIV/AIDS itself are obstacles to providing ART in sub-Saharan Africa. Workforce migration, both domestically and internationally, has disproportionately affected rural regions such as Northern Tanzania. Strategies to recruit and retain clinical staff are necessary to address this issue.

It has been demonstrated that the involvement of community organizations improves access to HIV/AIDS treatment services and results in enhanced adherence levels and improved clinical outcomes. The partnering of the majority of the respondents with community organizations bodes well for ART candidate selection and adherence and psychosocial support for people living with HIV/AIDS in the Northern Zone.

The WHO recommends that the following basic clinical capabilities be in place for ART monitoring in resource-limited settings: rapid HIV antibody testing and a means of resolving indeterminate rapid tests by a second serological method; full blood pictures and differentials; CD4 counts; alanine transaminase (ALT) tests; pregnancy testing; and sputum smears for acid-fast bacilli (AFB). Most or all facilities surveyed had the capacity to perform rapid HIV antibody testing, full blood pictures, and AFB for tuberculosis. However, there were gaps in capability with respect to ALT, CD4 count, and serological methods other than the simple, rapid HIV test. Our survey did not address capacity for performing pregnancy tests.

The lack of CD4 count capability is itself not a reason not to offer ART. Although it is recommended to follow therapy progress with a CD4 count every 6–12 months, simple clinical, and laboratory markers may be used as surrogate markers for CD4 count in resource-poor settings. Inability to follow LFTs such as ALT may lead to failure of early detection of ART-associated hepatotoxicity. Building capacity for or access to CD4 count and ALT assays is an important step for preparedness to deliver ART.

The proportion of health-care facilities stocking ART in our survey was low – not higher than 25%. Fixed-dose stavudine/lamivudine/nevirapine was by far the most common ART used on an outpatient basis.

Co-trimoxazole has been shown to reduce morbidity and mortality among HIV-1-infected adults and children living in sub-Saharan Africa. The prophylactic use of co-trimoxazole in people living with HIV/AIDS has been recommended as part of a minimum package of care by the Tanzania Ministry of Health. Although facilities’ patterns of co-trimoxazole prescription are unknown, the availability of co-trimoxazole in the pharmacies of all facilities surveyed means that the drug is available at the point of care.

In pooled analyses of both tuberculin skin test (TST)-positive and TST-negative patients, INH has been shown to reduce tuberculosis incidence in HIV-positive individuals by 42–43%, suggesting that all people living with HIV and suspected of having latent TB should receive preventive INH therapy after active tuberculosis has been excluded.15 The Tanzania Ministry of Health has
recommended that treatment of latent tuberculosis infection be offered to all HIV-infected adults after active tuberculosis has been excluded by acid-fast stain of sputum in patients with productive cough, chest radiography, and when appropriate, biopsy or acid-fast smear of a lymph node or pleural fluid. Since the vast majority of health-care facilities surveyed have capacity to do both acid-fast staining of sputum for mycobacteria and chest radiography, reasonable capacity for the exclusion of active tuberculosis is available at most facilities. Despite this capacity, the free availability of INH, and the National HIV/AIDS Treatment Guidelines, only 50% of facilities offering outpatient services to people living with HIV/AIDS make INH treatment of latent tuberculosis infection available. While this may represent a missed opportunity to prevent active tuberculosis, recently published data suggest that mycobacterial culture of sputum in HIV-positive patients with CD4 counts of $\geq 200$ may be necessary for effective diagnosis of subclinical active tuberculosis infection. Our study was not designed to evaluate laboratory capacity to perform mycobacterial culture. Further research is needed to evaluate the importance of mycobacterial culture to exclude active tuberculosis prior to INH use in settings like Tanzania.

Fluconazole is recommended in the Tanzania Ministry of Health National Guidelines for Clinical Management of HIV/AIDS for both severe or persistent candidiasis and for the treatment of Cryptococcus neoformans disease. Despite the provision to Tanzanian hospitals of fluconazole for the management of HIV-associated opportunistic infections through the 2003 Pfizer Diflucan Partnership Programme, fluconazole is not available at all health facilities in the Northern Zone. Strengths and limitations.

This study has several limitations. Selecting health-care facilities to provide geographic representation rather than taking a random sample may reduce the generalizability of our findings. The data described here are useful for targeting efforts to strengthen capacity and capability and for formulating referral guidelines.

In conclusion, in 2004, health-care facilities in the Northern Zone of Tanzania lacked staff trained to manage ART. Availability of co-trimoxazole prophylaxis was adequate, but INH was underutilized for treating latent tuberculosis. Laboratory facilities for ART monitoring were limited. Outpatient ART and PMTCT services were available at few sites in the Northern Zone. Efforts must be redoubled to strengthen health-care facilities, human resources, and services in these areas in order to ensure that the goal of universal coverage of ART for those who need it is achieved in Tanzania.

Acknowledgements: The Rapid Funding Envelope on HIV/AIDS (Award RFE 1083 CL2 KCMC) administered by Deloitte Touche, Tanzania, supported this study. We thank survey team members and health-care facility personnel of Northern Zone hospitals for participating in the study. We thank members of Kilimanjaro Christian Medical Centre AIDS Implementation Committee of for the guidance and administrative support (Henning Grossman, Zawadiel M Hillu, George W Kanza, Rehema A Kiwera, Redempta Mamsari, Eunice E Maringo, Gileard Masenga, Eva P Muro, Ole Sendue Nguyaine, Olola A Onoko and Noel E Sami).

References


(Accepted 27 July 2005)
5.29. **Antiretroviral treatment literacy among HIV voluntary counseling and testing clients in Moshi, Tanzania, 2003 to 2005**


**CONTRIBUTION**

My position as last author reflects my role as senior, supervising author on the paper. With Thielman, I conceived the research idea, assisted with seeking and obtaining funding, and provided on-site, day-to-day leadership of all aspects of the research including data collection, design of the analysis, and write up. I mentored the medical students, Ms. Landman and Ms. Tribble, as we conducted the analyses and wrote the paper together. Mgonja, H.J. Shao, and Itemba assisted with activities at the HIV counseling and testing site. Ndosi supervised the data management team. J.F. Shao and Bartlett provided technical input and managed interactions between partner institutions. All authors contributed to revisions of the manuscript.

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230
Antiretroviral Treatment Literacy Among HIV Voluntary Counseling and Testing Clients in Moshi, Tanzania, 2003 to 2005

Keren Z. Landman, MD, Nathan M. Thielman, MD, MPH, Anna Mgonja, Humphrey J. Shao, MD, Dafrosa K. Itemba, Evelyn M. Ndosi, Alison C. Tribble, John F. Shao, MD, PhD, John A. Bartlett, MD, and John A. Crump, MB, ChB, DTM&H

Antiretroviral treatment literacy leads to greater HIV testing and treatment and antiretroviral treatment adherence. Among northern Tanzanian subjects, antiretroviral treatment awareness was only 17%. Factors associated with low antiretroviral treatment literacy included having exchanged money or gifts for sex, living in rural areas, having more than 2 children, and having a primary education only. Previous HIV testing was protective against low antiretroviral treatment literacy. These results support refocusing HIV education efforts and increasing synergy between HIV prevention and treatment programs.

Methods
Subjects were recruited through a VCT center operated by Kikundi cha Wanawake Kilimanjaro Kupambana na UKIMWI (KIWAKKUKI: Women Against AIDS in Kilimanjaro) a women's HIV/AIDS advocacy, education, and home care organization based in Moshi. At the time of data collection, KIWAKKUKI's VCT center saw an average of 13 service users each weekday. A standardized questionnaire was administered to consenting adult clients at KIWAKKUKI between November 2003 and May 2005. The questionnaire included a question in Kiswahili, on whether clients knew of "medications to increase the body's resistance to HIV/AIDS." This question was worded in Kiswahili to best capture the concept of ART, and to avoid implications of the existence of curative HIV/AIDS treatment or drugs for the treatment or prevent of HIV co-infections.

For 2 decades, HIV has been a stigmatizing disease in sub-Saharan Africa for which no therapy has been routinely offered; however, the availability of antiretroviral therapy (ART) is increasing in the region. By the end of 2005, an estimated 810 000 people living with HIV/AIDS in sub-Saharan Africa were taking antiretroviral medications, representing a two-fold increase in patients receiving treatment in the region compared with the preceding year. Antiretroviral therapy availability and the accompanying clinical benefits can lead to greater uptake of voluntary counseling and testing (VCT), an essential step towards accessing HIV treatment and care programs. Knowledge about ART has also been linked with ART adherence and health status among patients living with HIV/AIDS in the developed world. Therefore, increasing HIV treatment literacy among the general public has the potential to increase early identification and effective treatment of people living with HIV/AIDS. To understand the extent and predictors of HIV treatment literacy in northern Tanzania, we assessed HIV treatment literacy among adult clients presenting for VCT in Moshi, Tanzania.

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before the midpoint with those after the midpoint. In addition, we compared responses on the first and second tests of a subgroup of KIWAKKUKI repeat testers.

Results

Of 3242 clients, 1721 (53%) were women, and 2182 (67%) had a primary school education only. The median age was 30 years (range, 18 to 83 years). A total of 590 (18%) were HIV seropositive, and 548 (17%) knew of medications to increase the body’s resistance to HIV/AIDS.

Factors associated with low HIV treatment literacy included having exchanged money or gifts for sex, living in rural areas, having more than 2 children, and having a primary education only. Having exchanged money or gifts for sex was a greater risk factor for low HIV treatment literacy among women than among men. Factors protective against low HIV treatment literacy included previous testing for HIV, having a sexual partner who had been ill or died, and testing on the basis of having had many sexual partners or suspicion that a sexual partner had been unfaithful (Table 1). Additional risk factors and protective factors for low HIV treatment literacy are listed in Table 1.

Of 3218 first-time testers, 1681 subjects tested before the study period time midpoint, and 1537 subjects tested on or after the midpoint. When groups testing before and after the testing period midpoint were compared, the proportion of women was similar between the early and late groups (53% and 54%, respectively), and the median age in both groups was 30 years. Characteristics that were significantly more common in the late group compared with the early group, included rural residence (odds ratio [OR], 1.26; *P* =.002) and treatment literacy (OR, 2.76; *P* <.001), whereas having more than 2 children (OR, 0.65; *P* <.001) and a history of exchanging gifts for sex (OR, 0.51; *P* <.001) were less common in the late group compared with the early group.

Among 455 repeat testers who responded to the question about treatment literacy, 68 subjects (15%) who responded negatively at their first test responded positively at their second test. This change in response was not found to be statistically significant.

Conclusions

In this northern Tanzania VCT population, levels of ART treatment literacy were low. Individuals living in nonurban settings and having a higher number of children were more likely to have low HIV treatment literacy. Possible explanations for lower HIV treatment literacy among rural individuals include decreased access to centers with VCT and HIV treatment services, less contact with others using such services, and less exposure to media where HIV services are promoted.

Individuals exchanging gifts or money for sex, including commercial sex workers, were at high risk

Table 1. Bivariate and Multivariate Analyses of Factors Associated With Low Treatment Literacy, KIWAKKUKI VCT Clients, 2003-2005

<table>
<thead>
<tr>
<th>Factor</th>
<th>Bivariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of exchanging gifts/money for sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.72 .001</td>
<td>2.26 &lt;.001</td>
</tr>
<tr>
<td>Male</td>
<td>1.30 .073</td>
<td>1.60 .020</td>
</tr>
<tr>
<td>Testing to plan for future</td>
<td>1.44 &lt;.001</td>
<td>1.90 &lt;.001</td>
</tr>
<tr>
<td>More than 2 children</td>
<td>1.53 &lt;.001</td>
<td>1.75 &lt;.001</td>
</tr>
<tr>
<td>Rural residence</td>
<td>1.91 &lt;.001</td>
<td>1.74 &lt;.001</td>
</tr>
<tr>
<td>Primary education only</td>
<td>1.40 .001</td>
<td>1.34 .029</td>
</tr>
<tr>
<td>Previously tested for HIV</td>
<td>0.73 .002</td>
<td>0.73 .001</td>
</tr>
<tr>
<td>Has had a sexual partner who has been ill or died</td>
<td>1.07 .663</td>
<td>0.65 .044</td>
</tr>
<tr>
<td>Testing for many sexual partners</td>
<td>0.57 &lt;.001</td>
<td>0.63 .006</td>
</tr>
<tr>
<td>HIV testing free of charge</td>
<td>0.65 .007</td>
<td>0.58 .024</td>
</tr>
<tr>
<td>Testing for suspected unfaithfulness of a sexual partner</td>
<td>0.50 &lt;.001</td>
<td>0.54 &lt;.001</td>
</tr>
<tr>
<td>Has experienced hemoptysis</td>
<td>2.04 .098</td>
<td>1.75 .571</td>
</tr>
<tr>
<td>Fever</td>
<td>1.42 .034</td>
<td>1.54 .061</td>
</tr>
<tr>
<td>Has left Kilimanjaro or Arusha region in past 3 years</td>
<td>1.41 &lt;.001</td>
<td>1.21 .185</td>
</tr>
<tr>
<td>Partner has left Kilimanjaro or Arusha region in past 3 years</td>
<td>1.44 .001</td>
<td>1.19 .222</td>
</tr>
<tr>
<td>Spouse’s primary source of income farming</td>
<td>1.18 .159</td>
<td>1.10 .658</td>
</tr>
<tr>
<td>Has had prior treatment for tuberculosis</td>
<td>0.95 .839</td>
<td>1.07 .937</td>
</tr>
<tr>
<td>Married</td>
<td>0.79 .020</td>
<td>0.93 .603</td>
</tr>
<tr>
<td>Female</td>
<td>0.84 .061</td>
<td>0.84 .262</td>
</tr>
<tr>
<td>More than 2 lifetime sexual partners</td>
<td>0.83 .056</td>
<td>0.79 .098</td>
</tr>
</tbody>
</table>
for low treatment literacy and are known to be at greater risk for HIV infection. It has been previously demonstrated that the rate of HIV seropositivity in some groups of multiparous women is higher than that in their primiparous counterparts. This is the first study to our knowledge that links low HIV treatment literacy to increased parity or to rurality.

People presenting to KIWAKKUKI for the first time who had previously tested for HIV elsewhere were less likely to have low HIV treatment literacy. A similar trend was observed among repeat testers at KIWAKKUKI, although this trend was not statistically significant. These findings suggest that VCT is a good setting for improving HIV treatment knowledge through education. Voluntary counseling and testing has previously been shown to increase HIV knowledge and condom use among men and women and has been shown to be a highly cost-effective intervention, especially in areas of high HIV prevalence. Subjects with sexual partners who had been ill or died were less likely to have low HIV treatment literacy. This may be a result of exposure to health education through an ill partner's interactions with the health care system.

Subjects tested in the second half of the study period were less rural, had fewer children, reported fewer exchanges of gifts or money for sex, and were more treatment-literate. Some of these differences may represent greater participation in VCT by urban adults with a greater baseline exposure to HIV education. However, because the increase in treatment literacy was more than twice the decrease in rural living, we suspect there was an increase in the entire population's understanding about HIV, whether through public health education efforts or through social contact.

Although ART treatment literacy needs to be improved in all groups in Tanzania, a greater focus of education programs in rural areas and among men and women exchanging gifts or money for sex is needed. The value of the VCT encounter as a tool for increasing treatment literacy is reinforced by this study, further strengthening evidence in favor of synergy between HIV prevention and treatment programs.

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References


5.30. Predicting CD4 lymphocyte count <200 cells/mm$^3$ in an HIV-1-infected African population


CONTRIBUTION

My position as second author reflects my role as co-principal investigator with Thielman on this project. Together we conceived the research idea, sought and obtained funding, designed the study, and supervised the research including the study team members responsible for participant enrollment, data collection, laboratory assessments, data analysis, and write up. We mentored the post-doctoral fellow, Dr. Morpeth. Morpeth, H.J. Shao, Ramadhani, Kisenge, Moylan, Naggie, Caram, and Landman managed day-to-day operations of the study. Morpeth wrote the first draft of the manuscript. Ostermann led statistical analysis. Sam oversaw laboratory evaluations. Itemba oversaw patient referral from the HIV counseling and testing site. J.F. Shao, Bartlett, and Itemba managed interactions with personnel at study sites and partner institutions. All authors contributed to revisions of the manuscript.

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Predicting CD4 Lymphocyte Count <200 Cells/mm$^3$ in an HIV Type 1-Infected African Population

SUSAN C. MORPETH,1,2 JOHN A. CRUMP,1,2,3 HUMPHREY J. SHAO,2 HABIB O. RAMADHANI,2 PETER R. KISENGE,2 CINDY A. MOYLAN,1 SUSANNA NAGGIE,1 L. BRETT CARAM,1 KEREN Z. LANDMAN,1,2 NOEL E. SAM,2,3 DAIFRAS K. ITEMBA,4 JOHN F. SHAO,2,3 JOHN A. BARTLETT,1 and NATHAN M. THIELMAN1

ABSTRACT

Clinical criteria are recommended to select HIV-infected patients for initiation of antiretroviral therapy when CD4 lymphocyte testing is unavailable. We evaluated the performance characteristics of WHO staging criteria, anthropometrics, and simple laboratory measurements for predicting CD4 lymphocyte count (CD4 count) <200 cells/mm$^3$ among HIV-infected patients in Tanzania. A total of 202 adults, diagnosed with HIV infection through community-based testing, underwent a detailed evaluation including staging history and examination, anthropometry, complete blood count, erythrocyte sedimentation rate (ESR), and CD4 count. Univariable analysis and recursive partitioning were used to identify characteristics associated with CD4 count <200 cells/mm$^3$. Of 202 participants 109 (54%) had a CD4 count <200 cells/mm$^3$. Characteristics most strongly associated with CD4 count <200 cells/mm$^3$ ($p$-value <0.0001) were the presence of mucocutaneous manifestations (72% vs. 28%), lower total lymphocyte count (TLC) (median 1450 vs. 2200 cells/mm$^3$), lower total white blood cell count (median 4200 vs. 5500 cells/mm$^3$), and higher ESR (median 95 vs. 53 mm/h). In a partition tree model, TLC <1200 cells/mm$^3$, ESR ≥120 mm/h, or the presence of mucocutaneous manifestations yielded a sensitivity of 0.85 and specificity of 0.63 for predicting CD4 count <200 cells/mm$^3$. The sensitivity of the 2006 WHO Staging system improved from 0.75 to 0.93 with inclusion of these parameters, at the expense of specificity (0.36 to 0.26). The presence of mucocutaneous manifestations, TLC <1200 cells/mm$^3$, or ESR ≥120 mm/h was a strong predictor of CD4 count <200 cells/mm$^3$ and enhanced the sensitivity of the 2006 WHO staging criteria for identifying patients likely to benefit from antiretrovirals.

INTRODUCTION

APPROXIMATELY 25 MILLION of the world’s 40 million HIV-infected individuals reside in sub-Saharan Africa, a region facing profound socioeconomic, political, and infrastructural challenges to the expansion of HIV care and treatment programs. Despite intensive efforts, only 23% of 4.6 million people in need of antiretroviral therapy (ART) had accessed treatment in sub-Saharan Africa by June 2006.1 With expanding initiatives to scale-up access to care, efforts to circumvent critical restrictions in health care infrastructure are increasingly necessary.

Initiation of ART among patients with CD4 counts <200 cells/mm$^3$ or with an AIDS-defining illness has been independently associated with a mortality benefit,2,3 and this threshold has been recognized by treatment guidelines for over a decade.4-6 In resource-poor settings, such as in rural Haiti, without capacity to measure CD4 lymphocytes, ART has been successful,7 and there is general consensus that access to this technology should not be a prerequisite for initiating ART.8 Strategies to accurately identify individuals most likely to benefit from therapy are needed. The World Health Organization (WHO) staging system is recommended to help identify those eligible for ART where CD4 counting is unavailable.8,9 Be-
PREDICTORS OF LOW CD4 COUNTS IN AFRICA

cause of both practical limitations (e.g., listing diagnostic criteria that are difficult or impossible to ascertain in resource-limited settings) and lack of sensitivity and specificity, some have recommended modifying this staging system to include simple laboratory and anthropometric measurements.5-11 We describe the performance characteristics of both the original WHO staging guidelines,8,12 and the recently released 2006 guidelines for identifying HIV-infected patients with CD4 counts <200 cells/mm3.9 Using decision tree analysis, we derive an algorithm based on simple clinical parameters and low-cost laboratory testing to identify such individuals.

MATERIALS AND METHODS

Population and clinical examinations

In Moshi, Tanzania, at the Kilimanjaro Christian Medical Centre (KCMC) medical outpatient clinic, 204 recently tested HIV-1-infected adult patients recruited from voluntary counseling and testing (VCT) centers were seen for staging evaluations from August 2004 until June 2005. Inclusion criteria were age ≥18 years and a positive rapid HIV-1 antibody test. Two patients taking ART were excluded from the analysis. After obtaining informed consent, structured histories and examinations targeting the original WHO staging criteria8,12 were conducted by trained healthcare workers. Patients were restaged retrospectively with the newly released 2006 WHO staging criteria.9 Anthropometric assessments included measurements of height and weight and skin fold thicknesses at the biceps, triceps, subscapular and suprailiac regions by caliper (The Body Calipers, Carson City, NV) and mid upper-arm circumference.

Laboratory assessments

Blood was drawn for complete blood count (CBC), erythrocyte sedimentation rate (ESR), and CD4 count. CBC was measured by a Coulter Ac.T diff Hematology Analyzer (Beckman Coulter Inc., Fullerton, CA). ESR was performed by the Westergren method. CD4 counts were performed using Coulter Manual CD4 Count Kits (Beckman Coulter, Inc., Fullerton, CA).

Ethical considerations

The protocol was approved by the Institutional Review Board at Duke University Medical Center, the Research Ethics Committee at KCMC, and the Tanzania National Institute for Medical Research. Patients were referred to the KCMC Infectious Diseases Clinic with their laboratory results for ongoing care, including access to ART once available and when appropriate.

Statistical analysis

Using previously published data of mean CD4 counts by WHO stage,13 we estimated that a sample size of 200 would prove sufficient to detect differences across groups with α < 0.05 and β > 0.80. In JMP 6.0 (SAS Institute, Cary, NC), median CD4 counts were compared across WHO stages by the Wilcoxon rank-sum test. Variables potentially associated with CD4 count <200 cells/mm3 were assessed by univariable analysis. Student's t-test and the Wilcoxon rank-sum test were used for continuous variables with normal and non-normal distributions, respectively. Chi-square tests were used for categorical variables. Using recursive partitioning modeling14 in JMP 6.0 we identified a cluster of clinical and laboratory characteristics strongly predictive of CD4 count <200 cells/mm3. Only characteristics with p < 0.1 on univariable analysis were entered into the model. Numerical variables [such as total lymphocyte count (TLC)] were entered as continuous data, allowing the statistical program to select the most discriminatory cut-offs at which to dichotomize the data. To explore the sensitivity and specificity of key hematological and anthropometric variables the receiver operating characteristics (ROC) for the TLC, ESR, hematocrit, and body mass index (BMI) were calculated. Using STATA 8.0 (College Station, TX), ROC plots of the for-

| TABLE 1. PREVALENCE OF COMMON DISCRIMINATORY WHO STAGING CRITERIA BY CD4 LYMPHOCYTE COUNT STRATUM AMONG RECENTLY DIAGNOSED HIV-1-INFECTED PERSONS IN MOSHI, TANZANIA |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Characteristic** | 2006 WHO Stage | CD4 < 200/mm³  | Prevalence (%) | CD4 ≥ 200/mm³  | Prevalence (%) | Relative risk (95% confidence interval) | p value |
| Muco-cutaneous manifestations | 2 | 63/88 (72%) | 25/88 (28%) | 1.8 (1.4-2.3) | <0.0001 |
| Herpes zoster | 2 | 27/42 (64%) | 15/42 (36%) | 1.3 (1.0-1.6) | 0.1644 |
| Weight loss >10% | 3 | 48/77 (62%) | 29/77 (38%) | 1.3 (1.0-1.6) | 0.0809 |
| Unexplained chronic diarrhea, >1 month | 3 | 21/30 (70%) | 9/30 (30%) | 1.4 (1.0-1.8) | 0.0735 |
| Oral candidiasis | 3 | 24/33 (73%) | 9/33 (27%) | 1.3 (1.0-1.7) | 0.1034 |
| HIV wasting syndrome | 4 | 23/32 (72%) | 9/32 (28%) | 1.4 (1.1-1.8) | 0.0331 |

**Conditions that were present but not discriminatory: generalized lymphadenopathy, weight loss <10%, recurrent upper respiratory tract infections, unexplained persistent fever, oral hairy leukopakia, pulmonary tuberculosis, severe bacterial infections, oral/genital ulcers, herpes simplex virus infection, esophageal candidiasis, Kaposi's sarcoma.

**Conditions that were present in up to two cases only: Pneumocystis jiroveci pneumonia, toxoplasmosis, cryptococcal meningitis, extrapulmonary tuberculosis, Pneumocystis carinii pneumonia, toxoplasmosis, disseminated mycobacteriosis, non-Typhi Salmonella septicemia, extrapulmonary cryptococcosis, lymphoma, HIV encephalopathy.

**Data for visceral leishmaniasis, unexplained anemia and/or neutropenia and/or chronic thrombocytopenia, HIV cardiomyopathy, HIV nephropathy; invasive cervical carcinoma and chronic isostiriosis were not collected.

**Oral candidiasis; data available for 138 of 202 patients.
**RESULTS**

**Subject population**

Overall the median age of subjects was 38 [interquartile range (IQR), 32–44] years; 75% were female; 79% were educated no further than primary school. The median (IQR) CD4 count by 2006 WHO Stage was Stage 1, 362 (142–588) cells/mm³, \( n = 19 \); Stage 2, 204 (103–345) cells/mm³, \( n = 41 \); Stage 3, 166 (91–304) cells/mm³, \( n = 75 \); Stage 4, 175 (94–278) cells/mm³, \( n = 67 \). CD4 counts were not significantly different between adjacent stages, but the median count for Stages 3 and 4 combined differed from that of Stages 1 and 2 combined (195 vs. 279 cells/mm³, \( p = 0.0136 \)).

Univariable analyses of WHO staging criteria, anthropometrics, and simple laboratory testing by CD4 lymphocyte count stratum

Univariable analyses performed between CD4 count of <200 cells/mm³ and variables selected from the history and examination for the WHO Staging schema are shown in Table 1 and simple laboratory tests in Table 2. Anthropometry tended not to yield clinically meaningful differences in median measure-
<table>
<thead>
<tr>
<th>Overall strategy</th>
<th>Area under ROC&lt;sup&gt;a&lt;/sup&gt; (95% confidence intervals)</th>
<th>Cut-off point</th>
<th>Sensitivity (95% confidence intervals)</th>
<th>Specificity (95% confidence intervals)</th>
<th>PPV&lt;sup&gt;b&lt;/sup&gt; (95% confidence intervals)</th>
<th>NPV&lt;sup&gt;c&lt;/sup&gt; (95% confidence intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original WHO Stage&lt;sup&gt;8,12&lt;/sup&gt; and TLC&lt;sup&gt;d&lt;/sup&gt; ≤1200/mm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.67 (0.59–0.74)</td>
<td>Original WHO Stage 3 or 4, or Stage 2 with TLC &lt;1200/mm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.82 (0.74–0.89)</td>
<td>0.37 (0.27–0.48)</td>
<td>0.61 (0.52–0.68)</td>
<td>0.64 (0.50–0.77)</td>
</tr>
<tr>
<td>2006 WHO Stage&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0.57 (0.49–0.65)</td>
<td>2006 WHO Stage 3 or 4</td>
<td>0.75 (0.66–0.83)</td>
<td>0.36 (0.26–0.47)</td>
<td>0.58 (0.49–0.66)</td>
<td>0.55 (0.42–0.68)</td>
</tr>
<tr>
<td>TLC&lt;sup&gt;e&lt;/sup&gt; ≤1200/mm&lt;sup&gt;3&lt;/sup&gt;, ESR ≥120 mm/h, and mucocutaneous manifestations&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.79 (0.73–0.85)</td>
<td>≥1 of TLC &lt;1200/mm&lt;sup&gt;3&lt;/sup&gt;, ESR ≥120/mm&lt;sup&gt;3&lt;/sup&gt;/h, mucocutaneous manifestations</td>
<td>0.85 (0.77–0.91)</td>
<td>0.63 (0.52–0.72)</td>
<td>0.73 (0.64–0.81)</td>
<td>0.78 (0.67–0.87)</td>
</tr>
<tr>
<td>2006 WHO Stage 3 or 4 and 1 of TLC 1200/mm&lt;sup&gt;3&lt;/sup&gt;, ESR 120 mm/h, mucocutaneous manifestations</td>
<td>0.63 (0.55–0.70)</td>
<td>2006 WHO Stage 3 or 4 or ≥1 of TLC &lt;1200/mm&lt;sup&gt;3&lt;/sup&gt;, ESR ≥120/mm&lt;sup&gt;3&lt;/sup&gt;/h, mucocutaneous manifestations</td>
<td>0.93 (0.86–0.97)</td>
<td>0.26 (0.17–0.36)</td>
<td>0.60 (0.52–0.67)</td>
<td>0.75 (0.57–0.89)</td>
</tr>
</tbody>
</table>

<sup>a</sup>ROC, receiver-operating characteristic curve. ROC curves and selected cut-off points are presented in Fig. 2.

<sup>b</sup>PPV, positive predictive value.

<sup>c</sup>NPV, negative predictive value.

<sup>d</sup>TLC, total lymphocyte count.

<sup>e</sup>ESR, erythrocyte sedimentation rate.

<sup>f</sup>Mucocutaneous manifestations: seborrheic dermatitis, papular pruritic eruption, prurigo, fungal nail infections, recurrent oral ulcerations or angular chelitis.
ments for subjects with CD4 counts above and below 200 cells/mm$^3$. Median BMI was 21.2 (IQR 18.7–24.0) vs. 20.3 (18.7–22.7) kg/m$^2$ for those with CD4 counts above and below 200 cells/mm$^3$, respectively, $p = 0.0981$. Low TLC, high ESR, mucocutaneous manifestations (including seborrheic dermatitis, popular pruritic eruption, prurigo, fungal nail infections, recurrent oral ulcerations, or angular cheilitis), low hemoglobin, lesser mid-arm circumference, lesser bicipital skinfold thickness, chronic diarrhea, weight loss of >10%, low BMI, and oral candidiasis were associated with a CD4 count of <200 cells/mm$^3$ with a $p$-value of ≤0.1.

**Recursive partitioning model**

The 200 subjects with complete CBC data were used for the partition tree analysis. TLC <1200 cells/mm$^3$, ESR ≥120 mm/h, or presence of mucocutaneous manifestations was able to correctly predict a CD4 count of <200 cells/mm$^3$ in 92 (85%) of 108 patients (Fig. 1). Sixteen subjects were incorrectly assigned to the group with CD4 count ≥200 cells/mm$^3$. Two of these had 2006 WHO Stage 4 disease and 6 had Stage 3 disease. Thirty-four subjects incorrectly assigned to the CD4 count <200 cells/mm$^3$ group had a median (IQR) CD4 count of 285 (240–346) cells/mm$^3$.

**Receiver-operating characteristics of selected parameters**

The AUROC for TLC was 0.7 [standard error (SE), 0.03]. A TLC cut-off of <1200 cells/mm$^3$ (selected by the partition tree) had a sensitivity of 0.38 and a specificity of 0.94 for a CD4 count of <200 cells/mm$^3$. The AUROC for ESR was 0.70 (SE, 0.04). An ESR cut-off of ≥120 mm/h (selected by the partition tree) had a sensitivity of 0.40 and a specificity of 0.88. The AUROCs for hematocrit and BMI were 0.67 (SE, 0.04) and 0.57 (SE, 0.04), respectively.

**Strategies for predicting CD4 counts <200 cells/mm$^3$**

The performance characteristics of different staging strategies for predicting CD4 counts <200 cells/mm$^3$ are detailed in Table 3. The formula derived from the study set had an AUROC significantly greater than each WHO staging-based criteria, Bonferroni-adjusted probability > $x^2 < 0.001$ for each (see Fig. 2 for the ROC plots).

**DISCUSSION**

The WHO staging system is a poor predictor of patients with CD4 counts <200 cells/mm$^3$ in an African setting, but it can be substantially improved by the use of simple laboratory tests. Because clinical staging is now used to determine who should start ART in regions without access to CD4 testing, we favor modifying algorithms to maximize sensitivity at the cost of specificity.

Recent iterations of the WHO treatment guidelines present varied recommendations on the use of TLC when CD4 counts cannot be performed.8,15 The correlation of TLC with CD4 and HIV RNA measurements has varied in different populations.16–19 In some settings, the direction of change of the TLC has been shown to predict CD4 count for monitoring patients taking ART, although it has been more useful in a positive than
a negative direction, and correlates poorly with virological response.\textsuperscript{11,20,21} Using TLC as a dichotomous variable will underestimate its usefulness, as the lower the TLC, the more predictive it is of a low CD4 count. The Rwandan Kigali staging system used a modified WHO stage together with hematocrit and ESR for prognosis.\textsuperscript{10} In the Multicenter AIDS Cohort Study (MACS) of men in the United States, a rapid decline in hemoglobin and an elevated C-reactive protein (an inflammatory marker similar to ESR) were independently associated with progression to AIDS.\textsuperscript{22,23} Venkatesh et al. associated ESR with tuberculosis incidence and low CD4 counts among an HIV-infected population in Tanzania.\textsuperscript{24} Although investigators in Cote d'Ivoire, Rwanda, and Malawi have described increased mortality among HIV-infected patients with lower BMI and fat mass,\textsuperscript{25,26} anthropometry was not particularly useful in our cohort and it is difficult to standardize.

Accumulating evidence for improved outcomes among patients initiating antiretrovirals at CD4 counts of 200–350 cells/mm\textsuperscript{3} supports strategies that start patients on therapy sooner.\textsuperscript{27–29} Strategies that sacrifice specificity for sensitivity will result in some patients with higher CD4 counts starting therapy sooner, which may be to their benefit. In our cohort, 34 subjects with CD4 counts \(\geq\)200 cells/mm\textsuperscript{3} would have received ART if the formula TLC \(\leq\)1200 cells/mm\textsuperscript{3} or ESR \(\geq\)120 mm/h or mucocutaneous manifestations was applied. Of these 34 subjects, the median CD4 count was 285 cells/mm\textsuperscript{3}. Thus, most if not all of them would benefit from ART anyway. Using the same formula, 16 subjects would have had therapy deferred despite having CD4 counts \(\leq\)200 cells/mm\textsuperscript{3}. This is the far greater problem of the two potential misclassification errors, and any guidelines attempting to substitute for CD4 counting need to aim to minimize this proportion. This is perhaps the strongest argument for including markers such as TLC and ESR with clinical staging criteria until CD4 counts are available more widely. Including our formula with WHO Stage 3 or 4 disease, 93% of those with CD4 counts \(\geq\)200 cells/mm\textsuperscript{3} would be classified as therapy eligible, compared to 75% of those with CD4 counts \(\leq\)200 cells/mm\textsuperscript{3} using WHO Stage 3 or 4 alone (see Table 3).

There are limitations to this study. The formula TLC \(\leq\)1200 cells/mm\textsuperscript{3}, ESR \(\geq\)120 mm/h, or mucocutaneous manifestations needs to be validated in other populations. In our experience, VCT is still accessed mostly by people with advanced HIV disease,\textsuperscript{30} and 54% of our subjects had CD4 counts \(\leq\)200 cells/mm\textsuperscript{3}. It is likely that with improved access to VCT services, the overall prevalence of CD4 count \(\leq\)200 cells/mm\textsuperscript{3} will be lower. In this circumstance, the negative predictive value of our derived treatment triage strategy would exceed 80% and the positive predictive value would drop.\textsuperscript{31} In addition, in some parts of Africa it may not yet be practical to perform even simple laboratory tests such as those described here.

The initiation of ART for patients with CD4 counts \(\leq\)200 cells/mm\textsuperscript{3} is life saving,\textsuperscript{32} and identification of HIV-infected individuals with this degree of immunodeficiency is an important priority as the availability of ART increases. In less developed regions where the measurement of CD4 counts is not yet feasible, practical tools to predict this threshold are needed. In our experience, the performance characteristics of the WHO treatment triage strategies for predicting this threshold were suboptimal. Our derived formula, when combined with 2006 WHO Stage 3 or 4 disease, offered the best balance between sensitivity and specificity in a population with a relatively high prevalence of advanced disease.

**ACKNOWLEDGMENTS**

We are very grateful to the staff of KIWA KUKI, Angaza, and the Rainbow Centre for referring recent VCT clients, to Dr. Mark Swai, Director of Hospital Services, KCMC, for making clinic space available, to Eline Ngomuo for performing manual CD4 counts, to David Shirima and Veneranda Mosha for performing ESR measurements, to Edward Livant, Ireen Kiwelu, Christine Misanga, and Dr. Saidi Kapiga for hematology support, and to Francis Karia. This study was supported by a Fulbright Research Award, U.S. Department of State (Dr. Thielman). Additional investigator support was obtained from AIDS Clinical Trials Group, National Institute of Allergy and Infectious Diseases, National Institutes of Health (U01 AI-39156, Drs. Bartlett and Thielman), the Fogarty International Center, National Institutes of Health (D43 TW006732, Drs. H. Shao and Ramadhani), the University of Rochester Medical Center Department of Infectious Diseases (Ms. Keren Landman), and the Hubert-Yeargan Center for Global Health, Duke University Medical Center (Dr. Morphet). Presented in part at the 12th Conference on Retroviruses and Opportunistic Infections, Boston, MA, February 22–25, 2005. Abstract 638a.

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MORPETH ET AL.


Address reprint requests to:
Nathan M. Thielen
Division of Infectious Diseases and International Health
Box 3152; Room 0371HS
Duke University Medical Center
Durham, North Carolina 27710

E-mail: n.thielen@duke.edu
5.31. Predictors of incomplete adherence, virologic failure and viral drug resistance among HIV-1-infected persons receiving antiretroviral therapy in Tanzania


CONTRIBUTION

My position as last author reflects my role as senior, supervising author in the paper. I conceived the research idea, sought and obtained funding, designed the study, and supervised the research including the study team members responsible for participant enrollment, data collection, data analysis, and write up. I mentored my junior Tanzanian colleague Dr. Habib Ramadhani. Dr. Ramadhani managed day-to-day operations of the research and wrote the first draft of the paper. Thielman, Landman, Ndosi, H Shao, Morpeth, McNeill, J Shao, and Bartlett participated in study design and design of questionnaires; Ndosi entered, cleaned and managed data; Gao, Kirchherr, Shah, and Morpeth led laboratory aspects of the research; Thielman contributed to statistical analyses; and H Shao and J Shao managed interactions with clinical teams and personnel at the study sites and partner institutions. All authors contributed to revisions of the manuscript.

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Predictors of Incomplete Adherence, Virologic Failure, and Antiviral Drug Resistance among HIV-Infected Adults Receiving Antiretroviral Therapy in Tanzania

Habib O. Ramadhan,1 Nathan M. Thielman,2 Keren Z. Landman,2 Evaline M. Ndosi,1 Feng Gao,4 Jennifer L. Kirchherr,4 Rekha Shah,4 Humphrey J. Shao,5 Susan C. Morpeth,6 Jonathan D. McNeil,7 John F. Shao,1,2 John A. Bartlett,1,2 and John A. Crump1,2

1Kilimanjaro Christian Medical Centre and 2Kilimanjaro Christian Medical College, Tumaini University, Moshi, Tanzania, and 3Division of Infectious Diseases and International Health and 4Human Vaccine Institute, Department of Medicine, Duke University Medical Center, Durham, North Carolina

(See the editorial commentary by Flanigan et al. on pages 1499-1501)

Background. Access to antiretroviral therapy is rapidly expanding in sub-Saharan Africa. Identifying the predictors of incomplete adherence, virologic failure, and antiviral drug resistance is essential to achieving long-term success.

Methods. A total of 150 subjects who had received antiretroviral therapy for at least 6 months completed a structured questionnaire and adherence assessment, and plasma human immunodeficiency virus (HIV) RNA levels were measured. Virologic failure was defined as an HIV RNA level >400 copies/mL; for patients with an HIV RNA level >1000 copies/mL, genotypic antiviral drug resistance testing was performed. Predictors were analyzed using bivariable and multivariable logistic regression models.

Results. A total of 23 (16%) of 150 subjects reported incomplete adherence. Sacrificing health care for other necessities (adjusted odds ratio [AOR], 19.8; P < .01) and the proportion of months receiving self-funded treatment (AOR, 23.5; P = .04) were associated with incomplete adherence. Virologic failure was identified in 48 (32%) of 150 subjects and was associated with incomplete adherence (AOR, 3.6; P = .03) and the proportion of months receiving self-funded antiretroviral therapy (AOR, 13.0; P = .02). Disclosure of HIV infection status to family members or others was protective against virologic failure (AOR, 0.10; P = .04).

Conclusions. Self-funded treatment was associated with incomplete adherence and virologic failure, and disclosure of HIV infection status was protective against virologic failure. Efforts to provide free antiretroviral therapy and to promote social coping may enhance adherence and reduce rates of virologic failure.

Access to antiretroviral drugs for all HIV-infected persons in need is a global health priority; currently, >2 million individuals are receiving antiretroviral therapy (ART), and a rapid scale-up in the number of individuals receiving ART is in progress [1, 2]. ART regimens in resource-limited areas commonly contain the nonnucleoside reverse-transcriptase inhibitor (NNRTI) nevirapine, which is frequently coformulated in a fixed-dose, 3-drug combination with 2 nucleoside reverse-transcriptase inhibitors (NRTIs). Several reports document suppression of plasma HIV RNA levels to below detectable limits, increases in CD4+ cell counts, and improved clinical outcomes and survival with the use of nevirapine-containing ART [3-7]. Adherence to ART, a principal determinant of therapeutic success, is excellent in African countries and may be higher than adherence levels measured in North America [8]. However, the sustained and successful delivery of ART in resource-limited areas involves many challenges, including drug supply, the durability of financial commitments from international donors, the limited financial resources of in-country health ministries, drug-related toxicities, and the need to monitor therapeutic success and recognize treatment failure, including the consequences of antiviral drug resistance [9]. Patients who receive NNRTI-containing regimens are particularly vulnerable to developing drug-resistant infection when virologic failure occurs, which could potentially result in broad resistance to NNRTIs and NRTIs, transmission of drug-resistant viruses, and future compromise of NNRTI- and NRTI-containing regimens among treatment-naive populations who become infected with drug-resistant virus [10-12]. Therefore, it is essential to identify patients starting ART or who are already receiving ART who are at risk for current or future treatment failure.
Previous studies in resource-limited areas have identified factors associated with decreased adherence to ART, the development of ART-resistant infection, and survival following ART. In Botswana, a study identified the cost of ART as a barrier to therapy adherence in 47 (44%) of 108 subjects, followed by other factors, including social stigma, migration and travel, and adverse effects [13]. A Ugandan study found that low monthly income and marital status were associated with decreased adherence [14]. A Malawian study assessed adherence, plasma HIV RNA levels, and antiviral drug resistance in a cohort of 1308 persons receiving ART (1279 [98%] of whom were receiving nevirapine-containing regimens) and observed plasma HIV RNA levels <400 copies/mL in 334 (84%) of 397 subjects. Self-reported adherence during the previous 4 days was the best predictor of detectable plasma HIV RNA levels. Of the 397 subjects with HIV RNA levels <400 copies/mL, 52 (13%) had plasma HIV RNA levels >1000 copies/mL. HIV genotyping was performed for 50 (96%) of these 52 subjects. Of these 50 subjects, 42 (84%) were infected with strains with NRTI mutations, and 47 (94%) were infected with strains with non-NRTI mutations [6]. In the ART in Lower Income Countries study, a strong relationship was found between receipt of ART and survival. The greatest survival benefit was realized when ART was administered free of cost to patients in resource-limited areas [15]. A meta-analysis of studies conducted in developing countries reported additional barriers to ART, including fear of disclosure of HIV infection status, concomitant substance abuse, forgetfulness, suspicion of treatment, complicated drug regimens, the large number of pills required, decreased quality of life, work and family responsibilities, falling asleep, and lack of access to medication [16]. To summarize, previous research in Africa has identified associations between poor adherence to therapy and financial constraints, detectable viremia, and antiviral drug-resistant infection, as well as an association between the availability of free ART and survival. However, no study has yet investigated the relationships between sociodemographic characteristics, economic status, adherence to ART, virologic outcome, and antiviral drug-resistant infection in a single cohort. We sought to understand the relationship between these factors and the outcomes of ART adherence, virologic failure, and the development of antiviral drug-resistant infection in a cohort of HIV-infected persons in northern Tanzania.

**PATIENTS AND METHODS**

**Study design and participants.** The Antiretroviral Drug Adherence and Resistance (ADAR) study was a cross-sectional cohort study to evaluate predictors of virologic failure among adult (>18 years of age) HIV-infected patients attending the Infectious Diseases Clinic at the Kilimanjaro Christian Medical Centre (Moshi, Tanzania), a referral hospital in northern Tanzania. Subjects were required to have been receiving the fixed-dose combination of stavudine, lamivudine, and nevirapine for >6 months. During the 6-month period before subjects entered the study, payment for ART transitioned from private payment by patients to free medications provided by the Tanzanian Ministry of Health. This transition offered a unique opportunity to study the effect of purchased versus free medications on the outcomes of treatment adherence, virologic failure, and the development of antiviral drug-resistant infection. After informed consent was obtained, subjects were administered standardized questionnaires translated into Kiswahili that assessed demographic, epidemiologic, clinical, and treatment information. Questions addressing potential predictors of incomplete adherence were developed through a literature search for established predictors of incomplete adherence, virologic failure, and the development of antiviral drug-resistant infection from both the developed and developing world. Locally relevant factors were identified through a series of focus group discussions with patients receiving ART. The questionnaires specifically gathered data on factors such as weekly expenditures as a measure of poverty, the frequency of interruptions in ART (defined as a period of >48 without therapy), and disclosure of their HIV infection status to family members or others. We used tools already validated in East Africa, including an asset survey for measuring wealth [17], the Hopkins Symptom Checklist-25 for symptoms of depression and anxiety [18], and an adherence assessment questionnaire [19].

**Sample collection and testing.** Twenty milliliters of whole blood were collected from each of the 150 subjects. The whole blood samples were centrifuged, and multiple plasma aliquots were frozen at −80°C. After samples for the study were fully accrued, stored plasma samples were shipped to Duke University Medical Center (Durham, NC) for measurement of plasma HIV RNA levels, genotypic antiviral drug resistance testing, and HIV subtyping. Plasma samples were assayed for the presence of HIV RNA using the Roche Amplicor assay, version 1.5 (Roche Molecular Systems), with a lower limit of detection of 100 copies/mL. If plasma HIV RNA levels were >1000 copies/mL, genotypic antiviral drug resistance testing and HIV subtyping were performed on sequences obtained by directly sequencing the PCR products using parallel allele-specific sequencing [20]. Key mutations were identified according to the November 2005 revision of the International AIDS Society–USA Drug Resistance Mutations in HIV document [21]. The pretreatment CD4+ cell counts for all subjects were recorded through chart review.

**Statistical analyses.** Statistical analyses were conducted using Stata software, version 8.2 (StataCorp). Incomplete adherence was defined as self-reported adherence <100%, virologic failure was defined as a plasma HIV RNA level >4000 copies/mL, and antiviral drug resistance was defined as >1 major
virologic failure, incomplete adherence, and antiviral drug-resistance mutations were calculated as proportions of subjects sampled. Parametrically continuous variables were compared among subjects with and without virologic failure using the Student’s t test for means and the sign test for medians. Non-parametric continuous variables were compared among subjects with and without virologic failure using the Wilcoxon rank-sum test. A multivariable logistic regression model was constructed for unmatched study design to determine factors associated with incomplete adherence and virologic failure.

**Research ethics.** The protocol for this study was approved by the Kilimanjaro Christian Medical Centre Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an Institutional Review Board of Duke University Medical Center.

**RESULTS**

From June through August 2005, 150 subjects were recruited into the study (table 1). Fifty-six subjects (37%) were male, and the median age was 41 years. The median CD4+ cell count at treatment initiation was 114 cells/mm³, and the median duration of ART at study entry was 12 months. The median walking time required to attend clinic visits was 20 min, exclusive of time spent in motor vehicles.

**Incomplete adherence.** One hundred twenty-six (84%) of the subjects reported not missing any doses of ART from the start of treatment. The remaining 24 subjects (16% of the study population) were defined as having incomplete adherence. Predictors of incomplete adherence on bivariable analysis were sacrificing health care for other necessities (e.g., food, clothing, children’s school fees, or housing; OR, 20.7; P<.01) and walking distance to clinic (OR per 10 min increment, 1.2; P =.05). Disclosure of HIV serostatus to persons other than health care providers (OR, 0.23; P=.07) and the proportion of months receiving self-funded treatment (OR, 4.9; P=.06) displayed trends toward significant associations, with the disclosure of HIV serostatus exhibiting a trend toward a protective effect (table 2). In multivariable analysis, sacrificing health care for other necessities (adjusted OR [AOR], 19.8; P<.01) and the proportion of months receiving self-funded treatment (AOR, 23.5; P=.04) were associated with incomplete adherence, and disclosure of HIV infection status again displayed a trend toward a protective effect (AOR, 0.16; P=.06) (table 2).

**Virologic failure.** Forty-eight (32%) of 150 subjects had plasma HIV RNA levels >400 copies/mL and met the definition for virologic failure. In bivariable analysis (table 3), virologic failure was associated with incomplete adherence (OR, 3.8; P<.01) and a greater proportion of months receiving self-funded ART (OR, 4.7; P=.03), and disclosing HIV status to someone other than the health care provider was protective (OR, 0.17; P=.04). In multivariable analysis (table 3), virologic failure remained associated with incomplete adherence (AOR, 3.6; P=.03) and the proportion of months receiving self-funded ART (AOR, 13.0; P=.02); disclosure of HIV infection status (AOR, 0.10; P=.04) and higher weekly household expenditures (AOR, 0.96; P=.03) were protective. To better understand the relationship between self-funded ART and virologic failure, further analyses were performed to investigate the role of incomplete adherence. Subjects who paid for ART were more likely than others to be maladherent (r = 0.54; P<.01).

**Antiviral drug-resistance mutations and HIV subtypes.** Among the 48 subjects with plasma HIV RNA levels >400 copies/mL, 35 (73%) had plasma HIV RNA levels >1000 copies/mL and had sequencing of their isolates attempted. Of these

### Table 1. Demographic and clinical characteristics of 150 patients with HIV infection who received antiretroviral therapy (ART) at Kilimanjaro Christian Medical Centre Infectious Diseases Clinic, Moshi, Tanzania, 2005.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 150)</th>
<th>Adherent to ART (n = 126)</th>
<th>Maladherent to ART (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median years (range)</td>
<td>41 (19-69)</td>
<td>41 (19-69)</td>
<td>41 (21-55)</td>
</tr>
<tr>
<td>Disclosure of HIV infection status, no. (%) of patients</td>
<td>143 (95)</td>
<td>122 (97)</td>
<td>21 (88)</td>
</tr>
<tr>
<td>Weekly ART expenditures per patient, median USD (range)</td>
<td>18.1 (0-104.4)</td>
<td>17.2 (0-104.4)</td>
<td>18.1 (1.7-30.2)</td>
</tr>
<tr>
<td>Male sex, no. (%) of patients</td>
<td>56 (37)</td>
<td>47 (37)</td>
<td>9 (38)</td>
</tr>
<tr>
<td>Duration of ART, median months (range)</td>
<td>12 (6-27)</td>
<td>12 (6-27)</td>
<td>12 (6-26)</td>
</tr>
<tr>
<td>Duration of self-funded treatment, proportion of treatment duration (range)</td>
<td>0.12 (0-0.9)</td>
<td>0.1 (0-0.9)</td>
<td>0.31 (0-0.8)</td>
</tr>
<tr>
<td>Depression, no. (%) of patients</td>
<td>31 (21)</td>
<td>24 (19)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>CD4+ cell count at ART initiation, median cells/mm³ (range)</td>
<td>114 (1-628)</td>
<td>116 (1-515)</td>
<td>94 (9-628)</td>
</tr>
<tr>
<td>Walking time to clinic, median min (range)</td>
<td>20 (0-180)</td>
<td>10 (0-90)</td>
<td>25 (5-180)</td>
</tr>
<tr>
<td>Sacrifice of health care for other necessities, no. (%) of patients</td>
<td>6 (5)</td>
<td>2 (2)</td>
<td>6 (25)</td>
</tr>
</tbody>
</table>

*Walking time was assessed in 10-min increments.*

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were associated with having to pay for medications for a longer duration. Incomplete adherence and virologic failure results demonstrate outstanding levels of adherence and good virologic response, and the link between adherence to therapy, virologic response, and antiviral drug resistance in a resource-limited setting. The ADAR study represents a novel attempt to rigorously assess antiviral drug resistance in a resource-limited setting. The results demonstrate outstanding levels of adherence and good virologic success among HIV-infected persons receiving fixed-dose combination ART in northern Tanzania. Conversely, maladherent subjects were more likely to have detectable viremia during treatment. Incomplete adherence and virologic failure were associated with having to pay for medications for a longer duration, and disclosure of HIV infection status to someone other than a health care provider was protective. These observations carry critical implications for policy makers in resource-limited areas during the remarkable, ongoing scale-up of ART delivery.

The ADAR study used an adaptation of a validated adherence questionnaire for measuring adherence [19]. Numerous studies have demonstrated high levels of adherence among persons receiving ART in resource-limited areas [14, 22-25], including among African cohorts. A recent systematic overview found that 77% of sub-Saharan Africans maintained 100% adherence with ART, compared with 55% of North Americans [8]. Therefore, the ADAR study results regarding adherence in a Tanzanian population are consistent with observations from other cohorts in resource-limited areas.

Other studies have also demonstrated high rates of virologic success among populations in resource-limited countries. Studies from India [26], Malawi [6], and Uganda [27] and the ART in Lower Income Countries study [15] found that 76%–84% of subjects had plasma HIV RNA levels <400 copies/mL, rates which are very similar to those among populations receiving ART in North America and Europe. Accompanying these high rates of virologic suppression have been marked improvements in CD4+ cell counts [6, 26] and reductions in HIV-related mortality [26]. These successes have been achieved predominately with nevirapine-based regimens, despite observations that these regimens may be less potent than the efavirenz-based regimens used commonly in less financially constrained countries.

Interruptions in ART with NNRTI-containing regimens may lead to increased rates of virologic failure and antiviral drug resistance [15, 25]. These observations reflect the relatively low genetic barrier to antiviral drug resistance for NNRTIs, as well as the prolonged plasma half-lives of NNRTIs, which lead to functional monotherapy as NRTI concentrations in plasma decrease. In resource-limited areas, treatment interruptions may increase. In resource-limited areas during the remarkable, ongoing scale-up of ART delivery.

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be more common because of the relatively high cost of the medications, the difficulty of accessing health care services, and challenges in the distribution and uninterrupted delivery of drug supplies [28].

The role of self-funded treatment in determining the success of ART has been identified by other studies [13, 14]. Presumably, self-funded treatment can result in more frequent treatment interruptions in vulnerable NNRTI-based regimens because of an inability to maintain the burdensome personal financial commitment involved in purchasing ART indefinitely. Within the ART in Lower Income Countries study cohort, access to free care, including ART, was associated with improved survival, which is certainly the most striking indicator of treatment success. The ADAR study results add depth to this understanding by demonstrating that a longer duration of self-funded treatment led to lower rates of 100% adherence and undetectable viremia, as well as a trend toward more antiviral drug-resistance mutations. Similar observations have been made regarding the use of voluntary counseling and testing services in northern Tanzania, where a modest co-payment restricted use and clear cost-effectiveness was identified with removal of financial barriers [29]. Because of the high cost of the failure of first-line ART, access to free ART and related services in resource-limited areas is very likely to achieve remarkable cost-effectiveness.

In the ADAR study, the protective role of disclosure of HIV infection status to persons other than health care providers was dramatic. This association may be a result of multiple factors, including social stigma, adherence coaching, and social isolation and depression. Strong social stigma regarding HIV infection exists globally and has been investigated for its effect on ART adherence. The effect of "coaching" on adherence has been identified in previous studies, and it may play a positive role. Finally, depression has been identified as a clear factor associated with decreased adherence in multiple studies, but it was not associated with incomplete adherence in the ADAR study. Although a validated tool was used to assess adherence in our study, the limited numbers of subjects with incomplete adherence may have prevented the identification of potential associations [13, 30].

The antiviral drug-resistance mutations found among ADAR subjects with plasma HIV RNA levels >1000 copies/mL fell into patterns of NNRTI mutations alone; NNRTI mutations plus M184V; and NNRTI mutations, M184V, and additional NRTI-associated mutations, including thymidine analogue mutations. These patterns have been previously recognized among persons

Table 3. Bivariable and multivariable logistic regression analyses of risk factors for virologic failure, Kilimanjaro Christian Medical Centre Infectious Diseases Clinic, Moshi, Tanzania, 2005.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bivariable analysis OR (95%CI)</th>
<th>P</th>
<th>Multivariable analysis Adjusted OR (95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>1.0 (0.97-1.0)</td>
<td>.71</td>
<td>1.0 (0.97-1.1)</td>
<td>.64</td>
</tr>
<tr>
<td>Disclosure of HIV infection status</td>
<td>0.17 (0.03-0.92)</td>
<td>.04</td>
<td>0.10 (0.01-0.94)</td>
<td>.04</td>
</tr>
<tr>
<td>Weekly expenditures, USD</td>
<td>0.98 (0.95-1.0)</td>
<td>.14</td>
<td>0.96 (0.93-1.0)</td>
<td>.03</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.3 (0.65-2.6)</td>
<td>.45</td>
<td>1.1 (0.49-2.6)</td>
<td>.79</td>
</tr>
<tr>
<td>Duration of ART, months</td>
<td>1.0 (0.96-1.1)</td>
<td>.49</td>
<td>0.95 (0.87-1.0)</td>
<td>.31</td>
</tr>
<tr>
<td>Portion of treatment that was self-funded, months</td>
<td>4.7 (1.2-18.7)</td>
<td>.03</td>
<td>13.0 (1.4-118.2)</td>
<td>.02</td>
</tr>
<tr>
<td>Depression</td>
<td>0.84 (0.35-2.0)</td>
<td>.69</td>
<td>0.73 (0.28-1.9)</td>
<td>.53</td>
</tr>
<tr>
<td>CD4+ cell count at ART initiation, median cells/mm^3</td>
<td>1.0 (1.0-1.0)</td>
<td>.68</td>
<td>1.0 (1.0-1.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Walking time to clinic, per 10-min increment</td>
<td>1.2 (0.99-1.4)</td>
<td>.08</td>
<td>1.2 (0.96-1.5)</td>
<td>.10</td>
</tr>
<tr>
<td>Sacrifice of health care for other necessities</td>
<td>1.3 (0.30-5.6)</td>
<td>.73</td>
<td>0.47 (0.07-3.1)</td>
<td>.43</td>
</tr>
<tr>
<td>Incomplete adherence to therapy</td>
<td>3.8 (1.5-9.3)</td>
<td>&lt;.01</td>
<td>3.6 (1.2-11.0)</td>
<td>.03</td>
</tr>
</tbody>
</table>

Table 4. Summary of genotypic antiviral drug-resistance mutations in isolates obtained from HIV-infected patients at Kilimanjaro Christian Medical Centre Infectious Diseases Clinic, Moshi, Tanzania, 2005.

<table>
<thead>
<tr>
<th>No. of mutations</th>
<th>No. (%) of isolates (n = 15)</th>
<th>Antiviral drug-resistance mutation(s) (no. of isolates), by type of antiviral drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 (13)</td>
<td>NRTI: None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NNRTI: K103N (1), Y181C (1)</td>
</tr>
<tr>
<td>2</td>
<td>7 (47)</td>
<td>NRTI: M184V (1), Y181C (1), K103N (3), Y181C (1), G190A (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NNRTI: K103N (3), Y181C (1), G190A (3)</td>
</tr>
<tr>
<td>&gt;=3</td>
<td>6 (40)</td>
<td>NRTI: Q151M (1), M184V (4), M184I (1), TAMs:M41L (1), K65R (1), L210W (1), T215Y (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NNRTI: K103N (3), V108I (1), Y181C (4), G190A (2)</td>
</tr>
</tbody>
</table>

NOTE. NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor.
receiving NNRTI-containing regimens, including among persons receiving ART in resource-limited areas [10]. We speculate that the marginal association between antiviral drug-resistance mutations and lower pre-ART CD4⁺ cell counts may be a reflection of higher pre-ART plasma HIV RNA levels, although baseline samples were not available for testing.

The ADAR study does have important limitations. First, the study is cross-sectional, and stronger conclusions would be possible with a longitudinal cohort. Because patients had received ART for ≥6 months at the time of evaluation, adherence was measured some time after ART was established. Furthermore, the study did not include those individuals who had defaulted from therapy or switched to second-line therapy prior to the time of inclusion. These 2 factors may have led to an overestimation of adherence. The size of the entire cohort was 150 subjects, but only 23 subjects were maladherent; therefore, the ability of the study to identify factors associated with incomplete adherence was limited. A relatively low proportion of subjects with plasma HIV RNA levels >1000 copies/mL had virus with antiviral drug resistance–mutations (15 of 35 subjects), which may have also diminished the ability of the study to recognize factors associated with antiviral drug resistance. It is notable that, despite these limitations in sample size, significant associations were identified. Finally, baseline samples were not available in this cohort, and their availability would have enhanced our ability to examine the impact of factors such as pre-ART plasma HIV RNA levels, preexisting drug-resistant viruses, and HIV subtypes on virologic failure and antiviral drug-resistance mutations.

In summary, the ADAR study has identified critical factors that are predictive of ART success and failure, and its findings should be incorporated by policy makers into practice. Structural barriers to care, including the time required to reach health care services and, especially, the personal financial burden of funding ART, must be removed. Social coping, including the disclosure of HIV infection status to others who are not health care providers, leads to higher rates of ART adherence and is protective against virologic failure. The availability of enhanced and accessible monitoring services, which promote the early identification of HIV-infected persons and their close monitoring before CD4⁺ cell counts reach extremely low levels, may lead to less antiviral drug resistance. In addition, sentinel programs designed for operational research that can identify detectable viremia in patients who are receiving ART and detect cases of antiviral drug–resistant infection provide valuable feedback to improve service delivery.

Acknowledgments


Potential conflicts of interest. All authors: no conflicts.

References

19. Ogugui JH, Byskika-Tusipine J, Charlebois ED, et al. Multiple validated measures of adherence indicate high levels of adherence to generic HIV

CSE THEME ARTICLE . CID 2007;45 (1 December) . 1497


5.32. Early versus delayed fixed dose combination abacavir/lamivudine/zidovudine in patients with HIV and tuberculosis in Tanzania


CONTRIBUTION

My position as second author reflects my role as co-principal investigator with Thielman on this project. Together with Bartlett, we conceived the research idea, designed the study, and supervised the research including the study team members responsible for participant enrollment, data collection, laboratory assessments, data analysis, and write up. We mentored the post-doctoral fellow, Dr. H.J. Shao. H.J. Shao, Ramadhani, Uiso, Ole-Nguyaine, Moon, and Kiwera managed day-to-day operations of the study. Woods oversaw HIV-1 RNA testing at a reference laboratory. Bartlett and Thielman sought and obtained funding. J.F. Shao and Bartlett managed interactions with personnel at study sites and partner institutions. All authors contributed to revisions of the manuscript.

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Early versus Delayed Fixed Dose Combination Abacavir/Lamivudine/Zidovudine in Patients with HIV and Tuberculosis in Tanzania

Humphrey J. Shao,1,2 John A. Crump,1,2,3,4 Habib O. Ramadhan,1,2 Leonard O. Uiso,5 Sendui Ole-Nguyaine,1,2 Andrew M. Moon,3 Rehema A. Kiwera,6 Christopher W. Woods,3,4 John F. Shao,1,2 John A. Bartlett1,2,3,4 and Nathan M. Thielman3,4

Abstract

Fixed dose combination abacavir/lamivudine/zidovudine (ABC/3TC/ZDV) among HIV-1 and tuberculosis (TB)-coinfected patients was evaluated and outcomes between early vs. delayed initiation were compared. In a randomized, pilot study conducted in the Kilimanjaro Region of Tanzania, HIV-infected inpatients with smear-positive TB and total lymphocyte count <1200/mm³ were randomized to initiate ABC/3TC/ZDV either 2 (early) or 8 (delayed) weeks after commencing antituberculosis therapy and were followed for 104 weeks. Of 94 patients screened, 70 enrolled (41% female, median CD4 count 103 cells/mm³), and 33 in each group completed 104 weeks. Two deaths and 12 serious adverse events (SAEs) were observed in the early arm vs. one death, one clinical failure, and seven SAEs in the delayed arm (p=0.6012 for time to first grade 3/4 event, SAE, or death). CD4 cell increases were +331 and +328 cells/mm³, respectively. TB-immune reconstitution inflammatory syndromes (TB-IRIS) were not observed in any subject. Using intent-to-treat (ITT), missing=failure analyses, 74% (26/35) vs. 89% (31/35) randomized to early vs. delayed therapy had HIV RNA levels <400 copies/ml at 104 weeks (p=0.6026). In an analysis in which switches from ABC/3TC/ZDV=failure, those receiving early therapy were less likely to be suppressed to <400 copies/ml [60% (21/35) vs. 86% (30/35), p=0.030]. TB-IRIS was not observed among the 70 coinfected subjects beginning antiretroviral treatment. ABC/3TC/ZDV was well tolerated and resulted in steady immunologic improvement. Rates of virologic suppression were similar between early and delayed treatment strategies with triple nucleoside regimens when substitutions were allowed.

Introduction

The clinical management of coinfection with HIV and tuberculosis (TB) presents a number of challenges, particularly in Africa, where 85% of incident coinfections occur and where TB is the leading cause of HIV-associated mortality.1-4 Many questions surround the optimal clinical management of HIV and TB coinfection; these include timing of initiation of antiretroviral therapy (ART), management strategies for overlapping toxicities of antituberculous and antiretroviral medications, and drug interactions between rifampicin-based antituberculous regimens with nonnucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs).

An important complication of initiating ART in coinfected patients is the paradoxical worsening of signs and symptoms of TB secondary to immunologic reconstitution. Commonly manifest by fevers, increased or initial appearance of adenopathy, new or worsening pulmonary infiltrates, serositis, cutaneous lesions, and new or expanding central nervous system mass lesions, this syndrome has been reported to occur in 8-43% of patients receiving ART.7,14 The risk is greatest among those with lower CD4 cell counts,7,8,14 a population for which prompt initiation of ART may be
life-saving, and for those with shorter intervals between initiation of ART and antituberculosis therapy.

Thus, in an individual patient, the risk of deferential therapy to avoid TB-associated Immune reconstitution inflammatory syndrome (TB-IRIS) must be balanced against the risk of death, particularly for patients with <200 CD4 cells/mm³. To date, no published prospective randomised study has examined the optimal timing of ART following initiation of anti-TB therapy.

Of the currently available NNRTIs and PIs, efavirenz is recommended as the drug of choice for patients requiring concomitant anti-TB therapy. However, non-efavirenz-based treatment strategies are needed for selected patients for several reasons: (1) efavirenz is not recommended for use in pregnancy, (2) perhaps in part because of the widespread use of nevirapine for the prevention of mother-to-child transmission, reports of virologic failure associated with NNRTI resistance are increasing, (3) a small but considerable proportion of patients in resource-poor settings discontinue this medication because of side effects, and (4) efavirenz-based regimens are more costly (approximately twice that of nevirapine-based regimens), limiting their availability in resource-poor settings.

Abacavir/lamivudine/zidovudine (ABC/3TC/ZDV) has been suggested as an alternate regimen for patients who cannot take efavirenz, but limited data describe its use among coinfected patients. In a small study of 34 subjects with HIV and tuberculosis and with CD4 cell counts >350 cells/ml at baseline, HIV RNA levels were suppressed to <400 and <50 copies/ml in 91% and 76% of subjects, respectively, at 24 weeks, and no cases of abacavir hypersensitivity reactions (HSR) or TB-IRIS were reported.

We designed a pilot study, the Tuberculosis and HIV Immune Reconstitution Syndrome Trial (THIRST), to further assess the efficacy and safety of ABC/3TC/ZDV among coinfected patients and to compare outcomes, including the emergence of TB-IRIS, between subjects randomized to early vs. delayed ART, relative to the initiation of anti-TB therapy.

Materials and Methods

Study subjects and design

Eligible patients were HIV-1-infected adults aged 13 years and older who had received no previous ART (unless received for the prevention of mother-to-child transmission of HIV), had been admitted to one of two hospitals within 56 days of a diagnosis of pulmonary or extrapulmonary TB based on acid fast smear positivity, had received <14 days of anti-TB therapy, and had a total lymphocyte count >1200/mm³. The study was approved by the Institutional Review Board of Duke University Medical Center, the Kilimanjaro Christian Medical Centre (KCMC) Research Ethics Committee, and the Medical Research Coordinating Committee of the National Institute of Medical Research in Tanzania, and all subjects provided written informed consent. A protocol-mandated interim safety review of serious adverse events was completed after 10 patients from each group had received at least 8 weeks of therapy, and results were reported to each ethical review board and the Tanzanian Food and Drugs Authority. This study is registered with ClinicalTrials.gov, number NCT00851630.

Screening evaluations included a review of HIV and TB treatment history, documentation of HIV testing, complete blood cell count with differential, alanine aminotransferase (ALT), and serum creatinine. Prior to initiating the trial, study subject numbers were randomly assigned 1:1 to early vs. delayed treatment groups by a computer algorithm, and study team members not involved in subject recruitment or enrollment placed assignments into sealed opaque envelopes that were opened once participants met eligibility criteria. Eligible, consenting subjects were thus randomized with equal probability to initiate fixed dose combination abacavir (300 mg)/lamivudine (150 mg)/zidovudine (300 mg) administered orally twice daily, either 2 weeks (early group) or 8 weeks (delayed group) after starting anti-TB medications.

The planned duration of this study was 104 weeks from initiation of ART. Baseline evaluations included a medical history, clinical assessment, and laboratory tests (see below) including CD4 cell count. Plasma was archived for HIV RNA testing.

All study subjects remained as inpatients from the time of initiation of anti-TB therapy until completion of their eighth week of ART. If subjects developed suspected abacavir HSR, fixed dose combination ABC/3TC/ZDV was discontinued, and patients were given tenofovir 300 mg by mouth once daily, lamivudine 150 mg by mouth twice daily, and zidovudine 300 mg by mouth twice daily. For study subjects suspected of zidovudine-related toxicities, fixed-dose combination ABC/3TC/ZDV was discontinued, and patients commenced abacavir 300 mg twice daily, lamivudine 150 mg twice daily, and stavudine 40 mg twice daily (30 mg for those weighing <60 kg).

Clinical assessments

Subjects were evaluated weekly for the first 8 weeks as inpatients following initiation of ABC/3TC/ZDV and monthly at KCMC thereafter. Subjects were assessed specifically for the development of TB-IRIS and abacavir HSR during each visit. TB-IRIS was defined by the protocol as (1) new persistent fever (temperature >101.5°F) developing after the initiation of ART, not believed to be associated with ART, and without an identifiable source, (2) marked worsening or emergence of intrathoracic lymphadenopathy, pulmonary infiltrates, or pleural effusions on radiologic examination, or (3) worsening or emergence of lymphadenopathy on serial examinations or worsening of other TB lesions. Study personnel received specific training to assess for abacavir HSR. Trained workers from the community-based organization Kikundi cha Wanawake Kilimanjaro Kupambana na Ukimwi (KIWA KKUKI) conducted home visits to encourage adherence.

Laboratory assessments

CD4 cell counts, complete blood cell counts (CBCs), ALT, serum creatinine, glucose, and erythrocyte sedimentation rate (ESR) measurements were taken at weeks 12, 24, 36, 48, 60, 72, 84, 96, and 104, and plasma was archived from each of these visits. More intensive toxicity monitoring during the first 2 months of ART included CBCs, creatinine, glucose, ALT, and ESR at weeks 4 and 8. Toxicities were graded using NIAID Division of AIDS criteria. CD4 cell counts were performed using Coulter Manual CD4 Count Kits (Beckman Coulter, Inc., Fullerton, CA). Plasma was archived for HIV RNA testing and shipped in three batches to the Durham Veterans Affairs Medical Center in Durham, NC, for HIV-1 RNA
EARLY VERSUS DELAYED ART IN HIV WITH TB


Statistical analysis

The primary objectives of this study were (1) to assess the feasibility and safety of a fixed-dose combination ABC/3TC/ZDV in HIV-infected subjects with TB in a resource-limited setting and (2) to assess the impact of delayed vs. early initiation strategy for fixed-dose combination ABC/3TC/ZDV on the rate of TB-IRIS. Based on literature available at the time the study was designed, the anticipated incidence of TB-IRIS for patients initiating antiretroviral and antituberculosis medications was 30%. Estimating that 30% and 5% of patients receiving early and delayed ART, respectively, would develop TB-IRIS, a sample size of 35 patients within each group (with allowance of up to seven patients to drop out of each group) was chosen to provide 80% power with alpha = 0.05 (one-sided).

Planned analyses included comparing the proportion of subjects demonstrating TB-IRIS and other adverse events in early vs. delayed groups, and descriptions of clinical, immunologic, and virologic responses. Safety in each arm was assessed using Cox proportional hazards modeling of time to first grade 3/4 event, serious adverse event, or death. The primary analysis of virologic responses to early vs. delayed strategies was intent to treat (ITT), missing = failure, in which protocol-defined substitutions were allowed. A secondary ITT analysis, missing = failure, was performed in which switches from fixed dose combination ABC/3TC/ZDV were considered failures.

Differences in proportions of subjects with HIV RNA levels <50 and <400 copies/ml between early vs. delayed groups were compared using Fisher’s exact test. Statistical analyses were calculated using JMP 7.0 (SAS Institute Inc., Cary, NC).

Results

Subject screening and baseline characteristics

Enrollment began in June 2004 and was completed in September 2005. The last subject completed 104 weeks of study therapy in September 2007. The disposition of study subjects from recruitment through 104 weeks of follow-up is shown in Fig. 1. Baseline demographic and disease characteristics at study entry (summarized in Table 1) were comparable across treatment arms with the exception of weight.

Subject disposition, safety, and TB-IRIS events

Of the 35 subjects randomized to receive early ABC/3TC/ZDV, two switched to abacavir, lamivudine, stavudine because of zidovudine-associated anemia, and three switched to tenofovir, lamivudine, zidovudine due to suspected abacavir HSR. Two subjects died, one at week 20 with a tension pneumothorax and underlying probable pneumonia and the other at week 35 with cerebral malaria; neither death was judged by the investigators at the time to be related to TB-IRIS. Altogether, 12 serious adverse events were recorded among patients in the early arm (see Table 2).

Among the 35 subjects randomized to delayed therapy, one switched to tenofovir, lamivudine, zidovudine for suspected abacavir HSR, one died of complications thought to be related to disseminated Kaposi's sarcoma at week 4, and another experienced overt clinical antiretroviral failure at week 86 and was removed from the study. This subject was eventually documented to have a bloodstream infection with M. smegmatis. A total of seven serious adverse events were documented among patients in the delayed arm. Overall five subjects in the early group and one subject in the delayed group were switched from their initial antiretroviral regimen of ABC/3TC/ZDV due to drug-related toxicity (p = 0.3565).

Cox proportional hazards modeling of time to first event (grades 3 or 4 toxicity, serious adverse event or death) showed no difference between early vs. delayed treatment arms (p = 0.6012). No participants from either arm of the study had a protocol-defined TB-IRIS event throughout the 1753 patient-months of follow-up, including inpatient monitoring for the first 8 weeks of ART for all subjects. At week 104, across both study arms, 60 (86%) of subjects remained on ABC/3TC/ZDV and 66 (94%) remained on study, receiving a protocol-defined regimen. Twenty-eight (80%) vs. 32 (91%) of subjects randomized to the early vs. delayed arms remained on ABC/3TC/ZDV at week 104 (p = 0.1719).

Immunologic and virologic responses

Figure 2 shows CD4 cell counts over time, by treatment group. The median increase in CD4 cell count from study entry to week 104 was 329 (IQR, 329–384) cells/mm³. There was no difference in the CD4 cell increases between subjects randomized to the early treatment arm, 331 (IQR, 313–376) cells/mm³, and the delayed treatment arm, 328 (IQR, 281–399) cells/mm³.

Plasma HIV RNA levels could not be measured on the samples obtained at study entry and week 24. Among samples from week 48, HIV RNA was detected in 34, not detected in 32, and could not be performed in one sample. In ITT analyses, 32 (91%) and 31 (89%) of subjects randomized to early and delayed arms, respectively, had HIV RNA levels <400 copies (p = 1.0), and 24 (69%) and 19 (54%), respectively, had HIV RNA levels <50 copies/ml (p = 0.3261). Among all 70 subjects at week 48, 63 (90%) were suppressed to <400 copies/ml and 43 (61%) to <50 copies/ml.

The proportions of subjects with undetectable plasma HIV RNA levels at <400 copies/ml and <50 copies/ml after 104 weeks are shown in Fig. 3. Plasma HIV RNA levels were <400 copies/ml in 26 (74%) and 31 (89%) of subjects randomized to early vs. delayed strategies, respectively (p = 0.2182), and <50 copies/ml in 23 (66%) vs. 26 (74%), respectively (p = 0.6026). Using intent to treat analyses, overall, 57 (81%) and 49 (70%) were suppressed to <400 copies/ml and <50 copies/ml, respectively, at 104 weeks. In an analysis in which switches from fixed dose combination ABC/3TC/ZDV were considered failures, those receiving early therapy were less likely to be suppressed to <400 copies/ml [60% (21/35) vs. 86% (30/35), p = 0.030].

Discussion

This pilot trial represents the first randomized study of early vs. delayed initiation of ART in patients infected with HIV and TB. No cases of immune reconstitution syndrome were seen in THRIST subjects in either arm, despite diligent monitoring throughout the study period. Virologic suppression and immunologic improvements on ABC/3TC/ZDV were robust, consistent with the clear mortality benefits for
94 patients screened

24 not enrolled
18 did not meet inclusion criteria
6 declined participation

35 randomized to early ART* with ABC/LMV/ZDV†

33 on ART at 48 weeks (28 on ABC/LMV/ZDV)

33 on ART at 104 weeks (28 on ABC/LMV/ZDV, 2 on ABC/LMV/d4T†, 3 on TDF/LMV/ZDV)†

35 randomized to delayed ART with ABC/LMV/ZDV

1 switched from ABC/LMV/ZDV

1 death

FIG. 1. Tuberculosis and HIV Immune Reconstitution Syndrome Trial (THIRST) profile and disposition of study subjects, postrandomization. *ART, antiretroviral therapy; †ABC/LMV/ZDV, fixed dose combination of abacavir, lamivudine, and zidovudine; ‡ABC/LMV/d4T, fixed dose combination of abacavir, lamivudine, and stavudine; §TDF/LMV/ZDV, fixed dose combination of tenofovir, lamivudine, and zidovudine.

initiation of antiretrovirals in HIV and TB-coinfected populations identified in cohort studies.34-37

Concerns about the occurrence of TB-IRIS have led to confusion regarding the optimal time to start ART following initiation of anti-TB therapy. Previous cohort studies have identified these syndromes in up to 43% of coinfected persons beginning ART.12-14 THIRST sought to actively identify manifestations of immune reconstitution syndromes by keeping subjects in-hospital for the first 8 weeks of ART and included daily monitoring of symptoms and vital signs and weekly physical examinations. Therefore it is unlikely that clinically important immune reconstitution syndromes were missed among THIRST subjects. The absence of subjects with these syndromes is consistent with a more recent retrospective series11 and two prospective trials32,38 suggesting that IRIS events occur less frequently than previously described. In
### TABLE 1. Baseline Demographic and Disease Characteristics at Entry of Subjects Enrolled in THIRST

<table>
<thead>
<tr>
<th>Condition</th>
<th>Overall (n = 70)</th>
<th>Early antiretroviral therapy (n = 35)</th>
<th>Delayed antiretroviral therapy (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>41 (59%)</td>
<td>23 (66%)</td>
<td>18 (51%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.2 (32.4–43.8)</td>
<td>36.0 (32.4–43.6)</td>
<td>36.7 (32.4–44.3)</td>
</tr>
<tr>
<td>CD4+ cell count (cells/mm³)</td>
<td>103 (55–155)</td>
<td>106 (58–151)</td>
<td>102 (51–203)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55.5 (50–61.2)</td>
<td>54 (46–60)</td>
<td>58 (51–64)</td>
</tr>
<tr>
<td>Hemoglobin (g/liter)</td>
<td>113 (50–61.2)</td>
<td>112 (105–120)</td>
<td>116 (108–121)</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (mm/h)</td>
<td>60.5 (43–78.2)</td>
<td>63 (50–72)</td>
<td>58 (42–84)</td>
</tr>
<tr>
<td>White blood cell count (cells/mm³)</td>
<td>5.4 (4.1–7.3)</td>
<td>5.2 (3.9–8.8)</td>
<td>5.6 (4.1–7.2)</td>
</tr>
<tr>
<td>Total lymphocyte count (cells/mm³)</td>
<td>1.2 (0.9–2.1)</td>
<td>1.5 (0.9–2.1)</td>
<td>1.1 (0.9–2.1)</td>
</tr>
<tr>
<td>Creatinine (µmol/liter)</td>
<td>94.5 (88–100)</td>
<td>92 (88–100)</td>
<td>96 (90–98)</td>
</tr>
<tr>
<td>Glucose (mmol/liter)</td>
<td>5.1 (4.4–5.6)</td>
<td>5.1 (4.6–5.7)</td>
<td>5.0 (4.1–5.4)</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/liter)</td>
<td>15 (14–16)</td>
<td>15 (14–15.2)</td>
<td>15 (14–16)</td>
</tr>
</tbody>
</table>

*Data are presented as median (interquartile range), unless otherwise stated.

ACTG protocol A5164, among 282 subjects not receiving ART and presenting with acute opportunistic infection other than TB, 20 (7%) of the subjects developed IRIS, and none of a recently described cohort of 34 subjects (with CD4 cell counts >350/mm³) initiating ABC/3TC/ZDV early in their course of anti-TB therapy developed TB-IRIS.

Three subjects died during the trial; two of them were assigned to the early ART group and had respiratory failure attributed to preexisting pulmonary disease. A third subject assigned to the delayed group died from cerebral malaria. Although it is possible that these deaths could have been directly related to or exacerbated by immune reconstitution syndromes, it is unlikely because of clearly identifiable alternative disease processes. Early mortality is a recognized complication of ART initiation in African cohorts, and the mortality rate of 4.3% among THIRST subjects is considerably lower than the rates of 8–26% in the first 12 months reported across multiple cohorts. It is also possible that the four subjects with suspected abacavir HSR could have been suffering from immune reconstitution syndromes and were mistakenly diagnosed with HSR. This is unlikely because they continued on ART with the substitution of tenofovir for abacavir, and their symptoms resolved rapidly during ongoing ART.

The antiretroviral and immunologic effects of the triple nucleoside regimen used in THIRST subjects were consistent with responses seen in other African cohorts. In the DART study, greater than 60% of subjects had plasma HIV RNA levels <50 copies/ml at 48 weeks; in THIRST this trend was sustained through 104 weeks with 70% at <50 copies/ml using a standard ITT, missing = failure, analysis. Immuno­logic improvement as measured by CD4 cell increases was comparable to that reported with zidovudine, lamivudine, and tenofovir in the DART trial, with a median increase of 140 cells/mm³ at week 48 (vs. 128 cells/mm³ reported in the DART trial). Overall, early CD4 cell increases were not as robust as has been reported in other trials in resource-limited settings. ART regimens containing zidovudine compared to those without zidovudine have been associated with smaller CD4 cell increases but not with more clinical events. It is possible that CD4 cell increases among THIRST subjects, all of whom were initiated on a zidovudine-containing regimen, occurred more slowly compared to patients receiving other regimens in similar settings and that a more gradual immunologic recovery favors fewer TB-IRIS events. Alternatively, patients in this study may have had smaller CD4 cell increases because of their coinfection with TB.

The risk of immune reconstitution inflammatory syndromes is greater among patients with lower baseline CD4 cell counts, and the median baseline count in the THIRST subjects was 104 cells/mm³. Therefore it is possible that immune reconstitution syndromes were not seen because of the relatively high baseline CD4 cell counts in the THIRST population. Finally, higher risk has been associated with a shorter duration of antituberculous treatment. To be eligible for THIRST, subjects could not have received more than 14 days of anti-TB therapy; this seems like an unlikely explanation for the lack of immune reconstitution syndromes.

### TABLE 2. Serious Adverse Events Occurring Postrandomization among THIRST Participants

<table>
<thead>
<tr>
<th>Condition</th>
<th>Week of antiretroviral therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>6⁷, 13⁸</td>
</tr>
<tr>
<td>Suspected abacavir</td>
<td>3⁶, 3⁶, 11⁹</td>
</tr>
<tr>
<td>Hypersensitivity reaction</td>
<td></td>
</tr>
<tr>
<td>Acute intestinal obstruction</td>
<td>14</td>
</tr>
<tr>
<td>Malaria (2)</td>
<td>7, 12, 35b</td>
</tr>
<tr>
<td>Tension pneumothorax (1)</td>
<td>20b</td>
</tr>
<tr>
<td>Syphilis (1)</td>
<td>12</td>
</tr>
<tr>
<td>Delayed</td>
<td></td>
</tr>
<tr>
<td>Allergic reaction to sulphanilamopyrimethamine (1)</td>
<td>-2⁶</td>
</tr>
<tr>
<td>Mycobacteremia with M. sherrisi (1)</td>
<td>86</td>
</tr>
<tr>
<td>Esophageal variceal bleeding (1)</td>
<td>9</td>
</tr>
<tr>
<td>Head trauma, grand mal seizure (1)</td>
<td>5</td>
</tr>
<tr>
<td>Disseminated Kaposi's sarcoma (1)</td>
<td>4⁶</td>
</tr>
<tr>
<td>Malaria (1)</td>
<td>-2⁶</td>
</tr>
<tr>
<td>Suspected abacavir</td>
<td>2⁹</td>
</tr>
</tbody>
</table>

*Data are presented as median (interquartile range), unless otherwise stated.

*Associated with death.

*Events occurred postrandomization, but prior to initiation of antiretroviral therapy.
Although triple nucleoside regimens have been shown to lack equivalent potency in comparison to three-drug regimens containing NNRTIs or PIs,\textsuperscript{15,42,43} they do have attributes that support their use in HIV/TB-coinfected persons. Their use is not complicated by potential drug interactions with rifampicin, and the fixed dose combination of ABC/3TC/ZDV can be prescribed as a simple regimen of one pill dosed twice daily. Potential disadvantages of this regimen in coinfected persons include the occurrence of the HSR syndrome, which may be difficult to differentiate clinically from immune reconstitution disease.

The virologic and immunologic responses to ABC/3TC/ZDV were consistent with previous trials of triple nucleoside-containing and double nucleoside plus nucleotide-containing regimens.\textsuperscript{15,42,43} There were no differences in immunologic or virologic responses between the early and delayed treatment strategies. In a separate analysis in which switches from ABC/3TC/ZDV were considered failures, significantly more subjects randomized to early therapy demonstrated plasma HIV RNA levels \(\geq 400\) copies/ml at 104 weeks, suggesting that without access to appropriate substitution antiretrovirals (e.g., stavudine and tenofovir), a strategy of early ABC/3TC/ZDV for HIV/TB-coinfected patients may be associated with greater rates of virologic failure. The regimen was well tolerated overall, with 86% (60/70) of subjects completing 104 weeks on ABC/3TC/ZDV.

Four subjects (6%) were diagnosed with suspected abacavir HSR and had abacavir switched to tenofovir; these subjects had plasma HIV RNA levels and CD4 cell counts that were similar to the subjects continuing on the triple nucleoside regimen. The suspected HSR diagnoses were made in three subjects randomized to early ART and one subject randomized to delayed ART; with such small numbers of subjects suffering HSR, no conclusions can be drawn concerning differences between the two groups. Abacavir HSR appears to be uncommon in African populations due to the low prevalence of the HLA-B-5701 phenotype,\textsuperscript{44} and therefore the rate in the THIRST subjects may be unexpectedly high. Presumably this is a reflection of potentially confounding illnesses among this population coupled with the conservative management of abacavir by the study investigators.

There was a trend toward more serious adverse events in the early ART group vs. the delayed group (12 events vs. 7 events). This trend may reflect the risk of earlier initiation of ART in coinfected persons. Both subjects with zidovudine-associated anemia had increases in hemoglobin concentration following a switch from zidovudine to stavudine, and at 104 weeks they had similar responses to the subjects continuing on their original medications.

There are limitations to the THIRST study. First, the sample size is relatively small as the trial was powered to detect differences in TB-IRIS events between the two arms based on early retrospective reports of TB-IRIS incidence. It was underpowered to detect small differences in immune reconstitution syndromes between the two groups, and therefore a type II error is possible. It is possible that immune reconstitution

![Graph showing median CD4 cell counts with interquartile ranges among THIRST subjects randomized to receive early vs. delayed antiretroviral therapy in relation to initiation of antituberculosis therapy, beginning at ART initiation through 104 weeks.](image-url)
EARLY VERSUS DELAYED ART IN HIV WITH TB

FIG. 3. Proportion of THIRST participants with plasma HIV RNA levels <400 copies/ml and <50 copies/ml at 104 weeks of ART.

 Syndromes were missed or misdiagnosed, although the diligent surveillance in a hospitalized setting, the continuous administration of ART with tenofovir and substitution for abacavir if HSR was diagnosed, and the complete and prolonged follow-up make these possibilities unlikely. It is also possible that the exclusion criteria excluded patients most likely to develop TB-IRIS (49% of subjects had CD4 cell counts <100 cells/mm³). Finally, sputum culture with definite identification of Mycobacterium tuberculosis was not performed, reflecting the current standard of diagnosis in most resource-limited settings.

In summary, the THIRST results suggest that early ART can be well tolerated by HIV/TB-coinfected subjects with a low risk of immune reconstitution syndromes, although there may be more adverse events necessitating regimen switches with early ART. Early initiation of ART led to predictable benefits in virologic suppression and increases in CD4 cell counts among THIRST study subjects receiving the triple nucleoside combination of ABC/3TC/ZDV with appropriate substitutions for drug toxicity. Larger, fully powered studies are needed to provide a definitive answer to the clinical question of when to start ART, but the THIRST results provide additional evidence to support the initiation of ART without delay.

Acknowledgments

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Disclosures

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Address correspondence to:
Nathan M. Thielman
Department of Medicine
Division of Infectious Diseases and International Health
Box 90519, Room 109, Trent Hall
Duke University Medical Center
Durham, North Carolina 27708
E-mail: n.thielman@duke.edu
6. DISCUSSION

6.1. Introduction

The research presented in this thesis represents a body of work that was responsive to data needs of the expansion of HCT and HIV care and treatment services in Tanzania. The growing complexity of the studies over time reflected the developing capacity of the KCMC-Duke University Collaboration to undertake larger projects as staff was trained, infrastructure developed, and resources secured through grants.

6.2. HIV voluntary counseling and testing

In countries with generalized HIV epidemics, widespread access to and uptake of HCT services is essential to communicate advice about HIV prevention and also to identify and link HIV-infected persons to care and treatment services. Simple, reliable, and rapid HIV antibody tests provide the means to make HIV testing available in even the most rural and remote parts of sub-Saharan Africa. As a consequence, research to evaluate and improve HCT services in northern Tanzania was identified as a high priority by our group. In parallel with careful validation of the HIV laboratory testing algorithm, our first HCT study was done in 2003 and sought to describe the characteristics of clients seeking HCT services at the KIWAKKUKI testing site in Moshi, Tanzania. This study demonstrated an HIV seroprevalence among clients of 16.7%, considerably higher than national HIV seroprevalence estimates at the time of 9.7%. Furthermore, almost half of HCT clients testing HIV seropositive were experiencing HIV-associated symptoms. Taken together these results suggested that HCT services in Moshi were reaching a high-risk group for HIV infection and that persons were seeking HCT services late in the clinical course of HIV infection.

Based on the results of the initial descriptive study, we sought to examine interventions that might lead to persons more representative of the general
population accessing HCT services, and to persons using HCT services earlier in the course of HIV infection. Health services in Tanzania typically required a patient co-payment, and this model was also applied to early HCT service delivery. Prior to international and national funding for HCT services, the co-payment was also considered to be an important component of sustaining the service. HCT providers were concerned that the co-payment might represent an important barrier for some to accessing services. Later in 2003, we studied the impact of offering free HCT services on HCT uptake both during and after a free HCT campaign and assessed the cost-effectiveness of offering free HCT services in Tanzania. We demonstrated that the provision of free HCT significantly increased the number of clients seeking testing per day and was also cost-effective. The findings of this study underpinned a policy change by the Tanzania Ministry of Health and Social Welfare to provide HCT services for free to all.

It has been hypothesized that the availability of free HIV care and treatment services is likely to encourage increased use of HCT services by providing clients access to ART and other potentially lifesaving interventions should they be found to be infected. In Tanzania, access to free ART began late in 2004. The KIWAKKUKI HCT cohort, established in 2003, provided us with the opportunity to conduct an ecologic study of the association between availability of HIV care and treatment services and HCT uptake over time. By combining HCT data from 2003 through 2007 with data on ART uptake over the same period, we developed a model that showed that concurrent with the scale-up of HIV care and treatment services, HIV seroprevalence and the prevalence of symptoms declined sharply among clients of the KIWAKKUKI testing site. These observations suggested that the expanding availability of HIV care and treatment services was associated with a lower risk, less ill population accessing HCT. The findings underscore a powerful synergy between HIV prevention and treatment efforts that has been confirmed with a range of novel strategies.
With increasing success in achieving higher rates of HCT uptake in the general population in Tanzania, we turned our attention to the issue of when repeat testing should be recommended for initially HIV seronegative persons. In the context of a generalized HIV epidemic and the limited influence of HCT on post-test sexual behavior,¹⁴ risk for acquisition persists after the initial HIV testing encounter. HCT guidelines in Tanzania and many other countries in sub-Saharan Africa recommended a single repeat test 1 to 3 months after an initial negative test.¹¹⁰ The recommendation for a single repeat test after 1 to 3 months is based on the concept of a single HIV exposure and the possibility that the patient might test seronegative during early primary HIV infection, prior to the development of the antibody response. We hypothesized that a single repeat test after 3 months with no subsequent testing might be less cost-effective than alternative re-testing strategies.¹¹¹ Using a mathematical model we demonstrated that alternatives to the single repeat test 3 months after the initial negative test were preferable. Higher risk populations merit more frequent HIV testing than low risk populations, but regular retesting is beneficial even in low-risk populations. Our data demonstrated benefits of tailoring testing intervals to resource constraints and local HIV incidence rates. The results of this study and those of others were used to inform changes to national HCT guidelines both in Tanzania and in a number of other countries in sub-Saharan Africa. Except for the situation of a well-defined single HIV exposure, repeat testing is now recommended annually for persons at high and ongoing risk for HIV acquisition.¹¹²

There has been considerable interest in the effect that an HCT encounter might have on sexual behavior change. A substantial portion of the HCT session is dedicated to counseling on risk reduction.¹¹⁰ However, meta-analysis suggest that HCT has a limited impact on subsequent risk behavior.¹⁴ We evaluated behavior changes and estimated HIV seroincidence among returning HCT clients at the KIWAKKUKI HCT service in Moshi, Tanzania.¹¹³ We showed that clients presenting for repeat HCT reported some reduction of risky behavior and improved knowledge of sexual practices and HIV serostatus of their
partners. However, HIV seroincidence among initially HIV seronegative repeat testers was 1.49 cases/100 person-years, consistent with considerable ongoing high-risk behavior.

Antibody based HIV testing is difficult to interpret in infants <18 months of age due to the persistence of maternal HIV antibody and the possibility of early primary infection related to delivery or breastfeeding. NAATs provide a means of diagnosing HIV infection in this age group by detecting HIV-1 RNA or DNA. We implemented and validated an HIV-1 RNA NAAT in Tanzania to support both early infant diagnosis of HIV and monitoring of HIV-1 RNA levels for patients receiving ART.\textsuperscript{114,115} HIV-1 RNA NAAT technologies are expensive and complex. In sub-Saharan Africa, services are available at a few tertiary and research centers. Since plasma must be tested within a few hours of collection or frozen at \(-80^\circ\text{C}\) and transported to the central laboratory on dry ice, HIV-1 RNA NAAT services are very difficult to access for persons living in rural and remote areas. We studied the technical and operational performance of a dried blood spot-based HIV-1 RNA service using the postal service for sample transport in Tanzania.\textsuperscript{116} We showed that under program conditions in Tanzania, dried blood spots provided HIV-1 RNA results comparable to conventional methods down to 5,000 HIV-1 RNA copies/mL. Our demonstration that dried blood spot RNA testing is an alternative to liquid plasma for HIV-1 RNA services in remote areas, led to a number of countries in sub-Saharan Africa considering adoption of a national dried blood spot program.

6.3. HIV home-based care

Community home-based care (CHBC) was a central component of HIV care provision in low resource settings, particularly prior to the widespread availability of free ART. After free ART became available, it soon became apparent that ART provision within the context of CHBC improved adherence.\textsuperscript{117} CHBC programs in East Africa are often managed and staffed by volunteers. The grass roots women's organization KIWAKKUKI was the main provider of CHBC in the Kilimanjaro Region of Tanzania. For the purposes of
CHBC program improvement, KIWAKKUKI wished to understand the extent to which CHBC clients were accessing free ART. In this study, structured questionnaires were administered to clients over two census rounds, one in October 2003 through February 2004 and the other in January 2005 through October 2005. At the time of the first interview only 27 (12.9%) clients were using ART and 108 (44.6%) were taking trimethoprim-sulfamethoxazole (SXT) prophylaxis. Compared with the first census round, ART use remained uncommon at the time of the second census round, with 15 (12.7%) of the clients using ART (p=0.970). By the time of the follow-up interviews, 102 (45.1%) of the clients included in the first round had died, for a mortality rate of 51/100 person-years of observation. The primary cause of death for 87 (85.3%) of the clients who had died was respiratory or gastro-intestinal infection, and the most common contributory causes of death were malnutrition (81.4%) and anemia (42.2%). These findings led to closer integration of CHBC services with HIV care and treatment centers. A liaison nurse was hired to ensure seamless referral of CHBC clients to facilities providing ART. Similarly, once clients were initiated on ART CHBC workers assisted with monitoring for adherence, toxicities, and co-infections.

6.4. HIV co-infections and their prevention

Most HIV-associated deaths in sub-Saharan Africa are due to HIV co-infections. HIV co-infections also place considerable strain on both community and hospital healthcare services. In addition to research on CHBC, clinicians with the Department of Medicine at KCMC were eager to have a picture of the contribution of HIV to admissions in the pre-ART era, the spectrum of HIV-associated disease, and patient outcomes. We reviewed the medical records of patients admitted to the medical wards of KCMC between 1990 and 2000. During this period, males represented more than half of HIV-infected inpatients at KCMC. Like other countries in sub-Saharan Africa, HIV is known to be more common among females than males in Tanzania. It is likely that males with HIV more frequently accessed healthcare at hospitals during this period, in
contrast with what we observed during inpatient studies conducted in 2007 and 2008. The inpatient case fatality rate for HIV-infected persons ranged from 15 to 21%. The most prevalent diagnoses on admission were found to be pulmonary tuberculosis (21%), malaria (14%), and diarrhea (12%) among adults, and non-tuberculosis pulmonary infection (21%), pulmonary tuberculosis (19%), and diarrhea (12%) among children. Furthermore, a single-day, point prevalence survey in September 2001 among the KCMC inpatients identified HIV infection in 21% of those surveyed. Many (44%) of the patients found testing positive were previously unaware of their infection. This finding pointed to the need for a policy of routinely offering HIV testing to hospital inpatients. The recommendation to offer routine, healthcare provider-initiated HIV testing was subsequently adopted by the WHO. Our findings of the early descriptive CHBC and KCMC morbidity and mortality studies informed much of our HIV co-infections research over the subsequent years.

Diarrheal disease was clearly a major contributor to HIV-associated illness and death among HIV-infected persons in both the community and the hospital in northern Tanzania. Waterborne enteric pathogens are known to contribute substantially to diarrheal disease in low-income countries. We conducted two studies in an area of high HIV seroprevalence in rural western Kenya to evaluate the impact of household-based water treatment on diarrheal disease illness and death. Persons living in the study area relied heavily on surface water as their primary source of drinking water. Surface water was often not only heavily contaminated with feces, but also highly turbid. Disinfection of drinking water with dilute sodium hypochlorite is used not only for water treatment in large, reticulated urban water supplies, but has also been proven to effectively render drinking water microbiologically safe and prevent diarrhea when used at the household level in developing countries. Unfortunately when sodium hypochlorite solution is added to highly turbid water it rapidly binds organic matter leaving little available to kill pathogens. The higher doses needed to disinfect turbid water may render the water safe to drink but the high chlorine levels adversely affect taste which may in turn diminish the willingness
of people to use the product. In the first study, we examined the performance of a novel combined flocculant-disinfectant household-based water treatment product compared with sodium hypochlorite and the locally used flocculant alum for both turbidity mitigation and disinfection. We showed that for source waters over a range of turbidities in western Kenya, the combined flocculant-disinfectant product effectively reduced turbidity to <5 nephelometric turbidity units and reduced *Escherichia coli* concentrations to <1 colony forming units per 100 mL, the WHO standards for drinking water quality.\textsuperscript{122} The second study was a cluster randomized, controlled trial of household-based flocculant-disinfectant drinking water treatment for diarrhea prevention in the setting of highly turbid source water in rural western Kenya.\textsuperscript{124} In this study 6,650 participants in 605 family compounds were randomized to receive either flocculant-disinfectant, sodium hypochlorite, or to continue usual water handling practice and were visited weekly for 20 weeks to assess diarrhea prevalence density and death. Among children <2 years old, compared with those in the control compounds, the absolute difference in prevalence of diarrhea was -25% in the flocculant-disinfectant arm (95% confidence interval -40 to -5) and -17% in the sodium hypochlorite arm (-34 to 4). In all age groups compared with control, the absolute difference in prevalence was -19% in the flocculant-disinfectant arm (-34 to -2) and -26% in the sodium hypochlorite arm (-39 to -9). There were significantly fewer deaths in the intervention compounds than in the control compounds (relative risk of death 0.58, \( P = 0.036 \)). Ours was the first study to demonstrate an impact of household-based water treatment on mortality as well as diarrhea. Household-based water treatment has become a routinely recommended component of integrated HIV care and treatment packages in sub-Saharan Africa.\textsuperscript{125}

Trimethoprim-sulfamethoxazole (SXT) has been shown to reduce hospitalization and death among HIV-infected patients not receiving ART in sub-Saharan Africa.\textsuperscript{80-83} Inexpensive and relatively safe, SXT and the related compound sulfadoxine-pyrimethamine play central roles in the management of...
common clinical syndromes in Africa. These drugs are frequently used to treat dysentery, lower respiratory tract infection, and fever in which *Shigella* spp., non-typhoidal *Salmonella enterica*, *Streptococcus pneumoniae*, and *Plasmodium* spp., respectively, play major roles. In 2000 when the World Health Organization (WHO) and the Joint United Nations Program on AIDS (UNAIDS) recommended the use of SXT prophylaxis for persons with symptomatic HIV disease or with CD4 T-lymphocyte counts (CD4 counts) <500 cells/mm$^3$ in Africa,$^{42}$ concern was raised that the widespread use of SXT may substantially increase the prevalence of antimicrobial resistance in common community-acquired pathogens. To understand the role that SXT prophylaxis might play in promoting antimicrobial resistance, we selected fecal *Escherichia coli* as an indicator organism for enteric pathogens. We then examined the hypothesis that initiation of SXT prophylaxis in HIV-infected individuals would lead to rapid and widespread resistance of fecal *E. coli* to SXT compared with HIV-infected and HIV-uninfected persons not receiving SXT. We showed that carriage of fecal *E. coli* nonsusceptible to SXT is common among HIV-uninfected persons and among HIV-infected patients before the commencement of SXT prophylaxis.$^{126}$ Furthermore, the initiation of SXT prophylaxis rapidly leads to further loss of susceptibility not only to SXT but also to other clinically important antimicrobial agents. These findings provide insights into the possible negative consequences of widespread use of life-extending SXT for HIV-infected individuals in Africa. It is notably that SXT prophylaxis appears to provide clinical benefit despite the high prevalence of SXT resistance among relevant pathogens,$^{127}$ presumably reflecting differences in minimum inhibitory concentration thresholds for prevention of infection compared with treating disease. Furthermore, 7 years on from our study the contribution of SXT prophylaxis relative to other uses of SXT and related drugs to SXT resistance at the population level among bacterial pathogens in Africa remains uncertain.
Tuberculosis is a major cause of HIV-associated illness and death in sub-Saharan Africa. A longstanding interest in tuberculosis in sub-Saharan Africa was sparked by my involvement with studies of tuberculosis molecular epidemiology in KwaZulu-Natal, South Africa in the mid-1990s. By combining tuberculosis subtyping by restriction fragment length polymorphism with field epidemiology at a district hospital, we were able to describe the importance of informal contacts in the community for tuberculosis transmission and to document nosocomial tuberculosis transmission. Patient encounters in London in the 1990s stimulated my interest in cryptic presentations of tuberculosis and disseminated tuberculosis in the context of HIV infection.

Disseminated tuberculosis is a major theme of this thesis. Our work on disseminated tuberculosis began in earnest at Duke University Medical Center (DUMC) in the United States in the late 1990s. We described 20 years of disseminated tuberculosis at DUMC, highlighting the role of HIV infection as a predisposing condition and the growing role of mycobacterial blood culture in diagnosis. Having described the growing role of mycobacterial blood culture in the diagnosis of disseminated tuberculosis at a tertiary referral center in the United States, we designed and implemented a study to examine the relative performance in terms of yield and time to positive of available blood culture systems for the detection of mycobacteremia. The resulting two-center study showed that the continuously monitored mycobacterial blood culture systems were as sensitive and faster for the detection of mycobacteremia than earlier manual systems. Because MAC is considerably more common than M. tuberculosis as a cause of mycobacteremia in the United States, this study focused on the performance of the systems for the detection of MAC bacteremia. There was a need to repeat the study in a setting where bacteremic disseminated tuberculosis was a greater public health problem.

When I moved to Tanzania in 2002 there was considerable interest in the diagnosis and management of disseminated tuberculosis. Earlier work showed
that the disease was a leading cause of hospitalization with fever in Dar es Salaam, Tanzania.\textsuperscript{135,136} We conducted a systematic review and meta-analysis that confirmed \textit{M. tuberculosis} as a leading cause of community-acquired bloodstream infection in sub-Saharan Africa, with most disease occurring in the context of HIV infection.\textsuperscript{67} After developing a full-service clinical microbiology laboratory on the KCMC campus, we were positioned to begin a program of clinical and laboratory research on disseminated tuberculosis. In two year-long studies, one among hospitalized febrile adolescents and adults\textsuperscript{119} and the other among infants and children,\textsuperscript{137} we confirmed that \textit{M. tuberculosis} was a leading cause of community-acquired bloodstream infection in the adolescents and adults group. Related work that is not part of this thesis confirmed that \textit{M. tuberculosis} bacteremia is uncommon or difficult to detect among infants and children.\textsuperscript{138,139} We also described the spectrum of invasive bacterial and fungal infections among both HIV-infected and HIV-uninfected inpatients beyond mycobacteremia. This research highlighted the role of cryptococcal disease, pneumococcal disease, and typhoid fever as causes of febrile illness among inpatients in northern Tanzania and led to the identification and official recognition of a novel non-tuberculous mycobacterium responsible for causing invasive mycobacterial disease in Africa.\textsuperscript{140,141} It also demonstrated the difficulty of diagnosing key febrile conditions without laboratory support.\textsuperscript{142,143} One notable finding of the research on invasive bacterial disease was that \textit{Salmonella} Typhi bloodstream infection was identified significantly less often among HIV-infected participants than HIV-uninfected participants, a surprising result that is nonetheless consistent with other research.\textsuperscript{67} This finding prompted an editorial\textsuperscript{144} that suggested that HIV-related immunosuppression could have blunted the febrile response in typhoid fever, resulting in patients with HIV and \textit{Salmonella} Typhi bacteremia being less likely to meet study eligibility criteria. Further work is needed to confirm and understand relationship between invasive \textit{Salmonella} infections, especially enteric fever, and HIV. The
findings on febrile illness underpin a stream of research that forms the basis of my current work, but that is not part of this thesis.

To improve the laboratory diagnosis of disseminated tuberculosis in Tanzania, we repeated and extended the mycobacteremia blood culture study done earlier in the United States. The Tanzania study showed no significant difference in yield but confirmed that the modern blood culture systems tended to have shorter times to positive than the older manual systems for *M. tuberculosis* bacteremia. We also described for the first time the magnitude of bacteremia in disseminated tuberculosis. The mean (range) magnitude of mycobacteremia was 30.0 (0.4, 90.0) CFU/mL and was correlated with the time to positivity in the continuously monitored blood culture system. Our prospective disseminated tuberculosis clinical study demonstrated that approximately half of patients with bacteremic disseminated tuberculosis died within one month of admission to hospital, even with provision of tuberculosis treatment and ART. Unfortunately, even in the continuously monitored blood culture systems, bottles flag positive with *M. tuberculosis* after a mean (range) time of 22.6 (9.4 to 37.5) days, so results are unlikely to influence clinical management decisions. We took two approaches to try to rapidly identify patients with disseminated tuberculosis for immediate treatment. The first focused on finding clinical predictors that might guide empiric treatment approaches. We showed that disseminated tuberculosis was associated with cough lasting >1 month, fever lasting >1 month, weight loss of >10%, lymphadenopathy, HIV infection, and lower CD4 cell count and total lymphocyte count. We then studied an *M. tuberculosis* NAAT following extraction of high volumes of peripheral whole blood to compensate for lower magnitude of mycobacteremia in some patients. Of 25 samples from patients with blood culture-confirmed *M. tuberculosis* bacteremia in this study, 9 (36.0%) were positive and 1 (1.5%) of 66 control samples was positive by NAAT. The NAAT shows promise, but future modifications should focus on improving sensitivity.
6.5. **Antiretroviral therapy**

The advent of provision of free ART in 2004 represented a major development in HIV care and treatment services in Tanzania. We sought to conduct research related to ART that would inform roll out of the national care and treatment program. In our first ART-related study, we studied the capacity of healthcare facilities in northern Tanzania to deliver HIV care and treatment services in 2004. Of 19 facilities surveyed, nine (47%) had staff trained to manage ART and three (16%) prescribed ART. Two (11%) offered CD4 counts, five (26%) offered liver function tests, 16 (84%) offered chest radiography, and 18 (95%) offered acid-fast sputum staining. Of 12 (67%) facilities offering outpatient HIV/AIDS services, 12 (100%) provided SXT to outpatients and six (50%) provided isoniazid (INH). All 19 (100%) facilities offered rapid HIV tests and full blood pictures. Overall in 2004, facilities needed strengthening to increase staff training in ART management and to implement INH for treatment of latent tuberculosis. Laboratory facilities for ART monitoring were inadequate, and outpatient ART was limited. The results of our study informed the national planning exercise on training and infrastructure development for HIV care and treatment programs in Tanzania.

Uptake of HIV counseling and testing, HIV care and treatment, and ART adherence is linked to awareness about ART or ART treatment literacy. To investigate HIV treatment literacy in northern Tanzania, we administered a standardized questionnaire to KIWAKKUKI HCT clients from November 2003 through May 2005. We showed that among northern Tanzania HCT clients, only 17% were aware of ART. Factors associated with low antiretroviral treatment literacy included having exchanged money or gifts for sex, living in rural areas, having more than 2 children, and having no more than a primary education. Previous HIV testing was associated with higher HIV treatment literacy. Our results supported a need to expand awareness of ART and highlighted the potential benefits of using HCT encounters as educational opportunities.
Our capacity survey in 2004 demonstrated that capability to measure CD4 count were limited among healthcare facilities in northern Tanzania. CD4 counts provide valuable information to inform decisions about when to initiate ART and about when ART might be failing. It was apparent that early ART roll out in much of Tanzania would take place prior to the widespread availability of flow cytometry to measure CD4 count. Since many clinicians would be relying on clinical evaluation of patients to make decisions about ART initiation, we evaluated the performance characteristics of WHO staging criteria, anthropometrics, and simple laboratory measurements for predicting CD4 count <200 cells/mm³ among HIV-infected patients in Tanzania. A total of 202 adults, diagnosed with HIV infection through community-based testing, underwent a detailed evaluation including staging history and examination, anthropometry, complete blood count, erythrocyte sedimentation rate (ESR), and CD4 count. Univariable analysis and recursive partitioning were used to identify characteristics associated with CD4 count <200 cells/mm³. Of 202 participants 109 (54%) had a CD4 count <200 cells/mm³. Characteristics most strongly associated with CD4 count <200 cells/mm³ (p-value <0.0001) were the presence of mucocutaneous manifestations of HIV disease (72% vs. 28%), lower total lymphocyte count (TLC) (median 1450 vs. 2200 cells/mm³), lower total white blood cell count (median 4200 vs. 5500 cells/mm³), and higher ESR (median 95 vs. 53 mm/h). In a partition tree model, TLC <1200 cells/mm³, ESR ≥120 mm/h, or the presence of mucocutaneous manifestations of HIV disease yielded a sensitivity of 0.85 and specificity of 0.63 for predicting CD4 count <200 cells/mm³. The sensitivity of the 2006 WHO Staging system improved from 0.75 to 0.93 with inclusion of these parameters, at the expense of specificity (0.36 to 0.26). The presence of mucocutaneous manifestations, TLC <1200 cells/mm³, or ESR ≥120 mm/h was a strong predictor of CD4 count <200 cells/mm³ and enhanced the sensitivity of the 2006 WHO staging criteria for identifying patients likely to benefit from ART. These findings were used to make ART initiation decisions at KCMC and elsewhere in northern Tanzania while flow cytometry instruments were installed and validated.

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ART was rolled out very rapidly in Tanzania, as it was in many countries in sub-Saharan Africa. ART program performance was measured initially in terms of the number of patients initiated and retained on therapy over time. However, the long-term success of an ART program is also closely related to patient outcomes. We sought to understand factors associated with ART adherence, virologic failure, and the development of antiviral drug-resistant infection in a cohort of HIV-infected persons. We studied 150 participants who had received ART for at least 6 months. Of participants, 23 (16%) reported incomplete adherence. Sacrificing health care for other necessities (adjusted odds ratio [aOR], 19.8; p<0.01) and the proportion of months receiving self-funded treatment (aOR, 23.5; p=0.04) were associated with incomplete adherence. Virologic failure was identified in 48 (32%) of 150 participants and was associated with incomplete adherence (aOR, 3.6; p=0.03) and the proportion of months receiving self-funded antiretroviral therapy (aOR, 13.0; p=0.02). Disclosure of HIV infection status to family members or others was protective against virologic failure (aOR, 0.10; p=0.04). Self-funded treatment was associated with incomplete adherence and virologic failure. Our findings confirmed that efforts to provide free antiretroviral therapy and to promote social coping might enhance adherence and reduce rates of virologic failure.

Recommendations on the use of ART were developed over more than a decade before their use in low-resource settings was widely considered possible. The application of ART in sub-Saharan Africa presented a number of unanswered questions related to drug selection and use that had not been addressed in the western ART research agenda. Chief among these was the selection and timing of ART in tuberculosis co-infected patients. Key issues include the best timing of initiation of ART relative to tuberculosis treatment to optimize survival while avoiding IRIS; managing overlapping toxicities of antituberculous drugs and antiretroviral medications; and interactions between rifampicin-based antituberculous regimens and NNRTIs and PIs. To contribute to resolving some of these questions, we conducted a randomized trial of early versus delayed ART with fixed dose combination...
abacavir/lamivudine/zidovudine (ABC/3TC/ZDV) among HIV-1 and *M. tuberculosis* co-infected patients. HIV-infected inpatients with smear positive tuberculosis and total lymphocyte count <1200/mm³ were randomized to initiate ABC/3TC/ZDV either 2 or 8 weeks after commencing antituberculous therapy and were followed for 104 weeks. Of 94 patients screened, 70 enrolled (41% female, median CD4 count 103 cells/mm³), and 33 in each group completed 104 weeks. Two deaths and 12 serious adverse events (SAEs) were observed in the early arm compared with one death, one clinical failure, and seven SAEs in the delayed arm (p=0.6012 for time to first grade 3 or 4 event, SAE, or death). CD4 cell increases were 331 and 328 cells/mm³, respectively. Tuberculosis-related IRIS was not observed in any participant. Using intent-to-treat (ITT), missing=failure analyses, 74% (26/35) versus 89% (31/35) randomized to early vs. delayed therapy had HIV RNA levels <400 copies/mL at 104 weeks (p=0.2182) and 66% (23/35) versus 74% (26/35), respectively, had HIV RNA levels <50 copies/mL (p=0.6026). Tuberculosis-related IRIS was not observed among the 70 co-infected participants beginning ART. We concluded that ABC/3TC/ZDV was well tolerated and resulted in steady immunologic improvement. Rates of virologic suppression were similar between early and delayed treatment strategies with triple nucleoside regimens when substitutions were allowed. This clinical trial was the first conducted by the KCMC-Duke University Collaboration and formed the basis for the subsequent successful competition by KCMC to become a site in the clinical trials networks on optimization of management of HIV infected adults (AIDS Clinical Trials Group; ACTG) and children (International Maternal Pediatric and Adolescent AIDS Clinical Trials group; IMPAACT). The ACTG and other groups have recently completed larger clinical trials that confirm and extend our findings.

6.6. Future directions

The work presented in this thesis represents a small contribution to the enormous and growing body of work on HIV prevention, treatment, and care in
sub-Saharan Africa. However, lines of investigation started through the thesis research are being developed in a number of directions to extend the work.

HCT services in sub-Saharan Africa have developed considerably from the concept of stationary testing facilities that we worked on initially. Ongoing and future research is focusing on delivering HCT in villages through mobile testing services\textsuperscript{157} and at the household level using door-to-door approaches.\textsuperscript{158,159} Work is needed to understand how to reach individuals and groups who have not accessed HCT services. Work on repeat HCT approaches has been informed by growing interest in ‘test and treat’ approaches to HIV control and elimination.\textsuperscript{160,161}

HIV CHBC remains an underutilized resource for improving HIV prevention and treatment. Designed during the pre-ART era, CHBC networks organized by volunteer workers exist in many countries in sub-Saharan Africa. CHBC programs have been shown to be excellent venues for delivery and monitoring of ART with high adherence,\textsuperscript{117,162} for reaching high-risk groups with HCT,\textsuperscript{159} and for delivery of a range of health services beyond those for HIV, including insecticide treated bed nets and household-based water treatment.\textsuperscript{125} Future work might include translational and implementation research linked to comprehensive CHBC roll out. However, Ministries of Health have not traditionally funded CHBC activities and further cost-effectiveness and policy research may be needed to make a case that funding CHBC programs is worthwhile.

Although HIV co-infections account for most illness and death among HIV-infected persons, over the past decade research has tended to focus much more on ART. There is great potential to make strides in improved prevention and management of HIV co-infections in sub-Saharan Africa. The place of SXT prophylaxis in the ART era in sub-Saharan Africa is incompletely understood. Research is needed to inform if and at what CD4 threshold it is safe to discontinue SXT once ART has been initiated. It would also be helpful to study the role of other antimicrobials, such as azithromycin, for preventing illness and
death among HIV infected patients who cannot tolerate SXT and in areas where resistance to SXT among relevant pathogens is common. Research on disseminated tuberculosis is needed on the place of empiric antituberculous treatment to avert early mortality. For early diagnosis of disseminated tuberculosis, *M. tuberculosis* NAATs show some promise and should be improved to increase sensitivity while maintaining specificity.

Future ART research for sub-Saharan Africa should include understanding the best choices for second- and third-line ART for patients in whom NNRTI-based first-line therapy has failed. Determining the role of genotypic resistance testing, if any, in regimen selection would be very helpful to inform policy. Research is needed on ART management in patients with HIV co-infections other than tuberculosis, particularly cryptococcal disease.

In the face of overwhelming health needs, a stretched health workforce, and limited facilities, it is essential that HIV prevention, treatment, and care research in sub-Saharan Africa stays closely attuned to the needs of patients and programs. Research activities must be closely integrated with service provision and training to ensure that patient care resources are used wisely and that the next generation of clinicians and researchers are trained and inspired.
7. REFERENCES


