THE IMPACT OF SEXUALLY TRANSMITTED INFECTIONS (STI) AND GENITAL TRACT INFLAMMATION ON HIV-1 ACQUISITION AND RATE OF DISEASE PROGRESSION IN SUBTYPE C INFECTED WOMEN.

Presented by

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AUTHOR'S DECLARATION

I, the undersigned, hereby declare that the work contained in this document is my own original work and that I have not previously, in its entirety or in part, submitted it at any university for a degree. The publications presented here originated from a cohort that I established, having led the clinical section of protocol writing as the protocol co-chair. As the Project Director, the study was run under my management.

This thesis constitutes work carried out by the candidate and complies with the stipulations set out for the degree of Doctor of Philosophy by research papers.

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ETHICS DECLARATION

The studies described in this thesis were approved by the Biomedical Research Ethics Committee of the University of KwaZulu Natal (E013/04).

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
CCR5	Chemokine receptor type 5
CD	Cluster of Differentiation
CI	Confidence Interval
DC	Dendritic cells
ELISA	Enzyme-linked immunosorbent assay
FSW	Female sex workers
gp	glycoprotein
HAART	Highly active antiretroviral therapy
HIV-1	Human Immunodeficiency Virus type 1
HSV-2	Herpes Simplex Virus Type 2
HLA	Human Leukocyte Antigen
HR	Hazard ratio
IFN-γ	Interferon gamma
IL	Interleukin
NAAT	Nucleic acid amplification test
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OR	Odds ratio
PCR	Polymerase Chain Reaction
POCT	Point of care testing
RNA	Ribonucleic acid
STI	Sexually Transmitted Infections
TNF-α	Tumour Necrosis Factor alpha
UNAIDS	The Joint United Nations Programme on HIV and AIDS
WHO	World Health Organisation

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Abstract

Introduction:

Women carry more than half the burden of HIV disease globally and this burden is even higher in sub-Saharan Africa (SSA). Young women, in particular, are at disproportionate risk of HIV infection in South Africa. Understanding risk behaviours and factors associated with ability to negotiate safe sex and condom use is one of the key elements in curbing the spread of HIV. Sexually transmitted infections (STI) and bacterial vaginosis (BV), which cause female genital tract inflammation, have been identified as key drivers of the HIV epidemic. This inflammation, which is also present in the absence of symptoms, is associated with increased susceptibility to HIV infection. Although syndromic management of symptomatic STIs or BV at the first encounter with a health care provider is an important public health measure, its effectiveness is minimised because a substantial proportion of individuals have either asymptomatic infections or fail to recognise signs and symptoms of STI and are therefore excluded.

Most new HIV infections in SSA occur among young people and particularly among young girls. Prompt diagnosis of acute HIV infection (AHI) is critical and benefits the individual as well as providing opportunities for public health intervention. In South Africa, the majority of HIV infections are due to infection with HIV-1 subtype C for which there is limited data compared to subtype-B HIV-1 infections.

The overall aim of this study was to assess the impact of BV, STIs, and associated genital tract inflammation on acquisition of HIV-1 subtype C infections; and evaluate the rate of subsequent disease progression in women.

The objectives were:

i. to investigate STIs and genital tract inflammation as risk factors for HIV infection in high risk women and adequacy of syndromic management;

ii. to evaluate the challenges associated with diagnosing recently acquired (acute) HIV infection in a subtype C prevalent population;

iii. and to evaluate the relationship between clinical disease progression and genital and or systemic inflammation in high-risk women who became infected with HIV.

We assessed the adequacy of syndromic diagnosis of STIs, compared with laboratory diagnosis of STIs, and evaluated the association between STI diagnosis and the risk of HIV acquisition in a cohort of high-risk women. Genital cytokine profiles and the degree of inflammation associated with common STIs and bacterial vaginosis were assessed. The most common signs and symptoms of acute HIV infection (AHI) were described and a clinical algorithm to identify acute HIV cases was developed. We investigated rates of HIV disease progression of subtype C–infected South African women.

Methods:

The CAPRISA 002 study was a prospective cohort study established to examine the pathogenesis and natural history of HIV-1 subtype C infection and to describe the immunologic, virologic and clinical characteristics of acute and early infection in KwaZulu Natal, South Africa. A total of 775 high-risk women were screened for HIV infection, and 245 HIV-uninfected women were enrolled into the study. At each monthly visit for a total of 24 months behavioural and clinical data were collected. Cervico-vaginal lavage (CVL) samples were collected at enrolment and at each six month follow-up visits and were tested for STI pathogens (including *Chlamydia trachomatis, Neisseria gonorrhoeae*, herpes simplex virus type 2 (HSV-2) and *Trichomonas vaginalis*) and bacterial vaginosis. Forty-two cytokines were measured from the CVL and 13 from the plasma samples at enrolment.

All women received monthly HIV-1 antibody and RNA testing and were assessed for AHI. Signs and symptoms at the visit with HIV-1 antibody or HIV-1 RNA positive test were compared to HIV negative visits. Logistic regression identified clinical predictors of AHI and a model-based score was assigned to each predictor to create a risk score for every woman. All women who seroconverted were followed up for more than five years and monitored for HIV disease progression. Rapid disease progression was defined as CD4+ T cell count decline to <350 cells/µl within two years post-infection. Serial clinical and laboratory assessments were compared using survival analysis and logistic regression models.

CAPRISA had established several cohorts of HIV negative women to determine the feasibility of establishing cohorts and sites for HIV biomedical prevention trials. Women seroconverting in these studies were referred to the CAPRISA 002 study for longitudinal follow-up for HIV post seroconversion.

Results:

In this study, the HIV-1 prevalence at screening was 59.6% (95% CI: 55.9% to 62.8%). During a total of 390 person-years of follow-up, 28 new infections occurred, yielding a sero-incidence rate of 7.2 (95% CI: 4.5 to 9.8) per 100 person-years. A total of 62 participants, including seroconvertors from other CAPRISA cohorts, were enrolled into the acute HIV infection Women from the HIV-1 negative cohort generally demonstrated a high level of knowledge on HIV/AIDS, and 60.3% reported use of condoms. Reported condom use at last sexual encounter varied slightly by partner type (57.0% with steady versus 64.4% with casual partners; $p = 0.36$), whilst self-perceived ability to choose to use a condom was significantly lower with steady partners compared to casual partners (20.8% versus 53.9%; $p=0.01$).

An important finding was that vaginal discharge was a poor predictor of laboratory-diagnosed STIs, as only 12.3% of women (25/204) who had a laboratory-confirmed discharge causing

pathogen had clinical evidence of a discharge (yielding a sensitivity of 12.3% and a specificity of 93.8%). CVL cytokine concentrations did not differ between women with asymptomatic or symptomatic STIs; and were elevated in women with either asymptomatic or symptomatic STIs compared to women with no STIs or BV. Women with chlamydia or gonorrhoea had the highest genital cytokine concentrations, with 17/42 and 14/42 of the cytokines measured in CVL being up-regulated compared with women with no infections, respectively. While BV was associated with elevated pro-inflammatory cytokine concentrations in CVL, women with BV had lower levels of chemokines and haematopoietic cytokines than women with no infections or BV. HSV-2 reactivation was inflammatory, but yielded a comparatively lower level of inflammation than Chlamydia or gonnorhoea. Trichomoniasis, despite being relatively common in this cohort, did not cause significant changes in genital tract cytokine concentrations compared to women with no infections or BV. Genital infections did not influence plasma cytokine concentrations, suggesting that the compartments were not linked with respect to cytokine responses. Although laboratory-diagnosed STIs were associated with increased risk of HIV infection [hazard ratio, 3.3 (95% confidence interval, 1.5 – 7.2)], clinical symptoms were not.

Of the women who became infected, factors predictive of AHI included age, 25 years (OR = 3.2; 1.4 – 7.1), rash (OR = 6.1; 2.4 –15.4), sore throat (OR = 2.7; 1.0 – 7.6), weight loss (OR = 4.4; 1.5 – 13.4), genital ulcers (OR = 8.0; 1.6 – 39.5) and vaginal discharge (OR = 5.4; 1.6 – 18.4). A risk score of 2 correctly predicted AHI in 50.0% of cases. The number of signs and symptoms correlated with higher HIV-1 RNA at diagnosis ($r = 0.63$; $p = 0.001$). The 62 acutely infected women were identified at a median of 42 days post-infection (IQR = 34 – 59). Mean CD4 count dropped by 39.6% at 3 months and 46.7% at 6 months post-infection in women with pre-infection measurements. CD4 decline to <350 cells/μL occurred in 31%, 44%, and 55% of women at 1, 2, and 3 years post-infection, respectively, and to <500

cells/μL in 69%, 79%, and 81% at equivalent time-points. Predictors of rapid progression were CD4 count at 3 months post-infection (hazard ratio [HR], 2.07; 95% confidence interval [CI], 1.31–3.28; P = .002), setpoint viral load (HR, 3.82; 95% CI, 1.51–9.67; P = .005), and hepatitis B coinfection (HR, 4.54; 95% CI, 1.31–15.69; P = .017). Conversely, presence of any of HLAB*1302, B*27, B*57, B*5801, or B*8101 alleles predicted non–rapid progression (HR, 0.19; 95% CI, .05–.74; P = .016).

Discussion/Conclusion:

This study showed that syndromic STI diagnosis, which is dependent on clinical signs of vaginal discharge, was poorly predictive of laboratory-diagnosed STIs or BV and missed a significant proportion of women with asymptomatic infections. However, the level of genital inflammation, as measured by cytokine concentrations in CVL, was similar in women with symptomatic and asymptomatic infections and therefore place women at increased risk for HIV infection. While laboratory-diagnosed STIs and the presence of inflammatory cytokines in the genital tract were associated with increased susceptibility to HIV acquisition, vaginal discharge was not. Chlamydial infection was associated with the highest genital cytokine concentrations, followed by gonorrhoea, HSV-2, trichomoniasis, and BV. In regions where HIV is prevalent and STIs are managed syndromically, targeted screening of populations at risk for STIs is critical and urgently needed.

Recognition of signs and symptoms of AHI is important for early diagnosis of HIV infection. The proposed algorithm of risk-stratifying individuals for AHI provides a useful clinical tool especially in resource-limited settings where there are limited or no routinely available tests for AHI. However, validation of the algorithm on another cohort is needed to assess its utility further. Point-of-care HIV antigen or viral load testing is needed, to detect asymptomatic, antibody negative cases enabling early interventions and prevention of transmission.

This cohort showed high rates of rapid disease progression, with nearly half of these subtype C–infected women progressing to a CD4+ T cell count of below 350 cells/μL within 2 years of infection. Implementing 2013 World Health Organization treatment guidelines (CD4+ T cell counts less than 500cells/μL) would require most individuals to start antiretroviral therapy within 1 year of HIV infection. The economic and health systems planning implicated by these findings need to be explored and addressed to guide policy makers as countries adopt the current WHO guidelines.

Chapter 1:

Introduction, Aims & Objectives and Thesis Framework

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1.1 Introduction, Aims and Objectives:

Sub-Saharan Africa continues to be the region most disproportionately affected by the HIV-1 epidemic, hosting more than half the globally infected population and home to 70% of all new HIV infections reported in 2012 [1]. Until recently, the natural history of HIV disease was based extensively on published data from subtype B infections most prevalent in better resourced countries, with very limited information being available from subtype C HIV infections, the commonest clade causing >95% of infections in Southern Africa [2, 3]. The Sub-Saharan African (SSA) epidemic is predominantly heterosexually transmitted with young women bearing the brunt of the disease. Even though the HIV prevalence amongst young men and women (18 – 24yrs) declined by 42% between 2001 and 2012 in SSA, prevalence remains more than twice as high in young women compared to men in this region [1]. This dissertation describes the establishment of a cohort of women from Durban, Kwa-Zulu Natal Province, South Africa at high-risk of HIV infection [2]. Women who self-identified as sex-workers and those who reported to have had more than 3 sex partners in the 3 months prior to enrolment, were recruited. In these women, clinical, behavioural, immunological and virological factors that influenced risk of HIV infection and subsequent disease progression were investigated and detailed studies of the clinical course of HIV infection were conducted. Prior to the data generated from the cohort described in this thesis, limited data existed on factors that influenced HIV-1 subtype C acquisition, establishment of viral set-point and impact of set-point on disease progression, if any, in the South African population.

The primary aim of this dissertation was to investigate the impact of sexually transmitted infections (STIs), bacterial vaginosis (BV) and genital tract inflammation on risk for HIV

acquisition and rate of disease progression in subtype C infected women. The specific objectives of the study were:

1. To investigate how STIs, BV, their clinical presentation and associated inflammation influenced the risk of HIV infection in women.
2. To define a clinical algorithm for diagnosing acute HIV infection in this population, by defining the acute seroconversion syndrome.
3. To describe clinical, immunological and genetic factors associated with HIV disease progression in this cohort.

1.2 Thesis Framework:

Chapter One: This chapter gives a brief introduction and rationale of the study, defining the aim and objectives. It also describes the framework of the thesis, summarising the objectives of each publication included in the thesis.

Chapter Two: This chapter contains the literature review that informs the conduct of this study. It describes the relative risk for HIV infection and HIV disease burden seen in young females of countries where HIV subtype C predominates. The challenges associated with diagnosing STIs and BV, especially in females in poorly resourced communities, are explored as well as the impact of genital tract inflammation linked to these genital infections on acquisition of HIV. Described herein, is the importance of and complexities in diagnosing HIV during acute infection, while these women have high viraemia and are at increased risk of transmitting the virus to their sexual partners. The natural history of HIV-1 infection, including an evaluation of the number of infectious virions required to establish an infection, is discussed as seen in various populations, as are known risk factors associated with rapid disease progression.

Chapter Three: This chapter contains the manuscript describing how the study cohort was established; the challenges associated with recruiting HIV negative cohorts in high prevalent settings and how this affects HIV prevention trials like HIV vaccines or microbicide studies (van Loggerenberg, Mlisana et al., PLoS One, 2009). This paper importantly sets the scene for the following manuscripts as it defines the methods used to enrol HIV-uninfected women, who were then followed longitudinally for HIV acquisition. A second manuscript defining the socio-behavioral characteristic of the cohort is included in this chapter (van Loggerenberg, et al., PLoS One 2012). All of the women who seroconverted in this study were confirmed to be infected with a subtype C strain of HIV.

Chapter Four: The poor performance of syndromic management of STIs and/or BV in women at high-risk of HIV infection is presented in this Chapter (Mlisana et al., Journal of Infectious Diseases, 2012). The current WHO recommendation of managing STIs syndromically excludes a significant percentage of individuals who do not have clinical signs or symptoms. This paper astutely demonstrates how, even though asymptomatic, these women have evidence of genital inflammation as shown by raised inflammatory cytokines in cervicovaginal lavages, and are at higher risk of HIV acquisition. This paper on the sensitivity of syndromic management to detect STIs or BV in a setting of high HIV risk attracted an editorial comment which is included in the thesis, highlighting the importance of the findings.

Chapter Five: This Chapter evaluated the genital cytokine profiles and the degree of inflammation associated with common STIs and BV (Masson, Mlisana et al., 2014). The genital cytokine profiles associated with distinct STIs and/or BV in women at risk of HIV infection are described. Asymptomatic genital tract infections were highly prevalent in this cohort, and were associated with significantly increased genital inflammatory cytokine concentrations, and a high HIV transmission or acquisition risk.

Chapter Six: The high prevalence and incidence of STIs and BV in this population increases the risk of HIV acquisition. As acute HIV infection (AHI) is associated with non-specific signs and symptoms, diagnosis poses a challenge. However, diagnosis of acute infection is critical as these individuals are at greater risk of transmission to their sexual partners because of the high viraemia associated with acute infection. In this chapter, a scoring system is defined for use in poorly resourced countries with limited laboratory resources (Mlisana et al., PLoS One, 2013). This clinical algorithm utilises commonly observed symptoms and signs at acute sero-conversion to identify clinical predictors of AHI using logistic regression analysis.

Chapter Seven: In this chapter, the relationship between genital inflammation during AHI and the subsequent rate of disease progression was evaluated (Roberts, Passmore, Mlisana, et al. Journal of Infectious Diseases, 2012). Elevated genital tract inflammatory cytokine concentrations during earlier HIV infection were associated with higher viral set-points and lower CD4 counts at 12 months post-infection, suggesting that genital inflammation around the time of infection may influence the rate of disease progression as well their risk of acquiring HIV in the first place. The mechanisms underlying this effect on disease progression is suggested to be increased HIV target cell availability at the genital mucosa during infection.

Chapter Eight: This chapter describes the rapidity with which disease progression occurred in this cohort of HIV infected women (Mlisana et al., 2014). The paper demonstrated how CD4 counts declined by 39.6% during the first 3 months of infection and 46.7% by 6 months post-infection in this cohort of women who became infected with HIV. About one-third of these women (30.6%) reached a CD4 count of <350 cells/µl within 6 – 12 months of infection, 43.5% within 24 months, and 54.8% within 36 months post-infection, highlighting how rapidly this cohort progressed and became eligible to start HAART according to our current South African national guidelines

Chapter Nine: This is the conclusion chapter which places the findings from this study in the broader context, highlighting the important findings, limitations of the study as well as recommendations for further development or policy changes.

1.3. Appendix 1:

All the manuscripts that have emanated from this cohort are listed in this appendix, highlighting the impact this AHI study has made and continues to make in advancing the body of HIV knowledge.

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Chapter 2:

Literature Review

Chapter 2: Literature Review

2.1 HIV-1 epidemic in sub-Saharan Africa

Sub-Saharan Africa (SSA) continues to be ravaged by the HIV epidemic despite significant achievements in prevention and understanding its pathogenesis over the past three decades. In 2012, an estimated 26.6 million HIV-1-infected individuals were in this region, which represents 67% of the global HIV burden and about 90% of the global infections in children under 15 years of age. In the same year, 1.8 million (67%) of the 2.7 million new HIV infections occurred in SSA [1]. Of the 1.9 million deaths estimated globally in 2012, 1.3 million (68%) occurred in SSA [1].

2.2 Disproportionately high rates of HIV in young women in SSA

Women continue to bear the brunt of HIV disease, with young women in particular being the most severely affected. [2]. In SSA approximately 60% of HIV-1-infected individuals are females [1] and young women (between the ages of 15 -24 years) are at >2-fold higher risk of infection compared to men in the same age group [1]. The peak prevalence of HIV in young women has typically been shown to occur 5 – 7 years earlier than their male counterparts [2, 3] (Figure 2.1). This disproportionate burden of HIV in women is likely to be linked to multiple factors, including gender disparities , cultural and sexual norms, intimate partner violence and abuse as well as poor socio-economic status seen in most African countries [4, 5]. Other factors thought to increase the risk of HIV infection amongst women in SSA include higher rates of sequential and concurrent sexual partnerships, vaginal insertion practices to enable dry sex by women and intergenerational sex [6-13]. The high

prevalence of HIV seen in adolescent girls and young women has been one of the significant factors that fuelled the epidemic in southern Africa [14-17].

Whilst the HIV epidemic in sub-Saharan Africa is generalised, pockets of high risk populations exist, as seen with high pooled prevalence rates of 36.9% in female sex workers (FSWs) [18] and 3.8 times more infections seen in men who have sex with men (MSM) compared to heterosexual men [19]. A recent meta-analyses of HIV burden in FSWs showed an odds ratio estimate of 13.5 (95% CI 10.0–18.1) for a FSW to be HIV positive compared to women in similar age groups from the general population in low and middle income countries [18]. In addition to increased risk for HIV infection, FSWs who had become HIV-infected were more likely to be infected with more than one HIV variant concurrently (dual infection) and were more likely to experience more rapid disease progression [20, 21]. Although the prevalence of HIV infection in FSWs varies geographically, the behavioural and biological risk factors are similar as evidenced by persistently high numbers of sexual partners and high prevalence of sexually transmitted infections (STIs) with the associated reproductive health complications [20, 21].

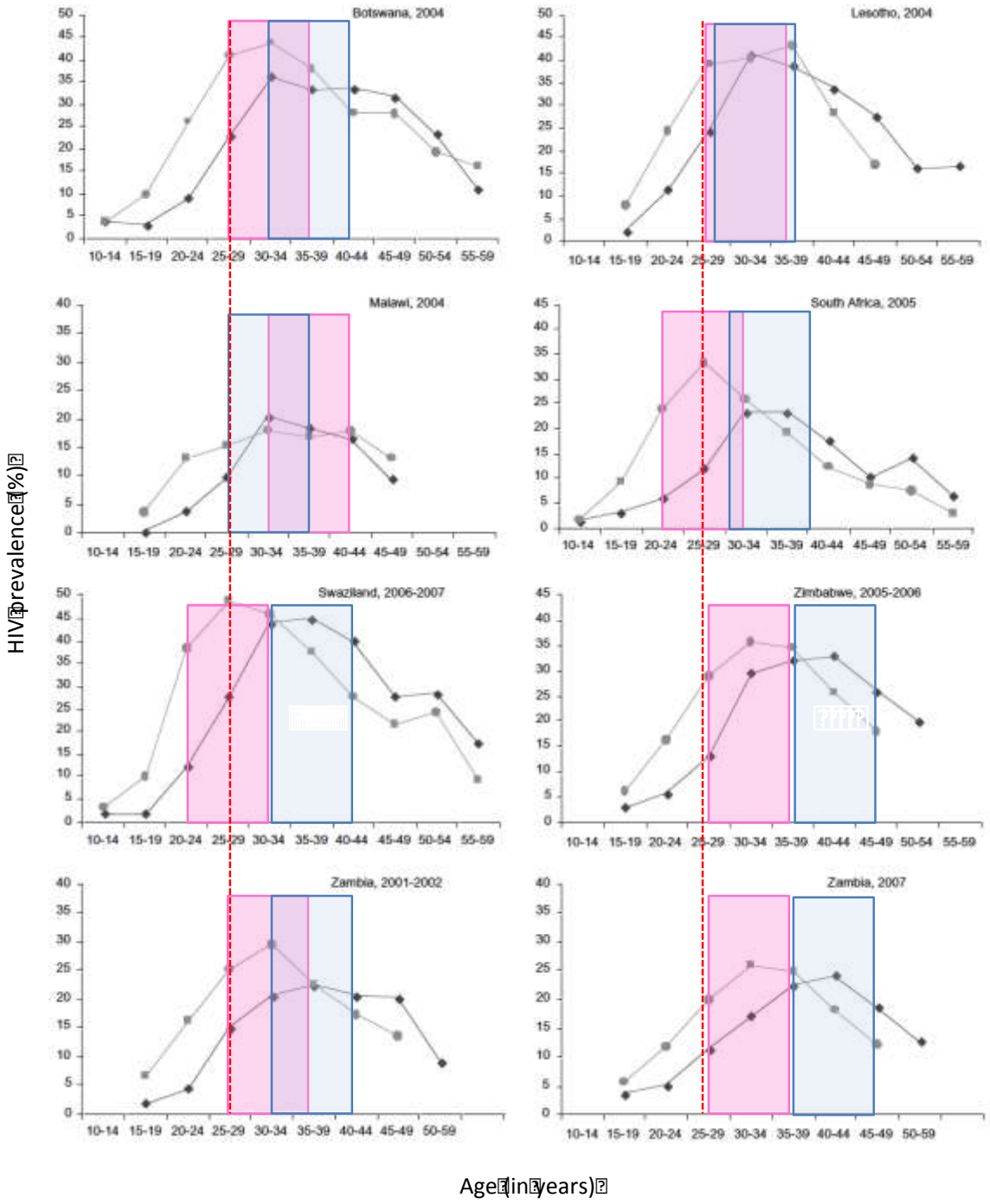

Figure 2.1. Age-specific HIV prevalence among men and women using data collected from national population based surveys in seven countries in southern Africa. Grey lines are for women and black lines for men. Pink and blue blocks indicate the age range of highest HIV burden in women and men per country, respectively. Red dotted lines indicate the peak prevalence for women living in South Africa (adapted from Gouws et al., 2008) [2].

2.3 Sexually transmitted infections (STIs), bacterial vaginosis (BV) and risk of HIV infection

Sexually transmitted infections (STIs) continue to present a major public health concern in both high and middle to low income countries, causing significant morbidity and mortality. In middle to low income countries, STIs and their complications are ranked in the top five disease categories for which adults seek health care [22]. BV, which causes the commonest type of vaginal discharge , results from an imbalance in the ecology of normal vaginal flora characterised by depletion of the hydrogen peroxide producing lactobacilli [23, 24]. Lactobacilli, by producing lactic acid, maintain the vaginal acidic pH which inhibits CD4+ T cell activation and therefore reduces the proliferation of HIV target cells in the vagina epithelium [25]. BV has been associated with an increased risk of HIV acquisition in HIV incidence studies with a risk rate of 1.61 (95 percent CI: 1.21, 2.13) and HIV seroprevalence ranging from was higher in most studies with BV positive women [26] The link between STIs and increased risk for HIV acquisition has been well described [26], with the mechanism for this increased risk for HIV infection facilitated through the disruption of genital epithelial barrier integrity (eg. genital ulcer diseases) or inflammatory modulation or changes to the epithelium [27]. Studies in various populations of both women and men of varying sexual orientations at risk for HIV infection have shown increased STI prevalence with concomitant high prevalence of HIV infection [28-30]. Presence of STIs increases HIV infectiousness and susceptibility with risk estimates ranging from 2 to 23.5 as observed in multiple studies from different continents [28].

STIs and BV increase the infectiousness of HIV-infected individuals and have been found to be the strongest determinate of the amount of virus being shed in genital secretions [31]. In the presence of STIs, localized genital inflammation is also known to significantly drive HIV shedding [32]. HIV viral load measurements in genital tract secretions from individuals

infected with an STI are higher than in persons without STIs, further increasing the risk of HIV transmission from an HIV-infected person to their uninfected partners [33, 34]. HIV levels in semen or genital secretions are directly linked to the number of white blood cells migrating to the genital tract [35]. The numbers of inflammatory cells in the genital tract have been shown to be directly proportional to HIV infectiousness [33]. Gonococcal and chlamydial infections have been associated with increased leucocyte migration, and high levels of HIV shedding. BV has also been associated with as high as six-fold increase in HIV shedding [36].

2.4 Syndromic management of STIs and BV in southern Africa

In SSA and other resource constrained settings within Africa and Asia, STIs are managed syndromically rather than by laboratory diagnosis of specific infections. Syndromic management is aimed at ensuring that patients presenting with signs and symptoms of STIs (commonly ulcers or genital discharge) receive high quality care at first point of contact without the delays of laboratory testing. This WHO approved approach has been simplified by use of easy-to-follow algorithms enabling primary healthcare nurses to manage these infections efficiently [37]. Syndromic management uses clinically distinct syndromes commonly associated with specific causative agents of STIs. Patient management includes therapeutic management for most pathogens producing the specific syndrome including education and counselling as well as condom provision and partner referral. Whilst clinical sign and symptoms of genital ulcers and urethritis in men with STIs are relatively straightforward and easily identified by patients, however, genital discharge in females is complicated by the fact that pathogens causing vaginal discharges differ from those that cause a cervical discharge higher up in the reproductive tract, and often discharges are undifferentiated by women from what they consider to be “normal” discharge. Furthermore many women remain asymptomatic further complicating the management of STIs. A

speculum examination, which is not readily available in resource constrained health settings, is necessary for diagnosis of cervical discharge in order to distinguish between cervical and vaginal discharge. A meta-analyses of syndromic management studies showed that algorithms for the diagnosis and treatment of urethral discharge and genital ulcer disease in men performed better than those for women, with sensitivities or cure rates in men ranging from 87 to 99% for urethral discharge and 68 to 98% for genital ulcer disease [38]. The sensitivities for vaginal discharge algorithms ranged from 73 to93% among symptomatic women and much less in asymptomatic women (29-86%) [38]. For syndromic management to be effective, it relies on an individual or health care provider noting the presence of symptoms of an STI (discharge and/or ulcers) as well as patients presenting to a health care facility. A recent South African study showed a statistically significant increase in reporting of STIs by young HIV infected females compared to males [39]. Thus, early detection and appropriate management of STIs is of significant public health benefit and potentially impacting on HIV transmission and acquisition.

By definition, syndromic management relies on the presence of symptoms of an STI. However, many STIs are asymptomatic with these asymptomatic infections being highly prevalent in communities with high HIV prevalence [39-41]. In a cohort study of MSM asymptomatic STI prevalence was around 14% with more than 20 incident cases per 100 person years of follow-up [41]. The associated risk factors in this cohort included young age, higher CD4 cell counts and the recreational use of marijuana. Whilst some genital ulcers may be missed, the highest occurrence of asymptomatic infections are seen in genital discharges with rates greater than 75% in females [42]. It has been demonstrated that even when STIs present asymptomatically, they continue to have serious health consequences. Undetected gonococcal and chlamydial infections in females, which would therefore be left untreated, increase the risk of pelvic inflammatory diseases (PIDs) and consequently risk for

ectopic pregnancies and infertility [43-45]. In addition to causing symptoms, like genital ulceration or discharge which increase susceptibility to HIV infection, STIs may be associated with sub-clinical manifestations, including up-regulated genital tract inflammatory cytokine responses [46, 47]. STIs are major causes of inflammatory cytokine upregulation and immune cell recruitment to the genital mucosa [48, 49].

2.5 Alternatives to syndromic management of STIs and BV

In comparison to syndromic management of STIs, aetiological diagnosis of STIs requires expensive laboratory equipment and well trained staff. Additionally, laboratory testing takes time to be performed and thus delays in the diagnosis and treatment of infected individuals [37, 50]. Since laboratory testing does not offer immediate results, transmission of STIs may persist while patients wait for their test results, and many patients do not return to the clinic for their results and remain untreated. In African studies of HIV testing, the return rate has been found to be as low as 37% in some areas [51].

Rapid point-of-care (POC) tests have been under development for several years and have been successfully used for HIV and syphilis diagnosis. The Chlamydia Rapid Test (CRT) for *Chlamydia trachomatis* has shown promise in a single study, with a sensitivity of 80% and specificity of 99% compared to nucleic acid amplification (NAAT) testing technology [52]. However, in another study, the CRT had a sensitivity of only 41% [53]. Rapid NAATs combining both chlamydia and gonorrhoea testing have also been developed and show comparable predictive value to laboratory NAATs [54]. Very recently, a highly sensitive and specific rapid NAAT test for *Trichomonas vaginalis* was also been described [55]. However these tests require an onsite GeneXpert Platform or PCR machine and may have cost implications and affordability for implementation in resource limited settings.

2.6 Population wide STI treatment as a tool to reduce HIV infection

Several strategies for enhanced STI management, using population-wide syndromic management algorithms, have been tested to determine its effectiveness in reducing HIV acquisition. However, with the exception of one successful syndromic management trial, these population-wide interventions (in which either improved syndromic management strategies were implemented or mass treatment of bacterial infections or therapy for HSV-2 were performed) were found not to be effective in reducing the rate of new HIV infections [17, 56-60]. In the first randomized controlled trial undertaken in Mwanza, Tanzania HIV incidence was reduced by almost 40% in communities randomized to receive enhanced syndromic treatment for STIs [60, 61], suggesting that intensified STI detection and treatment strategies could prevent HIV infection acquisition. In this trial, more than 12 thousand individuals from 12 communities were included, and followed for 2 years. Six of the intervention communities received enhanced STI syndromic management through active training of existing staff from local healthcare facilities in syndromic diagnosis and treatment of STIs; regular supply of drugs; regular supervisor visits and health education about STIs. The remaining 6 communities were part of the control group receiving the standard of care. At two year follow-up, the rate of HIV infection was significantly lower in the intervention group compared to the control group (risk ratio: 0.58, 95% CI 0.42-0.79, p= 0.007) [60]. However, there were no differences in chlamydia and gonorrhoea infection rates among men; and STIs among pregnant women were unchanged, although the prevalence of newly-diagnosed syphilis and male urethritis were lower in the intervention group [56].

Despite the promising results of the Mwanza trial, the two STI syndromic management interventions, implemented in Uganda and Zimbabwe, did not impact the rate of HIV acquisition [60, 62-64]. As a result, a growing skepticism about the value of STI treatment as an HIV prevention strategy currently prevails [65] whilst the importance of managing STIs

effectively cannot be underestimated. In Masaka, Uganda, nearly 100 thousand individuals were recruited from 18 communities and followed for 6 years between 1994 and 2000. The intervention in Uganda was similar to that in Tanzania and included training of health care workers in syndromic management of STIs, regular supply of drugs and supervision and health education about STIs. Although syndromic management reduced the incidence of active syphilis and gonorrhoea in this study, the incidence of chlamydia, genital ulcers, discharge and HIV-1 were unchanged [63]. Similarly, in Zimbabwe in which nearly 12 thousand participants were included, implementation of syndromic management of STIs was not associated with reduced incidence of self-reported STI symptoms or HIV incidence [64].

In an effort to improve on current syndromic STI treatment strategies, RCTs of periodic mass treatment of bacterial STIs and treatment for HSV-2 infection, however, did not demonstrate the effectiveness of these strategies in reducing HIV acquisition rates. In Rakai, Uganda, antimicrobial treatment of STIs resulted in only a modest decline in the prevalence of some infections, and all STIs and BV were persistent and the prevalence of symptoms of STIs was similar in both the treatment and control groups [56]. It has been suggested that persistence of BV and STIs may be due to recurrence [66] or re-infection by partners outside of the intervention communities. Monthly antimicrobial treatment of bacterial STIs in Kenya resulted in a more substantial reduction in the prevalence of STIs, but did not influence HIV-1 acquisition. HIV-1 infection was found to be associated with preceding *N. gonorrhoea* and *C. trachomatis* infections [57]. Treatment for HSV-2, which was highly prevalent in the participants of these studies, was not included in either the Uganda or the Kenya interventions and may have influenced HIV acquisition in these cohorts.

The conflicting results of these trials might be explained by various factors. The population characteristics varied substantially. At the time of conduct of the trials, the prevalence of HIV-1 in Tanzania was lower than the prevalence in Uganda, therefore the proportion of new

HIV- infections attributable to STIs may be lower in a more mature HIV epidemic, such as in Uganda [63, 67]. The Masaka trial was introduced at a more advanced stage in the Ugandan epidemic, when substantial behaviour change had already occurred, which could have accounted for marginal or no effect of STI treatment on HIV incidence [63]. The higher prevalence of HSV-2 in Uganda may also have reduced the effect of STI treatment since acyclovir was not included in the treatment regimen [62, 63]. More intensive STI interventions are likely to be necessary to achieve a more substantial reduction in STI and HIV-1 infection rates, perhaps integration of both syndromic management strategies and mass treatment targeting both bacterial and viral STIs.

More recently, Johnson et al. [67] modelled that 39% of heterosexually transmitted HIV infections in the early stages of South Africa's epidemic could be attributed to STIs; but that this has declined to 14% over time, partly as a result of increases in condom use and introduction of syndromic management protocols in the mid-1990's, which substantially reduced the prevalence of curable STIs. In women, the proportion of HIV infections attributable to STIs was higher, with an estimated 50% of new HIV infections due to other STIs in 2010 [67]. In South Africa, syndromic management was estimated to have reduced adult HIV incidence by more than 6% between 1994 and 2004 [67].

2.7 Female genital tract inflammation, inflammatory cytokines and their role in STIs and risk of HIV acquisition

There is a considerable heterogeneity in an individual's susceptibility to HIV infection during sexual transmission with some individuals remaining uninfected despite high levels of exposure [68]. The stratified epithelium of the female genital tract mucosa appears to provide an effective natural barrier to HIV infection, although this barrier is thought to be weakened by the presence of STIs which cause physical breaks (ulcers) in the barrier, or inflammation

[69]. Inflammation in the female genital tract has been identified as an important factor influencing risk of sexual HIV transmission and acquisition. Generally, inflammation is an essential innate process for control and/or clearance of infections but also an important mediator of tissue destruction [70, 71], and increased susceptibility to HIV infection [68, 72]. As part of this process, cytokines and chemokines, which are the main initiators of the inflammatory response, recruit immune cells from systemic circulation and activate these cells [73, 74]. Inflammatory cytokines that are involved in immune cell recruitment to the genital tract, or activation may increase risk for HIV infection, as HIV replication depends on the presence and density of immune cell targets, and the level of immune cell activation [75-78]. Although the inflammatory response to tissue injury is important for STI clearance, destruction of infected epithelial layers occurs in the process, allowing STI-associated microbes to access deeper tissues but also easier passage for HIV across the mucosa. This results in an influx of highly-activated HIV target cells to the genital mucosa and the presence of a generalized pro-inflammatory milieu which is more conducive for HIV acquisition and transmission [79].

2.8 HIV transmission, acute HIV infection and natural history of infection

HIV is transmitted through bodily fluids including semen, vaginal secretions; across the stratified squamous epithelium of the lower genital tract and the simple columnar epithelium of the endocervical canal, the transformation zone in females and the urethral simple epithelium, glans penis or foreskin in males [80, 81]. In females, CD4+ T cells and DC targets of HIV may migrate into the vaginal and ectocervical epithelium [82-84]. The main receptor that is used by HIV for host cell entry is the CD4+ T cell molecule [85]. All studies on sexual transmission have shown that new infections are established by viruses that use the CCR5 co-receptor for cell entry [86, 87]. Following genital mucosal transmission, experimental studies in macaques have shown that the simian immunodeficiency virus (SIV)

remains localized around the mucosa for the first 6 days before a productive systemic infection is established [88, 89].

Following infection, the clinical course of HIV infection has broadly been divided into three phases: the acute phase, chronic (asymptomatic) phase and the symptomatic phase of infection. A more detailed staging algorithm based on Feibig criteria has been used to further classify the acute (early) phases of HIV infection [90]. Fiebig staging proposes that the acute phase of HIV infection is further divided into 6 stages based on the emergence of host immune markers [91]. These stages are further defined in Figure 2.2.

The *acute phase* of HIV infection is characterized by rapid growth and spread of the virus within the host cell lymphoid tissues in the absence of host adaptive immunity, which leads to high titres of HIV virus in plasma and substantial loss of CD4+ T cells in the blood. At this stage, viral RNA can be detected in blood and corresponds to Fiebig stage I. During this early stage of infection, HIV spreads to the lymphoid tissues and viral reservoirs are established throughout the body. HIV p24 (making up the viral capsid) can be detected in plasma at approximately day seven of infection although host immunity cannot yet be measured (corresponding to Fiebig stage II). After this, an expanded population of HIV-1 specific cytotoxic CD8+ T cells (CTLs) and HIV-specific binding antibodies develop and are measurable. Emergence of these HIV-specific adaptive immune responses have been implicated in bringing viral replication under control, and lowering the viral load to a steady set point [92]. At this stage, HIV gp120 and gp41 antibodies can be detected by ELISA (corresponding to Fiebig stage III). Fiebig stage IV (antibody positive by ELISA but negative by western blot), stage V (antibody positive by ELISA and western blot) and stage VI (ELISA positive; western blot positive, p31 antigen positive using ELISA or western blot) follow.

The *asymptomatic or chronic phase* of HIV infection is characterized by the establishment of viral "set point", as a result of emerging host adaptive immune responses. Viral "set point" represents the sum of ongoing viral replication in the presence of this immunity and the ability of the host's immunity to retard viral replication. The duration of this asymptomatic or chronic phase of infection varies from person to person and from country to country, with typical duration ranging from 5-15 years. Despite host adaptive immune responses against HIV, a steady yet slow decrease in CD4+ T cell numbers continues during chronic infection, ultimately resulting in exhaustion of both CD4+ and CD8+ T cells [93]. Although the typical duration of this asymptomatic phase of infection is <10 years, some HIV-infected individuals may have no AIDS-defining symptoms for longer than 10 years in the absence of treatment[94].

The ***symptomatic phase*** or acquired immunodeficiency syndrome (AIDS) is characterized by a rapid decrease of CD4+ T cells, corresponding to a decline in HIV-specific T cell responses and rapidly increasing viraemia. In the absence of HAART, opportunistic infections usually appear leading to AIDS and death due to the collapse of host immunity [95] especially once CD4+ T cell counts decline to levels below 200 cells/ml blood. Since the introduction of anti-retroviral therapy, the number of HIV-infected individuals who progress to this stage of infection is on the decline as more individuals improve CD4+ T cell counts improve with HAART. Although 61% of individuals who need HAART are currently receiving treatment, and HAART rollout is increasing annually, most recent statistics report that 1.6 million people died of AIDS in 2012 [1].

In South Africa, 200 000 HIV-infected individuals died of AIDS in 2010 (UNAIDS, 2011). South African National Guidelines on antiretroviral therapy stipulate initiation of treatment once CD4+ T cell counts drop below 350 cells/ml except for pregnant women and TB

infected patients who are initiated on HAART irrespective of their CD4+ T cell counts (The South African Antiretroviral Treatment Guidelines, 2013).

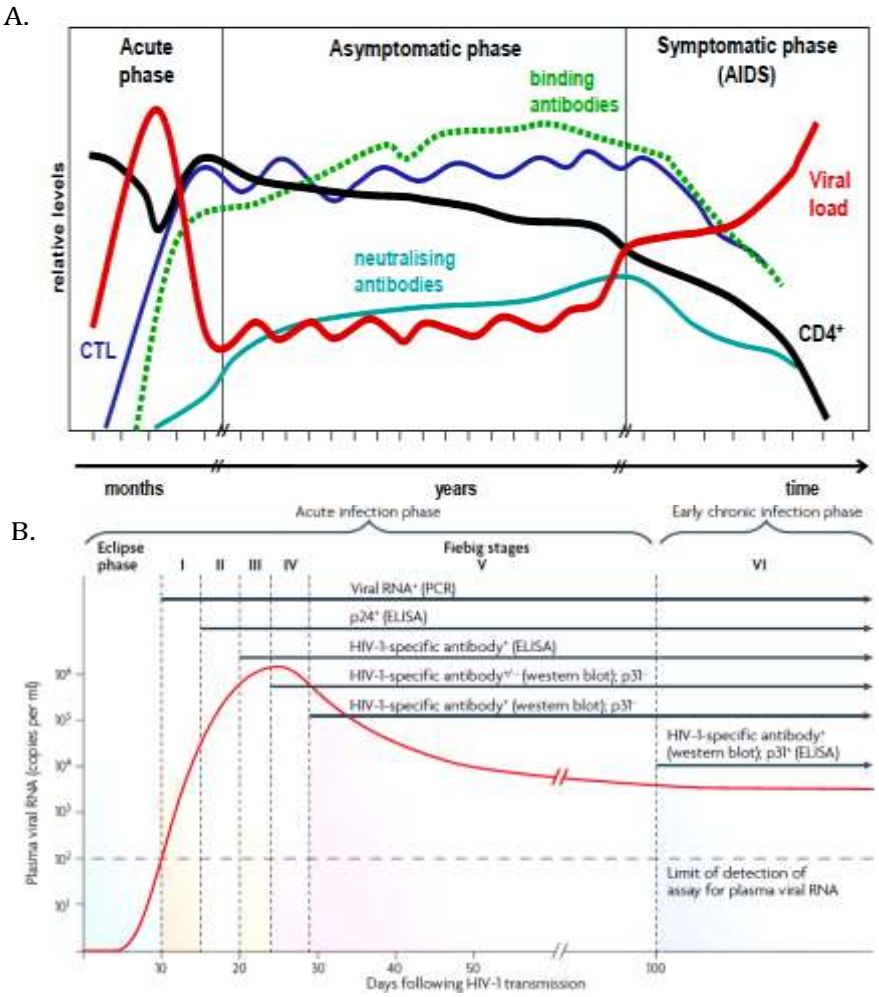

Figure 2.2. Typical clinical courses of HIV infection in humans. (A) Clinical course has broadly been divided into three phases: acute, asymptomatic and symptomatic infection phases. CD4+ T cell number (black line) drastically decreases during acute phase, rebounds slightly during the asymptomatic infection phase when host immunity emerges, achieving a relatively steady state. Viral loads (red line) increase during acute infection phase in the absence of host immunity, and decrease sharply when HIV-specific CTLs (blue line) and antibody responses emerge (green and turquoise lines, respectively). (B) Acute infection phase has further been divided into six Fiebig stages (I-VI) based on the detection of HIV-1 RNA, viral antigens and emergence of host immunity [96]

2.9 Importance of diagnosing acute HIV infection

The most common signs and symptoms of an acute HIV-1 infection include fever, fatique, morbilliform rash, headache, lymphadenopathy, pharyngitis, myalgia, arthralgia, aseptic meningitis, retro-orbital pain, weight loss, depression, gastrointestinal distress, night sweats and oral or genital ulcers [97, 98] . A maculopapular or morbilliform rash occurs in 40 to 80 percent of patients with symptomatic acute HIV-1 infection [97]. These signs and symptoms appear within days to weeks after initial exposure and may last from a few days to more than 10 days. Individuals in sero-discordant sexual relationships will have exposure to a person with known or possible HIV-1 infection and are presenting with signs and symptoms consistent with the diagnosis and history of exposure require an evaluation for acute HIV-1 infection. The risk of transmission appears to be much higher in patients with acute or early HIV infection compared to those with chronic and established infection [99]. This is partly due to the high viraemia associated with acute infection. As the probability (per person) of transmitting HIV-1 is closely associated with the viral burden in circulation, whenever there is a 10-fold increase in the viral load, the risk of transmission increases 2.5-fold [100]. During this early phase, the virus replicates rapidly unhindered by the immune responses, which are often delayed, reaching peak vireamic levels of >10 million copies/ml [101, 102].

Populations with relatively recently introduced HIV-1 epidemics attribute most of their recent infections to transmissions from recently infected individuals, i.e. there are higher rates of forward transmission events with acute or early infections [103]. Socio-behavioural patterns play a critical role in the risk of transmission as higher rates of changes in sexual partners increase the risk of encountering a partner with acute HIV-1 infection. Multiple concurrent sexual partners also increase the transmission risk. A recent comprehensive study conducted in Lilongwe, Malawi, using both behavioural and biologic data, ascribed 38% of HIV-1 cases to sexual exposure to individuals infected within 5 months [103], despite the

fact Malawi has a long-established epidemic. Studies in phylogenetically related isolates have further underscored the importance of acute HIV-1 infection by demonstrating that more than half of the individuals with newly diagnosed early HIV-1 infection in Montreal were infected with variants linkable through phylogenetic studies, suggesting the presence of transmission clusters, possibly due to individuals with acute and early infection [103].

2.10 Factors influencing the rate of HIV disease progression

It has been established that viral, immunologic and host factors generally influences the course of HIV-1 infection. In the transition from acute to early chronic HIV infection, the high viraemia observed during seroconversion declines to a virologic set-point which has been described as being predictive of the rate of disease progression [21, 104, 105]. This virologic set-point is determined by host factors that modulate the course of HIV disease. The CD4+ T cell count is the most significant predictor of disease progression and patient survival [106-108]. Whilst the direct cytopathic effect of HIV-1 on CD4+ T cells contributes to the gradual depletion of these cells, there is enough evidence to show that there are other indirect effects of viral replication that facilitate this progressive depletion [109-111].

Immune activation during HIV infection has been identified as a major contributor to HIV disease progression, and is the product of inflammatory responses to HIV-encoded Toll-like Receptor (TLR) ligands, microbial translocation and the homeostatic response to CD4+ T cell depletion [112, 113]. Cytokine profiles and mucosal inflammation in the genital tract during acute HIV infection are associated with lower CD4+ T cell counts and may thereby impact disease progression. We have previously shown that elevated levels of IL-1, IL-6, and IL-8 in the genital tract correlated with lower systemic CD4+ T cell counts during acute HIV infection [114]. A panel of HLA class 1 haplotypes has been demonstrated to be associated with different rates of HIV-1 disease progression [115, 116]. HLA* B35, B8, B45, and B53

have been associated with rapid progression, whereas HLA* B5701, HLA* B5703 and B27 are strongly linked with delayed progression [117-119].

The infecting HIV clade has also been associated with varying rates of disease progression. A study in East Africa showed that subtype D infected individuals had a shorter median to AIDS progression compared to subtype A infected [120-122]. Data from sub-Saharan Africa suggest rapid disease progression in this subtype C populated population [123].

This study was therefore undertaken to investigate the impact of sexually transmitted infections (STIs), bacterial vaginosis (BV) and genital tract inflammation on risk for HIV acquisition and rate of disease progression in subtype C infected women. The clinical presentation of these STIs, including BV, their associated inflammation and how these influence the risk of HIV infection in high risk women was studied. The research presented also describes the clinical symptoms in acute HIV subtype C infection, which has been proposed to be useful to develop a clinical algorithm for rapidly diagnosing acute HIV infection in this population, by defining the acute HIV seroconversion syndrome. To further understand the long term trajectory of HIV subtype C infection, the clinical, immunological and genetic factors associated with disease progression in this cohort have been clearly described.

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Chapter 3:

3.1 Establishing a Cohort at High Risk of HIV Infection in South Africa: Challenges and Experiences of the CAPRISA 002 Acute Infection Study.
Van *Loggerenberg, Francois, Koleka Mlisana, Carolyn Williamson, Sara C. Auld, Lynn Morris, Clive M. Gray, Quarraisha Abdool Karim et al" PloS one 3, no. 4 (2008): e1954.*

3.2 HIV Prevention in High risk Women in South Africa: Condom Use and the Need for Change.
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Establishing a Cohort at High Risk of HIV Infection in South Africa: Challenges and Experiences of the CAPRISA 002 Acute Infection Study

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Abstract

Objectives: To describe the baseline demographic data, clinical characteristics and HIV-incidence rates of a cohort at high risk for HIV infection in South Africa as well as the challenges experienced in establishing and maintaining the cohort.

Methodology/Principle Findings: Between August 2004 and May 2005 a cohort of HIV-uninfected women was established for the CAPRISA 002 Acute Infection Study, a natural history study of HIV-1 subtype C infection. Volunteers were identified through peer-outreach. The cohort was followed monthly to determine HIV infection rates and clinical presentation of early HIV infection. Risk reduction counselling and male and female condoms were provided. After screening 775 individuals, a cohort of 245 uninfected high-risk women was established. HIV-prevalence at screening was 59.6% (95% CI: 55.9% to 62.8%) posing a challenge in accruing HIV-uninfected women. The majority of women (78.8%) were self-identified as sex-workers with a median of 2 clients per day. Most women (95%) reported more than one casual sexual partner in the previous 3 months (excluding clients) and 58.8% reported condom use in their last sexual encounter. Based on laboratory testing, 62.0% had a sexually transmitted infection at baseline. During 390 person-years of follow-up, 28 infections occurred yielding seroincidence rate of 7.2 (95% CI: 4.5 to 9.8) per 100 person-years. Despite the high mobility of this sex worker cohort retention rate after 2 years was 86.1%. High co-morbidity created challenges for ancillary care provision, both in terms of human and financial resources.

Conclusions/Significance: Challenges experienced were high baseline HIV-prevalence, lower than anticipated HIV-incidence and difficulties retaining participants. Despite challenges, we have successfully accrued this cohort of HIV-uninfected women with favourable retention, enabling us to study the natural history of HIV-1 during acute HIV-infection. Our experiences provide lessons for others establishing similar cohorts, which will be key for advancing the vaccine and prevention research agenda in resource-constrained settings.

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Introduction

As we move into the third decade of the AIDS epidemic, the need to increase and improve the research being conducted in some of the hardest hit areas of the developing world has become increasingly clear. These regions pose unique challenges for scientific research as they are typically resource-poor settings with minimal existing infrastructure and populations that are not well integrated into a formal health care system. In order to conduct effective research into the HIV epidemic in southern Africa, it is critical to improve our ability to maintain longitudinal cohorts in the face of significant social, cultural, and logistical hurdles.

In order to determine which host and viral factors during the acute and early phases of HIV-1 infection have a significant impact on the subsequent course of disease, the Centre for the AIDS Programme of Research in South Africa (CAPRISA) initiated the CAPRISA 002 Acute Infection Study. This study will characterize HIV-1 subtype C viral load set point in heterosexual infection in South Africa, the role of specific T cell immune responses in the control of replication, and the relationship between viral set point, CD4+ T cell count trajectory and disease progression.

The design of this study overcomes several limitations of previous acute infection studies. While several earlier studies used

a large window period for identification of acute infection [1–5], others relied on patient recall of symptoms associated with acute retroviral syndrome [6]. Still other studies focused on one particular aspect of acute HIV infection, such as clinical signs and symptoms [7], without placing the clinical presentation in the context of virological and immunological responses. Furthermore, the majority of these studies were conducted in developed countries where both the context for the research and the viral subtypes are markedly different from those that are found in southern Africa and the developing world. The only prospective acute infection study that was conducted in Africa identified acute infections within a period averaging 1.1 months but was limited by its focus on clinical manifestations of acute infection [8]. As a prospective observational cohort study, this study will also provide a methodology for setting up and managing an observational cohort requiring frequent visits over an extended period of time against within a very challenging, resource-constrained environment with participants who tend to be highly mobile and difficult to contact by conventional means.

To our knowledge, this acute infection study will be the first in southern Africa to document acute infection in a prospective cohort with extensive follow-up on the natural history of HIV-1 subtype C infection. The aim of this paper is to provide a preliminary description of the demographic and clinical characteristics of the cohort of HIV-uninfected participants, the challenges in administering the cohort, and some of the successful strategies employed to overcome these obstacles. This paper provides important information with respect to the establishment of high-risk cohorts for acute HIV infection and other prospective observational studies. Establishment of cohorts in developing country setting have unique challenges which differ between locations and populations. Our cohort consists primarily of high risk HIV-uninfected women working as female sex workers, recruited from a large urban area. This cohort is of high potential impact as forms part of a larger acute infection study which measures virological, immunological and clinical events in acute and early infection. Results from this study are likely to provide valuable information to inform vaccine trials, as well as understanding host, immunological and virological correlates of disease progression in subtype C infection. This paper provides important information with respect to the establishment of high-risk cohorts for acute HIV infection and other prospective observational studies.

Materials and Methods

The CAPRISA 002 Acute Infection Study is being conducted at the Doris Duke Medical Research Institute (DDMRI) at the Nelson R Mandela School of Medicine of the University of KwaZulu-Natal in Durban, South Africa. The goal of this study is to identify acute HIV infection and, by prospectively following participants with acute infection, provide thorough information on the natural history of HIV-1 subtype C infection.

Cohort Development

Building on participatory research methods developed for earlier work in similar cohorts [9–11], a network of ten community liaison persons (CLPs) was established prior to initiation of the study to assist with study recruitment and retention efforts. Recruitment was based upon word-of-mouth and site visits by the CLPs. Initially we recruited directly within 5 kilometres of the clinic site, but CLPs did bring women in from as far as around 45 kilometres (approximately 3 to 28 miles). Most of the recruitment sites were associated with transport links into the city, and so participants were able to access the existing system of public transport. Participants received reimbursement for time, effort and their transport expenses when they visited the clinic. We limited our recruitment radius so that no woman would have to use her own money. Women from the community who were at least 18 years old and either self-identified as female sex workers (FSWs) or reported having had at least three partners in the 3 months prior to recruitment were screened for participation in the study. Young women in urban South Africa are already at high risk for HIV infection [12], and our goal was to identify women who would theoretically be at the greatest risk of HIV acquisition. Women who were pregnant at the time of screening or who planned to travel away from the study site for more than 3 months were excluded from participation.

The screening procedure for identifying HIV-negative women consisted of voluntary counselling and testing (VCT) followed by a blood collection for rapid HIV antibody testing and urine collection for pregnancy testing. If the first antibody test (Determine: Abbott Laboratories, Tokyo, Japan) was negative, the participant was enrolled into the HIV-negative cohort. If the first antibody test was positive, a second rapid antibody test (Capillus; Trinity Biotech, Jamestown, NY, USA) was administered. Those with a two positive antibody tests were referred for HIV follow-up care. Those with discordant antibody results were given a confirmatory HIV enzyme immunoassay (EIA) (BEP 2000; Dade Behring, Marburg, Germany) and were either enrolled into the HIV-negative cohort or referred for HIV follow-up care on the basis of those results.

In order to maximize participant retention, the study visit schedule was thoroughly explained during the informed consent process and was re-emphasized at each study visit. Detailed locator information was collected and was reviewed at each subsequent visit and a secure participant-tracking database was established to facilitate visit scheduling and prompt follow-up for missed visits. Additionally, participants were compensated (100 South African Rand, ~ $14USD, as approved by the ethics committee) for their transport to the study site and for their time at the clinic.

To improve communication with the cohort, a web-based short message service (SMS) is used to send pre-approved cell phone text messages to willing participants. This system is used to remind participants who generally do not have access to fixed-line telephone services about their study appointments. The service is cost effective (0.33 South African Rand per message) and also provides an electronic record of all messages sent and whether or not they are received.

If a participant misses a visit, the clinic nurse administrator attempts to call a participant for the first 3 days after the clinic visit. A SMS with the clinic contact number is also sent out on the second and third days. If these attempts at contact are unsuccessful by the second week, the study community liaison officer attempts person-to-person contact by travelling to the participant's documented contact site as well as to the site from which the participant was originally recruited.

In addition to the methods above, the study staff and community liaisons regularly communicate with the study community at large to increase awareness of HIV/AIDS and explain the purpose of HIV prevention research and the importance of completing research study visits. A Community Research Support Group (CRSG) comprised of the CLPs meets bi-monthly with study representatives to discuss and provide feedback on the progress of the study and to discuss challenges faced by the study team with regards to community and participant relations.

Participant Protection

The study protocol and informed consent documents were reviewed by the local ethics committees of the University of KwaZulu-Natal, the University of Cape Town, the University of the Witwatersrand in Johannesburg, and by the Prevention Sciences Review Committee (PSRC) of the Division of AIDS (DAIDS, National Institutes of Health, U.S.A.). The consent forms were translated into isiZulu and written informed consent is obtained at each stage of the study (screening, enrolment into HIV-negative cohort, enrolment into acute infection phase, and for sample storage). All women screened for participation, whether ultimately enrolled into the study cohort or not, receive HIV pre- and post-test counselling, risk reduction counselling, male and female condoms, access to clinical care, and treatment for sexually transmitted infections (STIs). HIV/STI risk reduction counselling, condom provision, and prevention education supplies are administered at each subsequent study visit. One-on-one counselling was provided by a non-governmental organization which offered the same service in the public sector in the region, to be consistent with the services in the region. Overcoming one of the key challenges to doing HIV-related work in this context [11], participants who were HIV infected during the study were referred to the CAPRISA Antiretroviral Treatment (CAT) programme where they were offered ongoing care, and treatment for HIV when clinically eligible.

Study Visits and Procedures

Once enrolled into the HIV-negative observational cohort, the participants underwent a baseline evaluation that included: a HIV behaviour risk assessment, a clinical evaluation, blood collection for routine laboratory assessment and HIV status, and a specimen collection for sexually transmitted infection (STI) diagnosis.

Following the baseline evaluation, participants in the HIV-negative cohort attend monthly follow-up evaluations for a maximum of 24 months. The monthly evaluations consist of a clinical evaluation, two HIV antibody rapid tests, HIV EIA, and pooled HIV-1 RNA testing (Amplicor v1.5: Roche Diagnostics, Rotkreuz, Switzerland). A urine dipstick is done on clinical suspicion of pregnancy, or if requested by the participant. In addition, a routine laboratory assessment, urinalysis, and STI screening are performed at 6-monthly intervals.

Women from the HIV-negative cohort are diagnosed with acute HIV infection by (1) the detection of HIV-1 antibodies within 5 months of a previously negative HIV-1 test; or (2) evidence of HIV-1 viral replication in the absence of HIV-1 antibodies. While women in this cohort were screened monthly allowing for rapid identification of acute infection, a 5 months window period was allowed to enable to recruit from other prevention cohorts where follow-up was only quarterly. Time of infection is defined as the mid-point between the last HIV antibody negative test and the first HIV antibody positive test; or if a positive RNA PCR assay is available on the same date as a negative HIV EIA, the date of HIV infection is estimated at 14 days prior to the first positive RNA PCR assay.

Staff Training and Infrastructure Development

Given the extended scope and longitudinal nature of this study, CAPRISA was required to expand the size of the staff and the ability of the organization to manage a large cohort. After hiring additional clinicians, nurses, counsellors, and laboratory personnel, all team members underwent extensive training in both good clinical practice (GCP) and in the particulars of this study with respect to the case report forms (CRFs) for documentation, the quality control plan, and Human Subjects Protections (HSP). Furthermore, the capacity of the laboratory had to be increased to handle the large quantity of specimens related to the study.

Data Management and Statistical Analysis

All data is entered onto case report forms (CRFs) at the study sites and faxed into the CAPRISA Data Management Centre, using the DataFax system (Clinical DataFax Systems Inc., Ontario, Canada). This capability did not previously exist at site, and had to be implemented in order to collect the data for this study. Source documents and original CRFs are maintained on site while an electronic version of the CRF is maintained by the Data Management Centre. Quality assurance and quality control are maintained and checked at specified intervals. All analyses are conducted using the SAS statistical package version 9.1 (SAS Institute, Cary, NC, U.S.A.).

Results

Establishing the Cohort

Between August 2004 and May 2005, 775 women who were classified as high-risk either by self-identification as sex workers or self-report of more than three sexual partners in the previous 3 months were screened for participation in the observational cohort. Recruitment was done at known FSW sites in the city, therefore the majority of these women (n = 193, 78.8%) were self-identified as sex workers. The cumulative number of women screened and enrolled over the entire 10-month period can be seen in Figure 1.

Of the 775 women screened, 59.6% (95% CI 55.9%–62.8%) were found to be HIV-positive. The mean ages of the women who were screened and found to be HIV-positive and HIV negative were 29.3 years (range 16–58) and 34.2 years (range 18–58), respectively ($p < 0.001$). The HIV prevalence among the screening participants changed dramatically over time from 83% in the first month of screening to 17% in the last month of screening (Figure 2).

Of the 313 women who were HIV-negative, 245 HIV-negative women were eligible and agreed to participate in the HIV-negative cohort. Exclusion criteria for women who were uninfected at screening were as follows: less than three sexual partners in the previous 3 months (n = 22), women who were pregnant at the time of screening (n = 16), women who planned to relocate (n = 4), women younger than 18 years (n = 3), and women

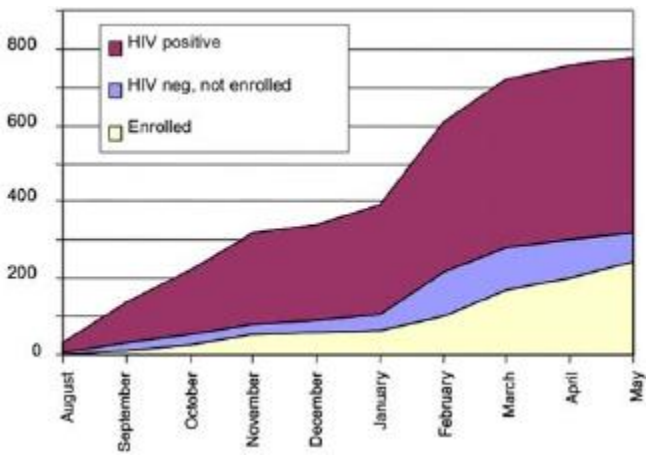

Figure 1. Cumulative HIV serostatus of screening participants over time.
doi:10.1371/journal.pone.0001954.g001

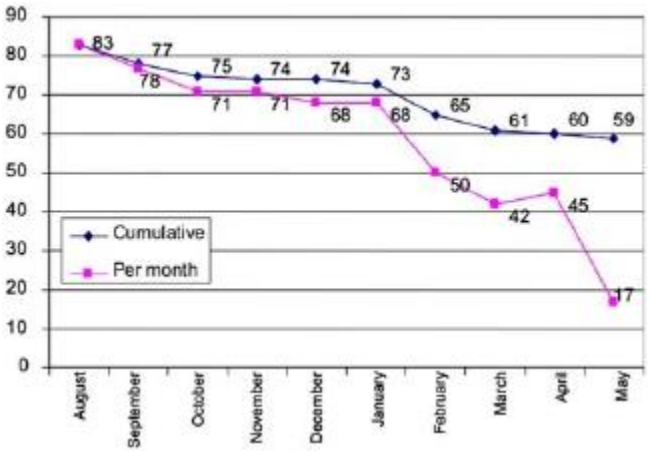

Figure 2. HIV prevalence in participants screened for enrollment into Phase I of the study over time, both cumulative and per month.
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who were afraid of the testing procedures (n = 2). There were twenty-one women who were eligible following screening but never returned for enrolment into the HIV-negative cohort.

The demographic characteristics of the 245 women enrolled in the HIV-negative cohort are presented in Table 1. The mean age of the overall cohort was 34.3 years (range 18–58). Approximately 42.0% of the cohort had 11 or 12 years of education or higher, 33.5% had between 8 and 10 years of education, and 24.5% had an education level less than 8 years.

The numbers of steady and casual partners reported by the women are listed in Table 2. Steady partners were defined as someone seen "most of the time, often over a period of time" while a casual partner was someone the women saw only occasionally or even only once (but not a client in the case of the FSWs).

All of the women in the cohort have engaged in peno-vaginal intercourse; 34.6% and 25.4% of the cohort have engaged in anal and oral sex respectively. The mean age at sexual debut was 17 years (range 12–26) and at the time of study enrolment the mean number of days since last sexual contact was 4.8 (range 1–45). While 58.8% of participants indicated that a condom was used at last sexual encounter, 13.9% and 34.3% of the women indicated that they were never able to insist on a condom being used with casual and steady partners respectively (Table 3). Additionally, a high proportion of the women (75.7%) provided economic support to adults (mean 0.9, range 0–7) or children (mean 2.8, range 0–14).

Among the women who self-identified as FSWs, the median length of sex work was 3 years (range 1 month–31 years) and the median age at which the women began sex work was 26 years

Table 1. Demographic characteristics of the HIV-negative observational cohort.

	Number (N = 245)	Percent		Number (N = 244)	Percent
Race/ethnicity			Marital status		
Black	241	98.4	Single, no steady partner	10	4.1
White	3	1.2	Married	16	6.5
Indian	1	0.4	Divorced	3	1.2
			Widowed	3	1.2
Education			Stable partner	75	30.6
Grade 11	103	42.0	Multiple partners	135	55.1
Grade 8–10	82	33.5	Separated	2	0.8
Grade 8	60	24.5			

(Note: where women refused to answer certain questions, the category total is less than the cohort total of 245.)
doi:10.1371/journal.pone.0001954.t001

Table 2. Sexual partner history for the 245 women in the HIV-negative observational cohort.

	Partners in the previous 3 months				Lifetime partners			
	Steady		Casual		Steady		Casual	
# of partners reported	Number	Percent	Number	Percent	Number	Percent	Number	Percent
0	7	2.9	4	1.7	2	0.8	0	0
1	158	65.6	14	5.9	9	3.7	0	0
2–3	62	25.7	166	69.5	42	17.4	32	13.4
4–6	8	3.3	33	13.8	80	33.1	31	13.0
7–12	1	0.4	3	1.3	20	8.3	17	7.1
.12	0	0	0	0	7	2.9	2	0.8
Too many to remember	5	2.1	19	7.9	82	33.9	156	65.5
Total	241	100	239	100	242	100	238	100

(Note: where women refused to answer certain questions, the category total is less than the cohort total of 245.)
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Table 3. Ease of condom use with casual and steady partners, showing a trend towards ease of condom use with casual partners, and lower and inconsistent condom use with steady partners (Fisher's exact test; p < 0.001).

Ease of Condom Use	Casual (% and n)	Steady (% and n)
Never	13.9% (34)	34.3% (84)
Sometimes	32.2% (79)	43.3% (106)
Every Time	53.9% (132)	20.4% (50)

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(range 14–53 years). The median number of days performing sex work per week was 3 days (range 1–7 days) with a median of 2 clients see per day (range 1–10), with a median of 2 clients (range 0–30) seen in the previous week.

Maintaining the Cohort

Enrolment for the Phase 1 HIV-negative cohort began in September 2004 and the last participant was terminated in May 2007. The retention rate for the HIV-negative cohort was 86.1%. Despite the high mobility and low socioeconomic status of this population, only 13 (5.3%) of the HIV-negative cohort were terminated because they could not be traced. Other reasons for termination included participant relocation (n = 13), inability to adhere to the study schedule (n = 4), participant withdrew consent (n = 4), and death due to causes unrelated to study participation (n = 5).

As part of the study protocol, the women in the cohort received regular clinical examinations. Key baseline characteristics are summarized in Table 4. On enrolment into the cohort, the HIV-negative cohort had a mean body mass index (BMI) of 31.0 kg/m^2. Of the 245 participants, 121 (49.4%) had a BMI greater than 30 kg/m^2 (defined as obese) and a further 58 (23.7%) were overweight with a BMI between 25 and 30 kg/m^2. Sixty-two participants (25.3%) had a normal BMI between 18.5 and 25 kg/m^2 and only five participants (2.0%) were underweight with a BMI less than 18.5 kg/m^2. Despite the high prevalence of obesity in this cohort, hypertension was not common. The mean systolic blood pressure was 117 mmHg and the mean diastolic pressure was 76 mmHg. Only 29 (11.8%) of the women had a systolic pressure greater than 140 mmHg or a diastolic pressure greater than 90 mmHg. Moderate anaemia was relatively common in this population and 48 (19.7%) of the women had a haemoglobin less than 12 g/dL at study enrolment.

On enrolment into the cohort and at 6-monthly intervals, these women underwent STI screenings for *Trichomonas vaginalis*, *Neisseria gonorrheae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, syphilis, and Herpes simplex virus-2. Overall, 29.4% of women were infected with an STI at baseline. This percentage increases to 62.0% if bacterial vaginosis is included. Further, while none of the women were pregnant on enrolment, there were 32 pregnancies in the cohort for an incidence of 8.5 per 100 person-years (95% CI 5.6–11.5). During this same period, there have been 5 deaths unrelated to study participation. Causes of death include: stab wound (1), clinically reported as idiopathic thrombocytopenia (1), and unknown (3).

Table 4. Baseline medical characteristics of the HIV-negative cohort (n = 245).

	Mean (SD)	Range
Blood pressure (mmHg)		
Systolic	117 (15)	90–200
Diastolic	76 (10)	55–110
Body mass index (kg/m^2)	31.0 (7.8)	17.2–54.5
Hemoglobin (g/dL)	12.7 (1.3)	7.7–16.1
Hematocrit (%)	37.3 (0.3)	25–46

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Finally, after 4784 monthly visits by 245 participants in this HIV-negative cohort, we identified 28 acute HIV infections. Twenty of the acute infections were among women who had self-identified as female sex workers and 8 of the acute infections were among women who had reported more than three partners in the preceding 3 months. The annual seroincidence after 390 person years was 7.2 per 100 person-years (95% CI 4.5–9.8).

We investigated if the number of new HIV infections reduced over time, which could be due to women who were most at risk of getting infected seroconverting early during follow-up or women reducing their risky behaviour over time and in response to repeated risk-reduction counselling. There was a significant trend over time in the rate at which women seroconverted (p-value = 0.0283), and this is graphically illustrated in Figure 3.

Discussion

This paper describes the recruiting strategies, screening methods, enrolment procedures and retention techniques for a cohort of high-risk South African women, consisting largely urban female sex workers, a typically transient and mobile population that formed the basis for the CAPRISA 002 Acute HIV Infection study. We encountered numerous challenges over the course of establishing and administering the cohort and this paper aims to describe our strategies for overcoming these challenges.

The first challenge was defining the inclusion and exclusion criteria for the cohort. The study design called for uninfected yet "high risk" women so that we could theoretically maximize the number of acute infections we were able to identify. As we began

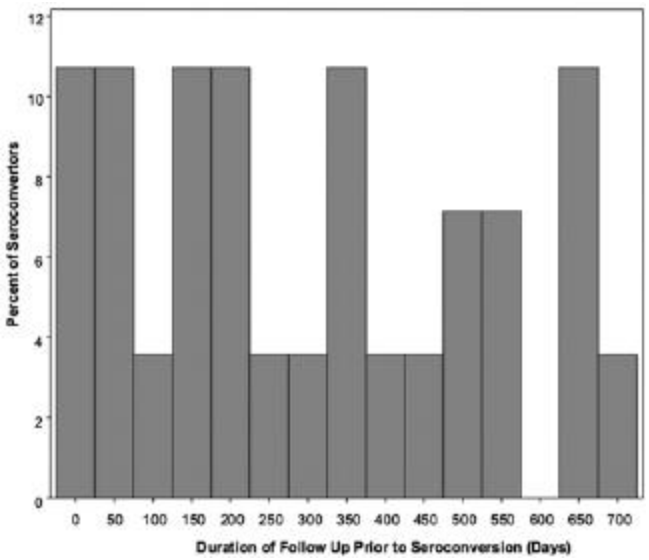

Figure 3. Percentage of acute HIV infections as a function of time in follow up (in days), showing a decrease in acute infections over time of follow up for the study.
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recruitment, we were confronted with cultural and social barriers to delineations within this population. While many women were involved in transactional sex of some form, they were often reluctant to identify themselves as FSWs. Transactional sex for money or goods with non-regular partners is common in this region and is often not explicitly described as commercial sex work [13]. Once these cultural taboos became apparent, our study staff made a concerted effort to ensure that transactional sex was asked about in an open and permissive manner. Close work with the CLPs, some of whom were self-identified FSWs, enabled the research team to systematize a non-judgmental approach. Future work in similar cohorts needs to take account of the fact that terms such as 'sex worker' or 'client' may be inappropriate, and that broader definitions that include multiple partners who provide material needs in exchange for sex should be included in behavioural risk assessments.

The screening was made more difficult because of the high prevalence of HIV (59.6%) in the screening population. This is an extremely high prevalence rate, even when compared with the reported prevalence rates of 13.3% for South African women, 16.5% for the highly-burdened KwaZulu-Natal province, and 33.3% for South African women between the ages of 25 and 29 years [14]. The change in HIV prevalence rates over the course of screening was quite dramatic – from 83% to 17% – with the majority of uninfected participants identified in the final quarter of recruitment. The decline in prevalence over the course of screening could reflect a desire of women who were most concerned about their status to come first to the clinic for HIV testing, or for women who were most active in sex work to come forward first. Regardless, this discrepancy highlights the fact that cross-sectional surveys of seroprevalence can be a highly variable, or even unreliable, indicator of true prevalence and underscores the benefits of having an extended screening period with adequate numbers of screening participants. Enrolling participants over time from different populations with different risk profiles could bias the results of many studies. This variation is particularly significant for prevention studies where care should be taken to design the randomization process to equally distribute these differences with respect to time and intervention. For example, it is important to use blocked randomization to ensure that equal numbers of participants are enrolled at each stage of the trial into the different treatment arms. Accordingly, any trends over time in these studies must be interpreted with caution as these trends could be due to baseline differences in the participants.

In our study there was a significant age difference between the HIV-positive women who were screened (mean 29.3 years) and the HIV-negative women (mean 34.3 years) who were enrolled into the study cohort. This difference may be a sign of the changing demographics of the HIV epidemic among high-risk women in South Africa and supports the view that the epidemic is being most acutely experienced by younger South African women. On the other hand, lower HIV infection susceptibility, potentially (but controversially) associated with factors such as HLA alleles, partner-specific alloimmunity, reduced CD4+ T cell susceptibility, variability of cellular proteins involved in HIV-1 replication, host antiviral cellular proteins, or some related host genetic factor [15–23], may explain why some of the women have remained negative despite high-risk exposures and therefore remain uninfected although significantly older. That is, the women who are susceptible to infection are infected at an early age, leaving the less susceptible women as the core of the HIV-negative cohort. This trend is also supported by our finding that women who remain the in cohort are statistically less likely to seroconvert as follow-up progresses. Further research has been initiated to examine some of these factors in the HIV-negative, high-risk women that remain uninfected at the end of their study follow-up.

Another major challenge of working with this cohort of HIV-1 negative women has been to reconcile the study goals of identifying acute infections with the ethical imperative to aggressively promote prevention. Prevention efforts in this cohort included the provision of male and female condoms along with monthly HIV risk-reduction counselling. In spite of these efforts, the high rate of pregnancies and STIs in this cohort underscores the difficulty of prevention promotion in this population. Similar to vaccine or microbicide studies that intensively promote prevention, our experience is that some women will remain at risk for HIV infection in spite of the best efforts of the research team.

Retention rates for the HIV-negative observational cohort have been extremely successful with close to 86% retention over the duration of follow-up. Given the highly mobile nature of this population, these rates are high and bode well for future microbicide and vaccine studies which could have even greater retention rates in light of the perceived benefits of the intervention to the participants. We believe that this success underscores the value of having a dedicated network of community liaisons and of making a coherent and concerted effort to engage the community on a structural level. Making use of all available contact mechanisms and channels, especially the cell phones and short message system technology, proved to be essential for maintaining follow-up in this highly mobile cohort in which fixed-line telecommunications access is not common. We have established the feasibility and utility of using these services to both remind patients about their study visits and to follow up with missed visits.

Additionally, while the study's CLP network was initially intended as an additional measure to assist with participant tracking and retention, we found that the designated CLPs were ultimately only effective in tracing women with whom they had pre-existing relationship or acquaintance. As the study progressed, the study staff would increasingly rely on friends and relatives to relay messages to participants. Other factors that have undoubtedly contributed to the high retention rates include the monetary compensation for study visits, the regular counselling sessions and subsequent relationships formed with the counsellors, the distribution of condoms, and the knowledge that participants will be referred for STI treatment and other medical services as necessary.

We have also presented here an overview of the demographic, behavioural, and clinical characteristics of this cohort. In reviewing the clinical profile of these women, while they are a relatively young population and would thought to be healthy, they have a number of comorbidities. The rates of hypertension (11.8%) are lower than reported rates for South Africa of 21.1% [24] but the cohort tended to be overweight or obese with a relatively high prevalence of anemia. The presence of these comorbidities will be important for the clinical management of these women and will need to be considered among HIV-infected South African women. They also have high rates of STIs and pregnancy despite intensive counselling and ready availability of prevention materials. Lastly, the non-study related deaths of five of the participants reveals that even HIV-uninfected women living in urban South Africa are at high-risk of death. As we continue to accrue longitudinal data on this cohort, we anticipate that these baseline characteristics will be expanded on, and specific results on anaemia during acute infection have been published [25].

The behavioural data provides an overview of the sexual history and potential exposures of this theoretically high-risk cohort, particularly the FSWs. A previous survey of sex workers in the

KwaZulu-Natal province of South Africa found that women working at truck stops averaged 22 sex acts per week, with coitus occurring between two to ten times in a 24 hour period [9]. This earlier survey also found that peno-vaginal sex was the only practice reported, with strong cultural sanctions against anal and oral sex. The sex workers in this urban cohort appear to have customers with whom they engage in fewer acts but see more regularly, averaging just three clients per week, and are also engaging in anal and oral sex. Consequently, they have a very different risk profile than what had been anticipated based on the prior studies. This difference in risk behaviour has likely impacted the HIV seroincidence in this cohort. Data are being analysed to determine the specific behavioural risk factors for HIV infection in this cohort.

The perceived ease of condom use in this cohort shows that women are significantly more likely to feel that they will be able to use condoms with casual partners than with their steady partners. This difference may explain why condom use has not led to a reduction in HIV risk as these women are still vulnerable due to inconsistent condom use with their steady partners. This observation suggests that condom use may be associated with perceptions of trust; whatever the case, condom use is inconsistent at best in this population. The behavioural risk data collected at baseline will be analysed after the full 24 month follow-up period to see which factors are most predictive of HIV acquisition in this cohort.

Based on previous data, the projected annual sero-incidence for this high-risk population was 18.2% [9,12]. The actual seroincidence in this cohort has been 7.2% which is a little higher than the national seroincidence estimates of 6.3% for all South African women between the ages of 15 and 49 [14]. The risk of HIV acquisition in the context of the maturing South African HIV epidemic seems to be highly generalized. Hence, our designations of "high risk" are seemingly less relevant in retrospect and perhaps need not be considered as rigorously when designing future studies. Furthermore, unmeasured susceptibility factors may have disproportionately influenced the observed infection rate in this cohort of highly exposed individuals, where the women who were more susceptible had acquired HIV prior to screening for this study. Furthermore, our data support the notion that high-risk cohorts may yield fewer seroconversions over time, and that this attrition should be factored into the design of such cohorts. Women who remain in the cohort at the end of the follow-up, may represent a group enriched with host genetic resistance factors, and this is currently being explored as this group of woman has been recruited into a highly exposed persistently negative cohort for additional study.

Conclusion and Significance

To the best of our knowledge, this is the first prospective cohort to be assembled in southern Africa for a comprehensive analysis of the behavioural, clinical, and immunological characteristics associated with acute HIV infection. The methods, as well the challenges, of recruiting and successfully retaining a HIV-negative cohort have been described and can provide guidance for others wanting to assemble similar cohorts. Given the characteristics of our cohort, these experiences are particularly relevant to the recruitment and retention of cohorts of urban female sex workers in South Africa for HIV prevention and pathogenesis research. The changing nature of the epidemic must be considered when recruiting high risk HIV-negative cohorts in prevention research, as demonstrated by the lower than anticipated seroincidence in this study.

Understanding natural history of HIV infection from as early a point following exposure to HIV as possible is key for the design, development and targeting of new prevention interventions as well as for the treatment of advancing HIV disease. Understanding how to identify those most at risk for HIV infection, whether this is due to high-risk behaviours, or due to the increased risk of infection due to high viral loads during acute or recent infection, or due to the increases risk conferred by concomitant sexually transmitted infections, has recently been suggested as the key concern for prevention science [26]. It is hoped that data from the Acute Infection study will contribute significantly to vaccine development by describing the natural history of HIV infection, the impact of host and viral factors during acute infection on viral load set point, as well as the impact of viral set-point on disease progression. Behavioural data collected before and after acute infection should assist in developing algorithms to identify those most at risk of infection. HIV screening algorithms [27] and clinical data collected during acute infection are potentially fruitful explorations of efficient and effective screening algorithms for identifying acute infection. These specific research areas will be addressed separately in publications from the CAPRISA 002 Acute Infection Study Team.

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Ethics: The Acute Infection study was reviewed and approved by the research ethics committees of the University of KwaZulu-Natal (E013/04), the University of Cape Town (025/2004), and the University of the Witwatersrand (MM040202). All participants provided written informed consent for screening, enrolment and specimen storage.

Author Contributions

Conceived and designed the experiments: LM CW CG SAK KM Fv QA. Performed the experiments: LM CW CG KM Fv II. Analyzed the data: AG. Wrote the paper: LM CW CG SAK KM Fv SA AG NB QA. Other: Conceptualized the paper and drove the paper writing and revision process: Fv. Study coordinator and behavioural co-investigator on the Acute Infection Study: Fv. Protocol co-Chair and clinical co-investigator: KM. Assisted in the conceptualization of the paper: AG KM. Commented on succeeding drafts of the paper: AG NB KM II CW LM CG SAK QA. Protocol co-Chair and virology co-investigator: CW. A Research fellow at CAPRISA: SA. Prepared the initial drafts of the paper, conducted the literature review, and assisted with subsequent redrafting of the paper: SA. An immunology co-investigator on the study: LM CG. Head of the laboratory core: LM. The study epidemiologist and a co-investigator: QA SAK. The study statistician: AG. Conducted the statistical analyses and produced the graphs for the paper: AG. The study community liaison officer and co-investigator: NB. Contributed the sections dealing with community: NB. The study clinician: II. Collected the clinical data for the study: II.

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HIV Prevention in High-Risk Women in South Africa: Condom Use and the Need for Change

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Abstract

Introduction: Young women are at disproportionate risk of HIV infection in South Africa. Understanding risk behaviors and factors associated with ability to negotiate safe sex and condom use is likely to be key in curbing the spread of HIV. Traditionally prevention efforts have focused on creating behavioral changes by increasing knowledge about HIV/AIDS.

Methods: This was a cross-sectional analysis from a prospective observational cohort study of 245 women at a high-risk of HIV infection in KwaZulu-Natal, South Africa.

Results: Participants demonstrated a high level of HIV/AIDS knowledge. Overall, 60.3% of participants reported condom use. Reported condom use at last sexual encounter varied slightly by partner type (57.0% with steady versus 64.4% with casual partners), and self-perceived ability to choose to use a condom was significantly lower with steady partners compared to casual partners ($p < 0.01$). In multivariate analysis, women who had high school education were more likely to use condoms at their last sex encounter compared to those with only primary school education (RR of 1.36 (95% Confidence Interval (CI) 1.06–1.75) and 1.46 (95% CI 1.13–1.88) for grades 8–10 and 11–12, respectively). Those who used condoms as a contraceptive method were twice as likely to use condoms compared to women who did not report using them as a contraceptive method. Greater perceived ability to choose to use condoms was associated with higher self-reported condom use at last encounter, irrespective of partner type (RR = 2.65 (95% CI 2.15–32.5).

Discussion: Self-perceived ability to use condoms, level of formal education and condom use as a contraceptive were all significantly associated with self-reported condom use at last sexual encounter. These findings suggest that that gender inequality and access to formal education, as opposed to lack of HIV/AIDS knowledge, prevent safer sexual practices in South Africa.

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Introduction

In the South African province of KwaZulu-Natal, an estimated 25.8% of all persons aged 15 to 49 years are HIV-positive. This prevalence rate is one of the highest in South Africa, a country that carries the highest number of HIV-infected persons in the world [1,2]. HIV is predominantly spread via heterosexual sex, with young women being the country's most vulnerable population [1,3].

Traditionally prevention efforts have focused on creating behavioral changes (i.e. safer sexual practices) within the general population by increasing knowledge about HIV/AIDS. Studies in the United States and other developed countries have demonstrated successful reduction in HIV infection rates with educational campaigns, while South Africa has continued to experience a rise in the prevalence of HIV despite increased public knowledge and awareness of HIV/AIDS [1,4,5]. Recent reductions in HIV incidence in South Africa have instead been attributed to increased access to HIV treatment [6]. Additionally, several studies have found that improved access to information about HIV risk is not translating into an effective behavioral change in women at high-risk of infection [1,7,8,9,10,11,12,13,14]. Researchers have investigated the possible causes of this disconnect and one theory is that behavioral changed is impeded by the 'social context' of this community [15].

Reported level of condom use has been used as way of identifying and understanding barriers to behavioral change.

Reported rates of condom use among sexually active populations in South Africa range from 32.8% to 78.4% [1,16,17,18,19, 20,21]. In high-risk women, such as commercial sex workers, the rate of condom use is low [13,14,15]. The negative stigma associated with condoms, lack of female power in sexual relationships, threat of physical violence if condom use is requested, and financial incentives to forego condom use are all potential barriers to their use [3,7,11,12,13,14,15,20,22,23,24, 25,26,27,28].

This investigation, as part of a larger behavioral study, focused on women at high-risk of contracting HIV/AIDS. It aimed to examine the impact of HIV/AIDS knowledge, as well as related sexual practices on self-reported condom use, and the influence these factors have on HIV acquisition in a high-risk population.

Methods

This study was a cross-sectional analysis of baseline behavioral data gathered from women enrolled into a prospective observational cohort study performed at the Centre for the AIDS Programme of Research in South Africa (CAPRISA), in Durban, KwaZulu-Natal Province, South Africa. This investigation, known as the CAPRISA 002 Acute Infection Study, began in 2004 to research the natural history of acute HIV infection and associated risk behaviors in a cohort of high-risk women, 78.8% of who were self-identified sex workers.

CAPRISA 002 study enrolled women $\geq$ 18 years of age who self-reported sex with more than three different partners in the previous three months, and who were HIV antibody negative on screening with rapid tests (first with the Determine test (Abbott Laboratories, Tokyo, Japan) and, if positive, confirmed with a Capillus test (Trinity Biotech, Jamestown, NY, USA)). Discordant rapid HIV tests were resolved with a confirmatory HIV enzyme immunoassay (BEP 2000; Dade Behring, Marburg, Germany). More detailed information regarding CAPRISA 002 study methodology, including recruitment methods and eligibility criteria, is described elsewhere [29].

Prior to study initiation, the protocol and informed consent forms were reviewed and approved by the research ethics committees of the University of KwaZulu-Natal (E013/04), the University of Cape Town (025/2004), and the University of the Witwatersrand (MM040202). Consent forms and information to patients were available in English and were translated into isiZulu, the local language. Written informed consent was obtained at each phase of the study, including collecting the behavioral risk data and to store the study specimens.

Participants completed a structured, in-person interview in either English or isiZulu (per participant preference) to identify their HIV risk behaviors. Upon enrollment, demographic, locator information and laboratory samples were obtained from each participant who received HIV pre- and post-test counseling, HIV and sexually transmitted infection (STI) risk reduction counseling, a behavioral risk assessment as well as a routine clinical evaluation. During the administration of the HIV/AIDS knowledge questions, participants were not prompted, but were asked to state their responses to the questionnaire administrator who ticked off the appropriate category according to the participant's response. Responses were then labeled with a participant identification number and entered into a data collection system. Each participant was paid for her participation in the study as a means of reimbursement for their time and travel expenses.

Descriptive and statistical analysis was performed using SAS software version 9.2 (SAS Institute Inc., Cary). Unadjusted and adjusted risk ratios, measuring the associations between the factors of interest, were calculated using generalized estimating equation regression models with the log link function. Variables in the unadjusted model with a p-value less than 0.20, or variables deemed as important to adjust for, were fitted to the multivariate model. The McNemar test was used to assess differences in perceived choice of condom use between participants' casual and steady partners.

Results

A total of 245 participants were enrolled in the study, the basic characteristics of whom are presented in Table 1. This was a cohort of high-risk women; 85.4% reported having 2–5 casual partners in the 3 months prior to enrollment and 78.8% self-identified as sex workers. Overall, 58.8% of women reported condom use at their last sexual encounter.

All participants reported that they had heard of HIV/AIDS at the time of study enrollment and, overall, participants demonstrated a good level of knowledge of HIV disease. These data are presented in Table 2.

Participants were knowledgeable about how HIV/AIDS is spread; almost all participants listed vaginal sex as a risk factor, but considerably smaller proportion listed anal or oral sex. When asked about the risk of contracting HIV via anal and oral versus penovaginal sex, slightly more than a third of participants answered that anal and oral sex carried more risk compared to vaginal sex.

Similarly, there was high level of awareness how to prevent acquisition or transmission of HIV. Almost all of the participants listed condoms as an effective method, while only 39% and 30% indicated abstinence and staying faithful to one partner as an effective HIV prevention method. Even though treatment was not widely available at the time of the interviews, the majority of participants knew that HIV could not be cured, while a similar proportion agreed that it could be treated.

Sexual behavior data is presented in Table 3, and indicate that participants were much more likely to have had sex with a steady partner than a casual partner (defined as one seen only occasionally, or even once, that was not a sex work client) at last encounter (75.9% steady versus 24.1% casual). In the three months prior to enrollment, over 85% of participants reported two to five casual partners. With respect to total lifetime partners, 54.1% reported more than five steady partners and 76.1%

Table 1. Participant demographic characteristics.

Mean age in years ± SD	34.2610.5
High school education % (n)	
- Primary	24.5% (60)
- Secondary to 10th grade	34.3% (84)
- Grades 11/12	41.2% (101)
Partner status % (n)	
- No partner	7.4% (18)
- One partner	35.7% (87)
- Many Partners	57.0% (139)
Race % (n)	
- Black	98.4% (241)
- Indian	0.4% (1)
- White	1.2% (3)

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Table 2. HIV/AIDS awareness and knowledge.

Have you ever heard of HIV/AIDS? % (n)	
- Yes	100% (n = 245)
- No	0%
How is HIV/AIDS spread? % (n)	
- Vaginal sex	99.6% (n = 244)
- Contact with blood	94.7% (n = 232)
- Anal sex	56.7% (n = 139)
- Oral sex	51.8% (n = 127)
- From mother to child	48.2% (n = 118)
- Breastfeeding	10.6% (n = 26)
- Touching someone	4.9% (n = 12)
- Mosquito bites	4.5% (n = 11)
- Sharing same cup	3.7% (n = 9)
- Using public toilets	2.9% (n = 7)
- Other	62.4% (n = 153)
How can you prevent the spread of HIV/AIDS? % (n)	
- Using condoms	99.6% (n = 244)
- Avoid sharing razors	75.1% (n = 184)
- Injections with clean needles	53.5% (n = 131)
- Abstaining from sex	39.2% (n = 96)
- Staying faithful	29.8% (n = 73)
- Having good diet	2.0% (n = 5)
- Other	35.5% (n = 87)
If you have an STI, do you think you are more likely to get HIV/AIDS? % (n)	
- Yes	71.8% (n = 176)
- No	1.6% (n = 4)
- Unsure	26.5% (n = 65)
Do you think that HIV/AIDS can be cured? % (n)	
- Yes	4.1% (n = 10)
- No	91.4% (n = 224)
- Unsure	4.5% (n = 11)
Do you think that HIV/AIDS can be treated? % (n)	
- Yes	95.1% (n = 233)
- No	2.0% (n = 5)
- Unsure	2.9% (n = 7)
Risk of HIV acquisition by route Anal sex compared to penovaginal sex? % (n)	
- Less risk	30.3% (n = 74)
- Same risk	27.9% (n = 68)
- More risk	34.0% (n = 83)
- Don't know	7.8% (n = 19)
Oral sex compared to penovaginal sex? % (n)	
- Less risk	23.9% (n = 58)
- Same risk	33.3% (n = 81)
- More risk	38.3% (n = 93)
- Don't know	4.5% (n = 11)

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Table 3. Sexual behavior.

Mean age at sexual debut 6 SD	17.062.4
Mean days since last encounter 6 SD	4.664.6
Last sex act partner type % (n)	
- With casual partner	24.1% (59)
- With steady partner	75.9% (186)
Number of casual partners in the last 3 months % (n)	
- 0–1	5.0% (12)
- 2–5	85.4% (205)
- _5	9.6% (23)
Number of lifetime casual partners % (n)	
- 0–1	0.0% (0)
- 2–5	23.9% (57)
- _5	76.1% (181)
Number of steady partners (last 3 months) % (n)	
- 0–1	68.4% (165)
- 2–5	29.1% (70)
- _5	2.5% (6)
Number of lifetime steady partners % (n)	
- 0–1	4.1% (10)
- 2–5	41.7% (101)
- _5	54.1% (131)
Mean number of penovaginal sex acts per month 6 SD	10.266.7
Mean number of oral sex acts per month % (n)	
- 0–1	79.9% (191)
- 1–4	14.2% (34)
- _4	5.9% (14)
Anal sex acts per month	
- Never	66.8% (159)
- Once or less	11.3% (27)
- $2	21.9% (52)
Douche after/between sex % (n)	
- Yes	9.4% (23)
- No	90.6% (221)
Proportion who ever had sex while drunk % (n)	26.9% (66)
Proportion who use any contraceptives % (n)	79.9% (195)
Proportion who use condoms % (n)	60.3% (147)
Contraceptives used: % (n)	
- Condom	32.0% (78)
- Dual (condom+other)	28.3% (69)
- Non-condom	19.3% (47)
- None	20.5% (50)
Proportion using injectable contraceptives % (n)	27.9% (68)

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reported more than five casual partners. Participants reported that they engaged in penovaginal sex an average of 10 times a month, with low levels of oral and anal sex. Approximately 80% of participants reported that they used contraceptives, with 60.3% listing condoms as a method of contraception. Importantly, none of these baseline characteristics or sexual practices were found to be statistically associated with subsequent HIV acquisition.

While frequency of contraceptive use was not assessed, condom use at the most recent sexual encounter varied depending on partner type (see Table 4). For example, 57.0% of participants

Table 4. Condom use.

Condom used at last sexual encounter % (n)	58. 8% (144)	
- Steady partner at last sexual encounter: condom use % (n)	57.0% (106/186)	Fisher's exact
- Casual partner at last sexual encounter: condom use % (n)	64.4% (38/59)	p = 0.36
Perceived ability to choose condom use with steady partner % (n)		
- Never	35.0% (84)	
- Sometimes	44.2% (106)	
- Every time	20.8% (50)	
Perceived ability to choose condom use with casual partner % (n)		
- Never	13.9% (34)	
- Sometimes	32.2% (79)	McNemar
- Every time	53.9% (132)	p< 0.01

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reported using a condom with a steady partner, compared to 64.4% with a causal partner (p = 0.36). Perceived ability to negotiate condom use with casual partners was significantly higher compared to steady partners, with only 20.8% of participants saying they felt they could always use condoms with their steady partner versus 53.9% with casual partners (p< 0.01).

Several factors were associated with condom use at last sexual encounter. For example, self-perceived ability to choose to use condoms was associated with self-reported use both in univariate and multivariate analyses (see Table 5). In the multivariate model, women who believed they had a high degree of control over condom use (able to choose always or over half the time whether or not to use condoms) were more than twice as likely to have used a condom than those who reported never having the ability to choose or who felt they could only insist on using a condom less than half the time (RR 2.21, 95% CI 1.79–2.72).

Education level was significantly associated with condom use. In multivariate analysis, women with Grade 8 to 10 education were approximately 40.0% more likely to report using condoms compared to women with primary school education (p = 0.02); and those with Grade 11 to 12 education were about 50.0% more likely to report using condoms (p< 0.01). Women who reported having no partner (single, divorced, widowed or separated) were 1.39 times more likely (95% CI 1.01–1.90) to have used a condom at their last sex act compared to women who were in a stable relationship or married (p = 0.04).

In univariate analysis, age was significantly associated with condom use, with older women being less likely to use condoms. It was no longer significant in the multivariate model, however, most likely because schooling was closely associated with age, with older women being less educated. Age was also associated with perceived choice or insistence on condom use; younger women reported being able to insist on condom use more often than older women. For every five year decrease in age, women were 1.03 times more likely (95% CI 1.01–1.05) to insist on condom use (p = 0.01), adjusting for education level.

Women who reported using condoms as a method of contraception were twice as likely to have used a condom at their last sex act compared to women who did not use condoms as contraception (RR 2.02, 95%CI 1.53–2.66, p< 0.01). However, women who said they were using hormonal methods as a contraception method were no more likely to have used a condom at their last sex act compared to women who were not on hormonal contraception (p = 0.51).

Discussion

This study provides information about the behavioral characteristics, level of HIV knowledge and predictors of condom use in a population of high-risk women in KwaZulu-Natal, South Africa. Specifically, this study illustrates that knowledge of HIV and HIV transmission does not correlate with high condom use in this population. These behavioral risk data are also supported by high STI and HIV infection rates in this cohort over time [29]. Previous studies also illustrated that lack of HIV specific knowledge does not explain low rates of condom use [7,8,11,12]. Of note, there was lower awareness of STI and HIV transmission risk with oral and anal sex, suggesting that this would be an important opportunity for more targeted education.

In our cohort, an important finding is the higher reported efficacy of condom use choice at last sexual encounter with casual rather than steady partners. Similar observations were made in other studies from South Africa and the proposed explanations include concern about perceived lack of trust and threat to intimacy and commitment between steady partners [12,13,14,15,30]. This highlights the fact that negotiating condom use with steady partners is challenging as it may represent infidelity or, conversely, that non-use of condoms may signal trust. Ironically this means that steady sexual partnerships may represent greater risk of HIV infection, for either partner.

In our cohort, several factors were significantly associated with reported condom use during last sexual encounter. We found an association between education level and increased frequency of reported condom use, a result consistent with other studies that show an association between schooling and reduced levels of HIV risk, particularly in young women [31]. Our results also suggests that using condom for contraceptive purpose seems to be a more acceptable motivation, and women are more likely to use condoms in their sex acts if this is being done as the primary means of contraception. It remains to be seen whether or not use of hormonal contraception in this social context would undermine condom use.

Importantly, we noted that women who perceived that they are able to make a choice about using a condom were twice as likely to report using one. It is therefore important to focus on female empowerment in sexual relationships. Previous literature suggests that encouraging self-agency and endorsing egalitarian gender roles in sexual relationships may have a positive impact on rates of condom use [22]. Similarly, a recent preliminary analysis from a randomized trial in South Africa has shown that interventions

Table 5. Perceived ability to choose to use a condom and predictors of condom use at last sexual encounter.

Variable	Univariate analysis			Multivariate analysis	
	% with condom use at last sex act (n/N)	Risk Ratio (95% CI)	p-value	Risk Ratio (95% CI)	p-value
Ability to insist on condom use (number of times)					
- Less than half/Never	36.9% (58/157)	1.00 (reference)	-	1.00 (reference)	-
- More than half/Always	97.7% (86/88)	2.65 (2.15–3.25)	<0.01	2.21 (1.79–2.72)	<0.01
Age (by 5 year increase)		0.92 (0.88–0.97)	<0.01	0.99 (0.94–1.04)	0.69
Age at sexual debut (by 5 year increase)		0.98 (0.78–1.24)	0.88		
Schooling					
- No schooling	28.6% (2/7)	1.00 (reference)	-		
- Any schooling	59.7% (142/238)	2.09 (0.64–6.77)	0.22		
Highest level of school					
- Primary	43.3% (26/60)	1.00 (reference)	-	1.00 (reference)	-
- Grade 8–10	63.1% (53/84)	1.46 (1.04–2.03)	0.03	1.36 (1.06–1.75)	0.02
- Grade 11–12	64.4% (65/101)	1.49 (1.07–2.05)	0.02	1.46 (1.13–1.88)	<0.01
Marital status					
- One partner	59.8% (52/87)	1.00 (reference)	-	1.00 (reference)	-
- Many partners	56.1% (78/139)	0.94 (0.75–1.18)	0.59	0.92 (0.76–1.10)	0.36
- No partner	72.2% (13/18)	1.21 (0.86–1.69)	0.27	1.39 (1.01–1.90)	0.04
Last sexual partner					
- Steady	57.0% (106/186)	1.00 (reference)	-	1.00 (reference)	-
- Casual	64.4% (38/59)	1.13 (0.90–1.42)	0.29	1.05 (0.87–1.26)	0.61
Sex worker					
- No	69.2% (36/52)	1.00 (reference)	-	1.00 (reference)	-
- Yes	56.0% (108/193)	0.81 (0.65–1.01)	0.06	0.96 (0.78–1.18)	0.69
Ever had anal sex?					
- No	63.5% (101/159)	1.00 (reference)	-	1.00 (reference)	-
- Yes	50.0% (42/84)	0.79 (0.62–1.00)	0.05	1.00 (0.82–1.24)	0.94
Ever had oral sex?					
- No	59.9% (109/182)	1.00 (reference)	-		
- Yes	54.8% (34/62)	0.92 (0.71–1.18)	0.50		
Ever had sex while drunk?					
- No	48.5% (32/66)	1.00 (reference)	-	1.00 (reference)	-
- Yes	62.6% (112/179)	0.77 (0.59–1.02)	0.07	0.89 (0.72–1.10)	0.29
Condom as contraceptive?					
- No	30.9% (30/97)	1.00 (reference)	-	1.00 (reference)	-
- Yes	76.9% (113/147)	2.49 (1.82–3.39)	<0.01	2.02 (1.53–2.66)	<0.01
Hormonal contraceptives?					
- No	60.0% (102/170)	1.00 (reference)	-		
- Yes	55.4% (41/74)	0.92 (0.73–1.17)	0.51		

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to empower a woman's ability to negotiate condom use can lead to an increase in self-reported condom use at the last sex act [32,33].

Negotiating condom use for women in our cohort is further complicated by the social and cultural context in which these relationships are developed. For although, it has been shown that women have positive attitudes toward condom use [11], South African men are traditionally the decision makers, especially regarding issues of sexual practice [12,15,24,26,27,34,35]. Therefore, despite high levels of knowledge about HIV transmission and prevention, some women are not able to protect themselves from infection.

A strength of this study is the prospective design, with a relatively large cohort, and the high response rate with almost all participants responding to each question. These findings are thus important as they represent unique data for a large cohort of HIV negative women at high risk for HIV infection, who had a broad range of risk factors, including both sex work and non-sex work partners. We believe these data contribute to greater understanding of the sexual networks and risk behaviors in this specific

context. Furthermore, this level of data is particularly important in designing culturally relevant risk reduction counseling messages in the context of, for example, HIV prevention studies.

A limitation of the study is that the HIV knowledge and behavioral risk questionnaires were only administered at baseline in the HIV negative cohort, limiting the analysis to cross-sectional data. For example, since data about factors associated with condom use are collected at a single time point, it is not possible to determine causality; prospective study would be needed to determine whether factors such as greater access to formal education would empower the woman to negotiate for condom use. Another notable weakness, shared by much behavioral research, is that the data are self-reported and therefore subject to reporting and recall bias. Attempts were made to minimize reporting bias by making the questions neutral and assuring participants of confidentiality.

Conclusions

Women at high-risk of HIV infection were found to have had a high level of knowledge about HIV acquisition and prevention of transmission. This indicates that education campaigns have reached women in need of this information. However, importantly, knowledge about the high risk of transmission during anal sex was lacking. Future HIV/AIDS educational campaigns therefore need to include messages regarding the risk of transmission via anal sex.

The self-perceived ability to choose to use condoms is significantly associated with reported condom use at last encounter, and furthers the argument that social barriers, particularly gender power imbalances in relation to sexual decision-making, as opposed to lack of knowledge, may be influential factors preventing safer sexual practices in South Africa. This information highlights the importance of female empowerment in prevention efforts. In particular, and supporting this empowerment hypothesis, our data show that women who attained higher levels of formal education, and those who choose to use condoms for contraception, are more likely to report that they use condoms during sex. Our findings, from a unique cohort of women at high risk for HIV infection with a range of partner types, suggest that focusing on ensuring higher levels of formal education in women and de-stigmatizing condom use, for example by emphasizing condom use as contraception, particularly among men who may view condom use for STI prevention as an indication of infidelity or a lack of trust, are essential components of HIV prevention efforts and promoting safer sexual practices in this part of the world.

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Author Contributions

Conceived and designed the experiments: FvL KM AG MES. Performed the experiments: FvL KM LW AG. Analyzed the data: LW AG AAD. Contributed reagents/materials/analysis tools: FvL KM AG LW. Wrote the paper: FVL AAD MES LW AG KM. Performed the literature review: AG.

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Chapter 4:

4.1 Symptomatic Vaginal Discharge Is a Poor Predictor of Sexually Transmitted Infections and Genital Tract Inflammation in High-Risk Women in South Africa. *Mlisana, Koleka, Nivashnee Naicker, Lise Werner, Lindi Roberts, Francois van Loggerenberg, Cheryl Baxter, Jo-Ann S. Passmore et al. Journal of Infectious Diseases 206, no. 1 (2012): 6-14.*

4.2 Classical Sexually Transmitted Diseases Drive the Spread of HIV-1: Back to the Future. *Cohen, Myron S. Journal of Infectious Diseases 206, no. 1 (2012): 1-2.*

MAJOR ARTICLE

Symptomatic Vaginal Discharge Is a Poor Predictor of Sexually Transmitted Infections and Genital Tract Inflammation in High-Risk Women in South Africa

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(See the editorial commentary by Cohen, on pages 1–2.)

Background. Diagnosis and treatment of sexually transmitted infections (STIs) is a public health priority, particularly in regions where the incidence of human immunodeficiency virus (HIV) infection is high. In most developing countries, STIs are managed syndromically. We assessed the adequacy of syndromic diagnosis of STIs, compared with laboratory diagnosis of STIs, and evaluated the association between STI diagnosis and the risk of HIV acquisition in a cohort of high-risk women.

Methods. HIV-uninfected high-risk women (n = 242) were followed for 24 months. Symptoms of STIs were recorded, and laboratory diagnosis of common STI pathogens was conducted every 6 months. Forty-two cytokines were measured by Luminex in cervicovaginal lavage specimens at enrollment. Human immunodeficiency virus type 1 (HIV-1) infection was evaluated monthly.

Results. Only 12.3% of women (25 of 204) who had a laboratory-diagnosed, discharge-causing STI had clinically evident discharge. Vaginal discharge was thus a poor predictor of laboratory-diagnosed STIs (sensitivity, 12.3%; specificity, 93.8%). Cervicovaginal cytokine concentrations did not differ between women with asymptomatic STIs and those with symptomatic STIs and were elevated in women with asymptomatic STIs, compared with women with no STIs or bacterial vaginosis. Although laboratory-diagnosed STIs were associated with increased risk of HIV infection (hazard ratio, 3.3 [95% confidence interval, 1.5–7.2)], clinical symptoms were not.

Conclusions. Syndromic STI diagnosis dependent on vaginal discharge was poorly predictive of laboratory-diagnosed STI. Laboratory-diagnosed STIs were associated with increased susceptibility to HIV acquisition, while vaginal discharge was not.

Sexually transmitted infections (STIs) impose a major health burden, particularly in developing countries such as South Africa, where the prevalence of human immunodeficiency virus type 1 (HIV-1) infection is high [1, 2]. In South Africa, most new HIV infections are sexually transmitted, and women are at higher risk of infection than men [3]. As STIs are associated with increased susceptibility to HIV infection, they have likely played a central role in facilitating the spread of HIV [4–11]. STI management is thus a key issue in preventing HIV infection in countries where both HIV infection and other STIs are prevalent.

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Developing countries such as South Africa manage most STIs syndromically, on the basis of signs and symptoms [12]. Etiological diagnosis is expensive and time-consuming and requires laboratory equipment and suitably trained staff, whereas syndromic management is easily implemented and inexpensive, with immediate treatment available [12, 13]. However, because the STI syndromes used for diagnosis are nonspecific and because a large proportion of STIs are asymptomatic, undertreatment, and sometimes overtreatment, are pervasive [14–18]. In South Africa, asymptomatic infections occur in almost 50% of STI-infected women who do not seek care and therefore remain untreated [19]. Untreated STIs are associated with significant direct sequelae, including pelvic inflammatory disease (PID), ectopic pregnancy, and tubal factor infertility [20]. On the other hand, a significant number of women with conditions such as vaginal discharge resulting from a derangement of normal vaginal flora may be treated for STIs that they do not have. The costs associated with overtreatment include the financial burden of supplying medicines, the potential development of antimicrobial resistance, and the social cost of misdiagnosis.

Several studies have investigated the influence of different strategies for STI management on the risk of HIV infection. Population-wide treatment of bacterial infections or therapy for herpes simplex virus 2 (HSV-2) was found to be ineffective at reducing HIV infection [21–25]. Two of 3 syndromic management interventions found no difference in HIV acquisition [26–29], suggesting that asymptomatic infections may play a role. In addition to causing visible clinical symptoms, certain STIs may be associated with subclinical manifestations, including elevated genital tract inflammatory cytokine responses [14, 30]. Although a direct association between genital tract inflammatory cytokines and susceptibility to HIV infection has not yet been demonstrated, previous studies have suggested that inflammatory cytokines may directly upregulate HIV replication in the genital tract by activating nuclear factor κB and by recruiting and activating various immune cells which act as targets for HIV infection [31–34]. Additionally, proinflammatory cytokines such as tumor necrosis factor α (TNF-α) may facilitate HIV infection by disrupting tight junctions between epithelial cells, reducing the integrity of this barrier [35–37].

The aim of this study was to establish the prevalence and incidence of STIs in a longitudinal cohort of HIV-uninfected women with multiple sexual partners in South Africa. The adequacy of syndromic management was evaluated by comparing clinical signs and symptoms to laboratory diagnosis of STIs. Inflammatory cytokine concentrations in cervicovaginal lavage (CVL) specimens were measured to determine whether women who had asymptomatic laboratory-diagnosed STIs also had subclinical inflammation that may facilitate HIV infection. Last, associations between STIs, cytokine concentrations, and HIV acquisition were investigated.

MATERIALS AND METHODS

Study Design

A total of 775 high-risk women were screened for HIV infection, and 245 HIV-uninfected women were enrolled into a prospective observational cohort study, the CAPRISA 002 Acute HIV Infection study, which was conducted in Durban, South Africa [38]. Participants attended the clinic monthly for risk-reduction counseling, condom provision, and HIV testing. STIs were assessed at enrollment and every 6 months thereafter. Acute HIV infection was diagnosed by detection of HIV RNA in the absence of HIV antibodies or on the basis of a reactive HIV antibody test within 5 months of a previously known negative antibody result. Laboratory diagnosis of STIs was conducted at enrollment and every 6 months thereafter. Ethics approval for the study was obtained from the University of KwaZulu-Natal and the University of Cape Town. Written informed consent was obtained from all participants.

Laboratory Diagnosis of STIs

A gynecological examination, including a speculum examination using an unlubricated sterile bivalve speculum, was performed at enrollment and every 6 months thereafter to visualize any cervical changes, and appropriate samples were collected from any suspicious lesions. Two vulvovaginal swab specimens were collected from the posterior fornices and lateral vaginal walls at each examination. Blood specimens were collected for serological testing for Treponema pallidum and HSV-2. All specimens were transported to the diagnostic laboratory (Medical Microbiology Laboratory, University of KwaZulu-Natal) within 1 hour of collection for same-day processing. One vulvovaginal swab specimen was rolled onto a glass slide for Gram staining for diagnosis of bacterial vaginosis, using Nugents criteria. The second swab specimen was used to extract DNA for detection of Neisseria gonorrhoeae and Chlamydia trachomatis, using the BDProbe Tec ET polymerase chain reaction (PCR) assay (Becton Dickinson Microbiology Systems, United States), and for detection of Trichomonas vaginalis, Mycoplasma genitalium, and HSV-2, using an in-house PCR assay [39].

Genital ulcers were diagnosed as previously described, on the basis of PCR testing for T. pallidum, Haemophilus ducreyi, C. trachomatis (lymphogranuloma venereum types), and HSV-2 [40]. An impression slide of ulcers for microscopy was collected for the diagnosis of Calymmatobacterium granulomatis, if suspected clinically. HerpeSelect-1 and HerpeSelect-2 enzyme immunoassays were used to confirm genital herpes diagnosis and to identify asymptomatic carriers. Syphilis screening was done using the Becton Dickinson Macro-Vue RPR (rapid plasma reagin [RPR]) card test, and positive reactions were confirmed by the T. pallidum hemagglutination test (Omega ImmuTrep TPHA test). The RPR test was done on serum samples that were undiluted or diluted at ratio of 1:8.

Collection of CVL Specimens and Cytokine Measurements

CVL samples for cytokine measurements were collected from study participants at enrollment into the study, as previously described [41]. Sterile normal saline (10 mL) was used to repeatedly bathe the cervix. The fluid was allowed to pool in the posterior fornix, where it was then aspirated into a plastic bulb pipette. Samples were centrifuged, and the supernatant was stored at −80°C. CVL samples were not collected from menstruating participants, for whom sampling was postponed to the following week. CVL samples for cytokine analysis were available for a subset of 227 of 242 women who were included in this study, of whom 66 had ≥1 STI (excluding bacterial vaginosis and HSV-2 serology findings). Prior to cytokine measurements, CVL samples were prefiltered by centrifugation using 0.2 µm cellulose acetate filters (Sigma, United States). Concentrations of epidermal growth factor, eotaxin/CCL11, fibroblast growth factor 2, fms-like tyrosine kinase 3 ligand (Flt-3L), fractalkine/CX3CL1, granulocyte colony-stimulating factor (CSF), granulocyte macrophage CSF, growth related oncogene family (CXCL1-CXCL3), interferon α (IFN-α), interferon γ (IFN-γ), interleukin 1α (IL-1α), interleukin 1β (IL-1β), interleukin 1 receptor antagonist, interleukin 2 (IL-2), interleukin 3, interleukin 4, interleukin 5, interleukin 6 (IL-6), interleukin 7, interleukin 8 (IL-8)/CXCL8, interleukin 9, interleukin 10, interleukin 12p40, interleukin 12p70 (IL-12p70), interleukin 13 (IL-13), interleukin 15, interleukin 17 (IL-17), IFN-γ–induced protein 10/CXCL10, monocyte chemotactic protein 1/CCL2, monocyte chemotactic protein 3/CCL7, macrophage-derived chemokine/CCL22, macrophage inflammatory protein 1α/CCL3, macrophage inflammatory protein 1β/CCL4, platelet-derived growth factor AA, platelet-derived growth factor AB/BB, RANTES/CCL5, soluble CD40 ligand (sCD40L), soluble IL-2 receptor α, transforming growth factor α, tumor necrosis factor α (TNF-α), tumor necrosis factor β (TNF-β), and vascular endothelial growth factor (VEGF) were measured using Human Cytokine LINCOplex kits (LINCO Research, MO), according to the manufacturer's protocol. The sensitivity of these kits ranged from 0.01 and 18.3 pg/mL for each of the 42 cytokines measured. Data were collected using a Bio-Plex Suspension Array Reader (Bio-Rad Laboratories), and a 5-parameter logistic regression formula was used to calculate sample concentrations from the standard curves. Data were analyzed using BIO-plex manager software (version 4). Cytokine levels that were below the lower limit of detection of the assay were reported as the midpoint between the lowest concentration measured and zero.

Statistical Methods

Data was analyzed using SAS, version 9.2 (SAS Institute, Cary, NC), GraphPad Prism version 5 (GraphPad Software, San Diego, CA), and STATA (StataCorp, TX). Demographic and baseline behavioral characteristics are described overall and by STI and genital symptom profile at enrollment. The Fisher exact test and the Kruskal–Wallis test were used to compare characteristics between the groups. STI incidence was calculated during follow-up for participants who were STI negative at baseline or for whom resolution of a previous infection allowed detection of a newly acquired STI. Sensitivity, specificity and positive and negative predictive values were calculated for STI diagnosis by discharge and genital ulcers, using laboratory results as the true diagnosis. A Mann–Whitney U test was used to compare cytokine concentrations at enrollment in unmatched women with asymptomatic and symptomatic STIs and women with none of the assessed STIs. Hierarchical clustering was used to cluster women according to the relatedness of their cytokine expression profiles (Qlucore Omics Explorer). Principal component analysis (PCA) was used to simplify the data set and generate new variables (component estimates), which are representative of the unique and common variance of each of the included cytokines. P values were adjusted using a false discovery rate step-down procedure in order to reduce false-positive results when multiple comparisons were made [42]. The hazard ratios (HRs) for HIV infection were calculated using proportional hazards regression models with STIs as time-varying covariates. A multivariate proportional hazard model was also fitted, with adjustment for all laboratory-diagnosed STIs, clinical symptoms, and demographic and behavioral factors. Only STI infections diagnosed prior to the estimated date of HIV infection were included in the models predicting HIV infection. Demographic and behavioral factors associated with HIV risk will, however, be explored in more detail in a separate analysis. Participants lost to follow-up were censored at the last contact visit, while all other participants were censored at the time of the last clinic visit.

RESULTS

High Prevalence and Incidence of STIs in this Cohort of High Risk Women

A total of 245 high-risk HIV-uninfected women were enrolled into this study and followed for up to 24 months. Of these, 242 were included in the STI analysis, as 3 participants did not have preinfection data available. At enrollment, 57.0% of women reported having multiple "stable" partners, while 35.7% had 1 stable partner or were married. However, most women (95.0%) reported having had >1 casual sex partner within the last 3 months, and 78.8% were self-identified female sex workers (Table 1) [38]. The prevalence of laboratory-diagnosed STIs at enrollment was high: 20.3% had T. vaginalis, 5.4% had N. gonorrhoeae, 4.2% had C. trachomatis, 1.2% had M. genitalium, and 3.7% were HSV-2 positive on PCR. Of these, 25.3% of women received a diagnosis one of 1 STI, and 5.8% received a diagnosis of ≥2 STIs. HSV-2 antibodies were detected in 86.0% of the women, while the prevalence of

Table 1. Demographic and Behavioral Characteristics of Study Participants

Characteristic	Total (n = 245)	No STI, BV, or Symptoms (n = 83)	Laboratory-Diagnosed STI		P
			Asymptomatic (n = 65)	Symptomatic (n = 12)	
Education					
Primary school	60 (24.5)	18 (21.7)	14 (21.5)	2 (16.7)	.7827
Secondary school to grade 10	84 (34.3)	29 (34.9)	28 (43.1)	6 (50.0)	
Secondary school grade 11 and higher	101 (41.2)	36 (43.4)	23 (35.4)	4 (33.3)	
Marital status					
Single	18 (7.4)	9 (11.0)	6 (9.2)	0 (0.0)	.1336
Stable partner/married	87 (35.7)	32 (39.0)	22 (33.9)	1 (8.3)	
Many partners	139 (57.0)	41 (50.0)	37 (56.9)	11 (91.7)	
No. of casual sex partners in last 3 mo					
0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.0475
1	12 (5.0)	5 (6.2)	2 (3.1)	0 (0.0)	
2–5	205 (85.4)	73 (90.1)	51 (79.7)	10 (83.3)	
>5	23 (9.6)	3 (3.7)	11 (17.2)	2 (16.7)	
No. of steady sexual partners in last 3 mo					
0	8 (3.3)	1 (1.2)	5 (7.8)	0 (0.0)	.0717
1	157 (65.2)	59 (72.0)	35 (54.7)	10 (83.3)	
2–5	70 (29.1)	20 (24.4)	22 (34.4)	1 (8.3)	
>5	6 (2.5)	2 (2.4)	2 (3.1)	1 (8.3)	
No. who reported condom use at last sex act	144 (58.8)	54 (65.1)	36 (55.4)	8 (66.7)	.4390
Mean age in years (SD)	34.2 (10.5)	34.6 (9.7)	34.2 (11.5)	31.6 (8.0)	.5974
Mean age of sexual debut in years (SD)	17.0 (2.4)	17.1 (2.4)	16.4 (1.9)	16.4 (2.6)	.2830

Abbreviations: BV, bacterial vaginosis; STI, sexually transmitted infection.

bacterial vaginosis was 52.7% (Table 2). During the study period, the incidence of any STI (excluding bacterial vaginosis and HSV-2 serology findings) was 26.7 cases per 100 person-years, and the incidences of T. vaginalis, N. gonorrhoeae, C. trachomatis, M. genitalium, and T. pallidum were 14.7, 3.3, 4.8, 3.8, and 2.1 cases per 100 person-years, respectively.

Most Women With Laboratory-Diagnosed STIs Were Asymptomatic

Occurrence of genital ulcer disease and abnormal vaginal discharge was compared to laboratory diagnosis of STIs. Only 3 participants presented with genital ulcers during the follow-up period, and none presented at enrollment. Each of these women tested positive for HSV-2 IgG antibodies prior to the ulcer episode. However, only one of these women tested positive for HSV-2 by PCR after receiving an etiological diagnosis. The other 2 women were negative for all of the ulcer-causing organisms assessed (T. pallidum, H. ducreyi, C. trachomatis, HSV-2, and C. granulomatis).

At enrollment, 15.3% of women presented with vaginal discharge, while only 1.5% and 4.8% reported vaginal discharge at their 6- and 12-month study visits, respectively (Table 2). Contrary to this, at enrollment 27.5% of women tested positive for a vaginal discharge–associated STI (T. vaginalis, N. gonorrhoeae, M. genitalium, or C. trachomatis), with 18.5% and 23.9% testing positive at their 6- and 12-month visits, respectively.

Overall, only 34.3% of vaginal discharge incidents were accompanied by a positive laboratory test for ≥1 discharge-associated STI (Table 3). Meanwhile, a total of 87.7% laboratory-diagnosed STIs (discharge associated) had no accompanying clinical symptoms. Assuming laboratory testing of the above STIs to be the reference standard, the presence of discharge for screening had a sensitivity of 12.3%, a specificity of 93.8%, a positive predictive value of 34.3%, and a negative predictive value of 80.2%. Therefore, only 12.3% of women with a confirmed laboratory-diagnosed STI would have been appropriately treated using vaginal discharge as an indicator for syndromic treatment.

Asymptomatic Laboratory-Diagnosed STIs Were Associated With Elevated Genital Tract Inflammatory Cytokine Concentrations

The concentrations of 42 cytokines were measured in CVL samples from study participants in order to determine whether women who had asymptomatic STIs also had

Table 2. Prevalence and Incidence of Laboratory-Diagnosed Sexually Transmitted Infections (STIs) and Clinical Symptoms

Variable	Prevalence, % (Proportion)			Incidence, Cases/100 py (95% CI)
	Enrollment (n = 242)	6 mo (n = 260)	12 mo (n = 189)	
Laboratory diagnosis				
HSV-2 serology	86.0 (208/242)	90.1 (183/203)	91.0 (171/188)	26.0 (11.2–51.2)
HSV-2 PCR	3.7 (9/241)	1.5 (3/205)	2.2 (4/185)	4.1 (2.2–6.9)
T. pallidum	2.9 (7/242)	3.0 (6/202)	1.6 (3/184)	2.1 (.8–4.3)
BV	52.7 (127/241)	46.8 (96/205)	42.9 (79/184)	50.5 (40.9–61.6)
T. vaginalis	20.3 (49/241)	13.2 (27/205)	17.3 (32/185)	14.7 (10.7–19.7)
N. gonorrhoeae	5.4 (13/240)	2.0 (4/205)	2.2 (4/184)	3.3 (1.6–5.8)
C. trachomatis	4.2 (10/240)	4.4 (9/205)	3.3 (6/184)	4.8 (2.7–7.7)
M. genitalium	1.2 (3/241)	1.5 (3/205)	3.8 (7/185)	3.8 (2.0–6.5)
Any STI (excluding BV and HSV-2 serology)	31.3 (75/240)	21.7 (44/203)	27.5 (50/182)	26.7 (20.8–33.7)
1	25.3 (61/241)	17.6 (36/205)	24.3 (45/185)	…
>1	5.8 (14/241)	3.9 (8/205)	2.7 (5/185)	…
Clinical diagnosis				
Vaginal discharge	15.3 (37/242)	1.5 (3/203)	4.8 (9/189)	14.2 (10.6–18.5)

Abbreviations: BV, bacterial vaginosis; CI, confidence interval; C. trachomatis, Chlamydia trachomatis; HSV-2, herpes simplex virus 2; Mycoplasma genitalium, M. genitalium; N. gonorrhoeae, Neisseria gonorrhoeae; PCR, polymerase chain reaction; py, person-years; T. pallidum, Treponema pallidum; T. vaginalis, Trichomonas vaginalis.

subclinical genital inflammation. Cytokine concentrations in CVL specimens were compared in (1) women who tested negative for all assessed STIs and bacterial vaginosis, (2) women who had vaginal discharge and tested positive for ≥1 STI (excluding bacterial vaginosis and HSV-2 serology findings), and (3) women who had no clinical symptoms but tested positive for ≥1 STI (excluding bacterial vaginosis and HSV-2 serology findings; Figure 1A). The concentrations of IL-1α,

Table 3. Sensitivity and Specificity of Discharge and Other Symptoms of Sexually Transmitted Infections (STIs) in Detecting STIs

Variable	Clinical Symptoms	Laboratory Diagnosis		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		+	–				
Any discharge-causing STI (including BV)	+	55	18	10.5	96.0	75.3	48.1
	–	468	433				
Any discharge-causing STI (excluding BV)	+	25	48	12.3	93.8	34.3	80.2
	–	179	723				
BV	+	44	29	10.0	94.4	59.5	55.9
	–	398	504				
T. vaginalis	+	20	54	13.8	93.5	27.0	86.2
	–	125	778				
N. gonorrhoeae	+	5	68	17.9	92.8	6.9	97.5
	–	23	879				
C. trachomatis	+	5	68	13.9	92.8	6.9	96.6
	–	31	871				
M. genitalium	+	2	72	10.5	92.5	2.7	98.1
	–	17	886				
HSV-2 (by PCR)	+	2	72	8.7	92.5	2.7	97.7
	–	21	882				

Abbreviations: BV, bacterial vaginosis; C. trachomatis, Chlamydia trachomatis; HSV-2, herpes simplex virus 2; Mycoplasma genitalium, M. genitalium; N. gonorrhoeae, Neisseria gonorrhoeae; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; T. vaginalis, Trichomonas vaginalis.

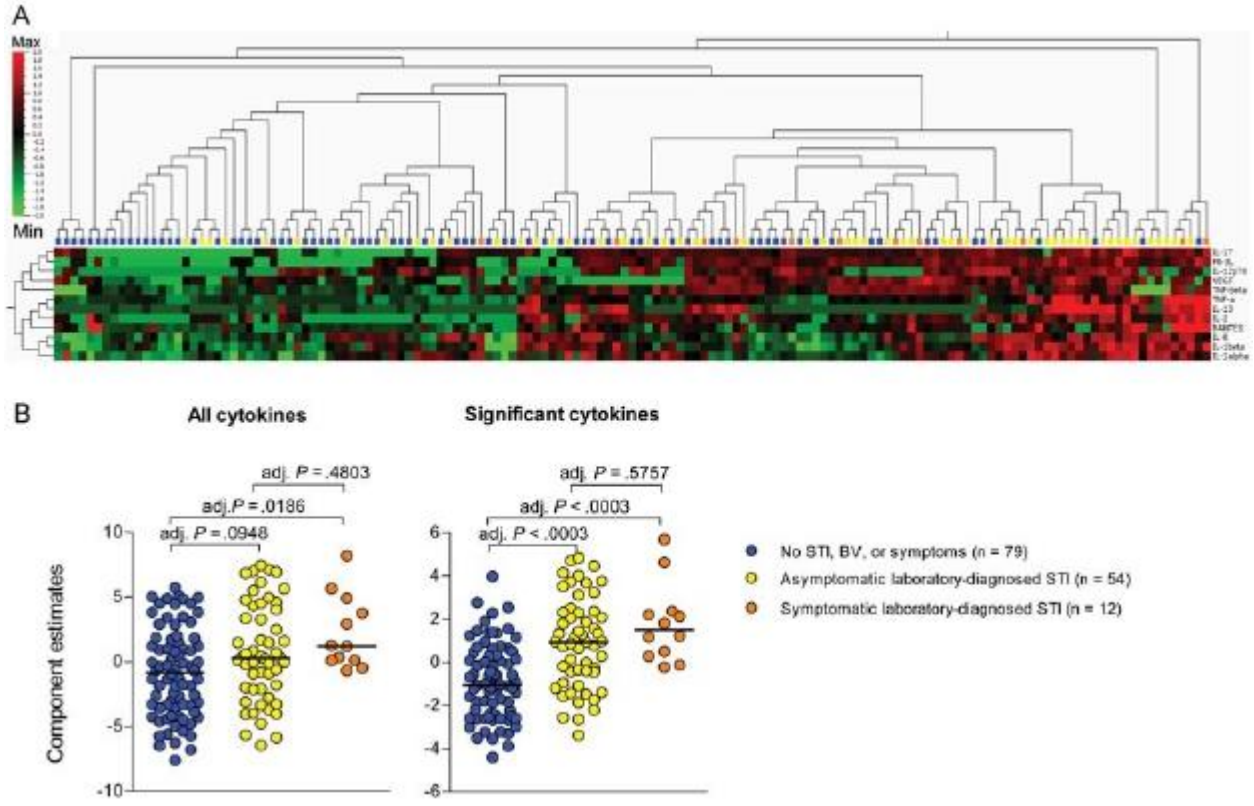

Figure 1. Cervicovaginal (CVL) cytokine profiles of women who did not have a sexually transmitted infection (STI), compared with women who had asymptomatic or symptomatic infections. A, Cytokine concentrations were measured in CVL samples that were available for 227 of 242 participants in this study. Women who did not have an STI, bacterial vaginosis (BV), or vaginal discharge (blue dots/blocks), women who had an asymptomatic STI (yellow), and women who had a symptomatic STI (orange) were clustered according to their genital cytokine concentrations. Only the cytokines that differed significantly between these groups after adjustment for multiple comparisons were included in this analysis. Abbreviations: Max, maximum standardized cytokine concentration measured; Min, minimum standardized cytokine concentration. B, Principal component analysis was used to group either (1) all cytokines or (2) cytokines that differed significantly between the groups into single components and generate estimates representative of each component. P values were adjusted for multiple comparisons, using a false discovery rate step-down procedure in order to reduce false-positive results when multiple comparisons were made. Adjusted (adj.) P values < .05 were considered statistically significant.

IL-1β, IL-12p70, TNF-α, TNF-β, RANTES, Flt-3L, VEGF, IL-2, and IL-17 were significantly elevated in CVL specimens from women who had vaginal discharge and a laboratory-diagnosed STI, compared with women who had no STI or bacterial vaginosis, after adjustment for multiple comparisons (adjusted P values: .0043, .0016, .0016, .0152, .007, .0064, .0039, .014, .012, and .0294, respectively). Levels of 7 of these cytokines (IL-1α, IL-1β, IL-12p70, TNF-α, VEGF, IL-2, and IL-17), as well as levels of IL-8 and IL-13, were elevated in women who had ≥1 active STI but no clinical symptoms (adjusted P values: < .0016, < .0042, .012, .0027, .0429, .0115, .0204, .0084, and .0462, respectively). Cytokine concentrations did not differ significantly between women who had asymptomatic STIs and those with symptomatic STIs. It was found that women who had an STI, either symptomatic or asymptomatic, clustered separately from women who did not have an STI (Figure 1A). PCA was used to further reduce the complexity of the data set by defining new variables that were representative of either (1) the unique and common variance of each of the 42 cytokines assessed or (2) the variance of only those 12 cytokines that had significantly elevated levels in CVL from women who had an STI (Figure 1B). It was found that, although the groups of women were not clearly differentiated using the cluster of 42 cytokines, the estimates of a component including only the 12 significant cytokines were significantly higher in women who had either a symptomatic STI or an asymptomatic infection, compared with women who did not have an STI. Therefore, women who had asymptomatic STIs had subclinical inflammation that may increase their susceptibility to HIV infection.

Asymptomatic Laboratory-Diagnosed STIs Were Associated With Increased Risk of HIV Infection

Of the enrolled 245 HIV-seronegative participants, 28 became HIV infected, yielding an HIV incidence of 7.2 cases per 100 women-years (95% confidence interval [CI], 4.5–9.8) [38]. As

Table 4. Sexually Transmitted Infections (STIs) and Risk of Human Immunodeficiency Virus (HIV) Type 1 Infection

Variable	Unadjusted Analysis		Adjusted Analysis	
	HR (95% CI)	P	HR (95% CI)	P
Symptom				
Genital ulcers	0	…	…	…
Discharge	1.04 (.24–4.58)	.9594	0.59 (.12–3.00)	.5283
Laboratory diagnosis				
BV	2.04 (.90–4.63)	.0892	1.69 (.71–4.06)	.2375
HSV-2 serology	1.30 (.31–5.52)	.7234	2.12 (.43–10.50)	.3558
HSV-2 (by PCR)	1.96 (.26–14.59)	.5108	1.42 (.18–11.44)	.7426
T. pallidum	0[a]	…	…	…
T. vaginalis	1.78 (.71–4.50)	.2203	1.74 (.62–4.92)	.2935
N. gonorrhoeae	7.74 (2.82–21.24)	<.0001	4.62 (1.34–15.93)	.0154
C. trachomatis	3.99 (1.19–13.39)	.0250	0.90 (.18–4.63)	.9006
M. genitalium	4.49 (1.04–19.44)	.0446	4.08 (.83–20.19)	.0846
Any STI (excluding HSV-2 and BV)	3.27 (1.49–7.21)	.0033	…	…
No. of concurrent STIs (excluding HSV-2 and BV)				
1	2.93 (1.26–6.82)	.0124	…	…
≥2	6.15 (1.72–22.03)	.0053	…	…

The multivariate model adjusted for all laboratory-diagnosed STIs and clinical symptoms, as well as for demographic and behavioral factors (data not shown).

Abbreviations: BV, bacterial vaginosis; C. trachomatis, Chlamydia trachomatis; HR, hazard ratio; HSV-2, herpes simplex virus 2; Mycoplasma genitalium, M. genitalium; N. gonorrhoeae, Neisseria gonorrhoeae; PCR, polymerase chain reaction; T. pallidum, Treponema pallidum; T. vaginalis, Trichomonas vaginalis.

[a] Participants who tested positive for T. pallidum remained HIV uninfected, and a HR could not be calculated.

3 of these 28 did not have preinfection data, they were excluded from analysis. Among the STIs tested, N. gonorrhoeae, C. trachomatis, and M. genitalium were associated with HIV acquisition (Table 4). After control for demographic and behavioral factors, clinical symptoms, and other STIs, only N. gonorrhoeae remained significant (adjusted HR, 4.62 [95% CI, 1.34–15.93]). The presence of any STI (excluding bacterial vaginosis and HSV-2 serology findings) was also significantly associated with HIV seroconversion (HR, 3.27 [95% CI, 1.49–7.21]). Additionally, the number of STIs was predictive of HIV infection, with women who had 1 STI having a 3-fold increased risk of acquiring HIV (HR, 2.93 [95% CI, 1.26–6.82]), compared with women with no STI, and those who had ≥2 concurrent STIs had a 6-fold increased risk of HIV infection (HR, 6.15 [95% CI, 1.72–22.03]). HSV-2 seropositivity, bacterial vaginosis, and vaginal discharge were not found to increase the risk of HIV acquisition. Genital ulcers were also not associated with increased risk of HIV infection, although the prevalence of ulcers was low in this cohort (n = 3).

Despite the long period between cytokine measurements in CVL specimens (at enrollment) and HIV infection (median, 302 days prior to infection [range, 14–686 days]), several inflammatory cytokines were associated with risk of HIV infection, before adjustment for multiple comparisons. Elevated concentrations of IL-1β, IL-6, IL-8, and sCD40L were associated with greater risk of HIV infection (HR [95% CI], 1.25 [1.05–1.50], 1.28 [1.07–1.54], 1.39 [1.04–1.87], and 1.45 [1.02–2.07], respectively, per 1 $\log_{10}$ pg/mL increase in cytokine concentration). Of these cytokines, IL-1β and IL-8 were elevated in women who had asymptomatic STIs, relative to women who had no STIs.

DISCUSSION

The prevalence of STIs is generally very high in sub-Saharan Africa [1, 2], and these infections are associated with increased susceptibility to HIV acquisition and secondary transmission [4–11]. There is conflicting evidence regarding the use of syndromic management of STIs for reducing HIV incidence, and it is thought that this approach may underestimate STI prevalence, as many infections are asymptomatic [26–29, 43]. The prevalence and incidence of laboratory-diagnosed STIs in this cohort were high; however, only 12.3% of women who tested positive for ≥1 STI had visible clinical symptoms. As a result, 87.7% of STIs in this cohort of high-risk women would have been left untreated in a syndromic management setting. The presence of any laboratory-diagnosed STI was associated with a 3-fold increased risk of HIV infection, and this association was independent of clinical symptoms. The STI most significantly associated with HIV infection was N. gonorrhoeae, increasing the risk of HIV infection by almost 5-fold.

No associations were found between abnormal discharge or genital ulcers and susceptibility to HIV-infection, although only a small proportion of women presented with ulcers in this study. Furthermore, HSV-1 was not tested for in this study but may be considered in future testing algorithms, given the increasing prevalence of this organism [44]. The prevalence and incidence of STIs were much higher than the values of approximately 50% reported in most studies. This might reflect a higher frequency of reinfection due to the higher number of partners in this cohort of mainly commercial sex workers.

Genital tract inflammatory cytokine concentrations were similar in women who had symptomatic and asymptomatic laboratory-diagnosed STIs and were elevated in these women, relative to women who had no STIs, bacterial vaginosis, or symptoms. Previous studies have suggested that elevated genital tract inflammatory cytokine concentrations may facilitate HIV transmission by directly upregulating HIV replication, by recruiting and activating immune cell targets for HIV infection, and by disrupting tight junctions between epithelial cells [31–37]. Although confounded by the long interval between CVL cytokine measurements and time of HIV infection and by the fact that women were treated for STIs in the period between enrollment and the time of HIV infection, we found that higher concentrations of inflammatory cytokines (IL-1β, IL-6, IL-8, and sCD40L) in CVL specimens were associated with increased risk of HIV infection, albeit weakly. This supports our finding that women who had no clinical symptoms but had subclinical STIs were at increased risk of HIV acquisition, with N. gonorrhoeae remaining associated after adjustment for behavioral factors. Although this suggests that underlying genital tract inflammation may be a biological mechanism for HIV transmission in these women, it is also possible that the relationship between elevated inflammation and risk of HIV infection is indirect. Elevated cytokine concentrations were found to be associated with STIs, which may be markers of high-risk sexual activity and thus increased risk of HIV infection due to behavior.

The finding that clinically evident and syndromically managed STIs declined during follow-up while the prevalence of laboratory-diagnosed STI remained relatively high confirms that syndromic management does not address subclinical STI infections, which may contribute to genital inflammation and risk of HIV infection. This has important implications not only for HIV prevention strategies, but also for complications that are associated with untreated inflammatory STIs, including pelvic inflammatory diseases, ectopic pregnancy, and infertility [20, 30].

These data create a compelling argument for readdressing the STI management strategy in high-risk populations in which negotiating condom use is a challenge for women. Healthcare systems should include regular screening for STIs by means of laboratory testing in these groups rather than relying on symptoms only. The increased diagnostic power of PCR technology and the related potential to impact the risk of HIV acquisition could outweigh its high costs. This strategy would lead to treatment of greater numbers of STIs, reducing prevalence and incidence. Another diagnostic approach in underresourced areas would be to explore point-of-care STI testing as an alternative to laboratory testing. Some of these point-of-care tests have proven to be sensitive diagnostic tools in trials and would offer significant benefit in both rural and urban poor-resourced settings that do not have good access to a larger STI sentinel screening laboratory [45, 46].

Findings from this study and those from studies of other high-risk cohorts in South Africa [13] suggest that the current symptom-driven syndromic management system is untenable for high-risk populations and underscores the need for a paradigm shift in diagnosing STIs. Only when more effective STI treatment is achieved are we likely to see STI management playing a role in HIV prevention.

Notes

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Classical Sexually Transmitted Diseases Drive the Spread of HIV-1: Back to the Future

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(See the major article by Mlisana et al, on pages 6–14.)

The transmission of human immunodeficiency virus type 1 (HIV-1) depends on the infectiousness of the index case (ie, vector) and the susceptibility of the host [1]. The probability of the transmission event has been extensively studied [1–3], and the risk is often described as about 1 in 1000 coital events [4]. However, large numbers of exposures like these are often derived from studies of stable, heterosexual, discordant couples [4, 5]. By definition, the HIV-1–negative partners in these couples can be defined as "exposed and uninfected" at the time of enrollment. The transmission of HIV-1 is almost certainly often more efficient than reflected in studies of couples and is likely enhanced by amplifying factors [6].

Perhaps no other HIV transmission cofactor has attracted as much attention as sexually transmitted diseases (STDs). More than 20 years ago, Wasserheit and colleagues described the transparent and omnipresent relationship between classical STDs and HIV-1, coining this unfortunate marriage of pathogens "epidemiologic synergy" [7]. We subsequently showed that infection with Neisseria gonorrhoeae greatly increased shedding of HIV-1from the male genital tract in seminal plasma, offering a biological view of such synergy [8]. In recent years, however, interest in the relationship between STDs and HIV-1 has waned, primarily because it has proven nearly impossible to reduce the spread of HIV-1 through directed or empirical treatment of STDs [9].

In this issue of The Journal of Infectious Diseases, Mlisana et al [10] contribute to this consideration. Because of the limited laboratory infrastructure in low- and middle-income countries, treatment of STDs in women often depends on the recognition of signs and symptoms of vaginal discharge, leading to empirical treatment with antibiotics [11, 12]. Syndromic management is important but often suboptimal since a substantial number of people using this method are over- or undertreated [11, 12].

Mlisana and colleagues [10] have further expanded our concerns about syndromic management. Two-hundred forty-two women at risk for HIV-1 infection were enrolled in a prospective cohort. Four things were measured: the presence or absence of a vaginal discharge, detection of ≥1 STD pathogens, vaginal cytokine concentrations, and HIV-1 acquisition. The results offered a stark reality for HIV-1 prevention and demonstrate yet again that STDs represent a "hidden epidemic," the title of a compelling Institute of Medicine report published more than a decade ago [13]. Only 12.3% of women infected with a pathogen that might cause a vaginal discharge had signs or symptoms of infection. Women with STDs were >3-fold more likely to acquire HIV-1 than those who harbored no pathogens. Women with gonococcal infections, among the most inflammatory of the classical STD agents [14], had had an eye-opening 7-fold increased risk of HIV-1 acquisition, bringing us full circle to earlier reports [8]. Surprisingly, inflammatory cytokines were not significantly different in women with symptomatic STDs, compared with asymptomatic infections, although they were greater than in women with no STDs or with bacterial vaginosis. Passmore et al [15] have reported that some unique inflammatory cytokine profiles predict risk for HIV acquisition.

How can we fit these observations into sensible HIV-1 prevention strategies? Padian et al [9] have provided an exhaustive summary of interventions designed to prevent HIV-1 transmission, emphasizing the general lack of prevention benefit with treatment of classical STDs. The failure of this approach, in my opinion, is not because STDs are not critically important. Rather, we are simply unable to treat the right infections with the right drugs at the right times, and so the results of the interventions prove disappointing. Sadly, except for hepatitis B virus vaccine and HPV vaccine, STD vaccines are not available.

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Where do we go from here? Mlisana and colleagues argue for more frequent STD testing, using point-of-care assays where possible. This recommendation stems directly from our inability to know which women have an STD, as demonstrated in their report. The problem, of course, is that missing healthcare infrastructure and high relative costs for STD testing led to syndromic management of vaginal discharge in the first place, and these limitations have not been resolved. Mlisana et al also indicate that in the absence of some other strategy, the amplification of HIV-1 transmission by STDs—both infectiousness and acquisition—will continue unabated. Perhaps ironically, this past year was filled with great optimism in HIV-1 prevention, leading The Economist to focus on "The End of AIDS" [16] and Secretary of State Hillary Clinton to describe an "AIDS-Free Generation" [17]. But the "hidden epidemic" [13] of classical STDs is squarely blocking optimal prevention of HIV-1 transmission. These STDs—symptomatic or asymptomatic—simply cannot be ignored. As we commit to combination HIV-1 prevention, we must redouble our efforts to think of every possible way to recognize and treat classical STDs. Surely this problem is no more impossible to attack or less important than any other part of the HIV-1 pandemic.

Note

Potential conflicts of interest. Author certifies no potential conflicts of interest.

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Chapter 5:

5.1 Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study. *Masson, Lindi, Koleka Mlisana, Francesca Little, Lise Werner, Nonhlanhla N. Mkhize, Katharina Ronacher, Hoyam Gamieldien et al. Sexually transmitted infections (2014): sextrans-2014.*

ORIGINAL ARTICLE

Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study

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ABSTRACT

Objectives Sexually transmitted infections (STI) and bacterial vaginosis (BV) cause female genital tract inflammation. This inflammation, which is often present in the absence of symptoms, is associated with increased susceptibility to HIV infection. We aimed to evaluate genital cytokine profiles and the degree of inflammation associated with common STIs and BV.

Methods HIV-uninfected women (n=227) were screened for BV, Chlamydia trachomatis, Neisseria gonorrhoeae, Herpes simplex virus type 2 (HSV-2), and Trichomonas vaginalis. Concentrations of 42 cytokines in cervicovaginal lavages and 13 cytokines in plasma were measured using Luminex. Changes in cytokine profiles were evaluated using Mann–Whitney U test, logistic regression and factor analysis. p Values were adjusted for multiple comparisons using a false discovery rate step-down procedure.

Results Women with chlamydia or gonorrhoea had the highest genital cytokine concentrations, with 17/42 and 14/42 cytokines upregulated compared with women with no infection, respectively. BV was associated with elevated proinflammatory cytokine concentrations, but lower chemokine and haematopoietic cytokine concentrations. HSV-2 reactivation was associated with lower levels of inflammation, while trichomoniasis did not cause significant differences in genital cytokine concentrations. Genital infections did not influence plasma cytokine concentrations. Although certain STIs, in particular chlamydia and gonorrhoea, were associated with high genital cytokine concentrations, only 19% of women with an STI/BV had clinical signs.

Conclusions Chlamydia was associated with the highest genital cytokine levels, followed by gonorrhoea, HSV-2, trichomoniasis, and BV. In regions where HIV is prevalent and STIs are managed syndromically, better STI/BV screening is urgently needed, as certain infections were found to be highly inflammatory.

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INTRODUCTION

Sexually transmitted infections (STI) and bacterial vaginosis (BV) are prevalent in South African women at high risk of HIV infection.[1 2] The lower female genital tract is a unique, non-sterile environment that, while tolerant to allogeneic sperm, must respond rapidly to infectious agents.[3] Inflammation is an immune process essential for microbial control and clearance that is initiated and sustained by cytokine production in response to pathogen recognition.[4] STIs are major causes of inflammatory cytokine upregulation and immune cell recruitment to the genital mucosa.[5–9] Although inflammation can play an important role in STI clearance, it may also cause destruction of infected epithelial layers, allowing STI-associated microbes to access deeper tissues.[4 10] Relatively few women spontaneously clear an infection without treatment, and STIs are often recurrent or persistent.[11 12] If untreated, STIs such as Chlamydia trachomatis, Neisseria gonorrhoea and Trichomonas vaginalis are associated with reproductive complications, including pelvic inflammatory disease (PID), ectopic pregnancy, miscarriage, preterm delivery and infertility.[13]

BV is a syndrome characterised by displacement of healthy vaginal flora, predominantly consisting of Lactobacillus species, by Gram-positive and Gram-negative bacteria.[14 15] While BV can upregulate genital proinflammatory cytokines, downregulation of some cytokines may also occur.[7 16 17] Untreated BV is also associated with sequalae including PID, preterm delivery and postpartum infection.[13]

STIs, BV and associated inflammation influence susceptibility to HIV infection.[18–20] We have shown that C. trachomatis, N. gonorrhoeae and Mycoplasma genitalium infections and elevated cervicovaginal lavage (CVL) concentrations of interleukin (IL)-1β, IL-6, IL-8 and soluble CD40L (sCD40L) were associated with increased risk of HIV acquisition.[20] Elevated cytokine concentrations in the genital tract may facilitate HIV infection by recruiting and activating HIV target cells, reducing epithelial barrier integrity and promoting HIV replication through NF-kB activation.[10 21–24]

The aims of this study were to evaluate whether common STIs or BV were associated with specific changes in genital cytokine profiles in women at high risk of HIV infection, and to compare the relative degree of cytokine production associated with each infection.

METHODS

Study participants

A cohort of 242 HIV-uninfected women at high risk of HIV infection was established in Durban, South Africa.[20] For this study, CVLs were available from 227 women and plasma was available from 142 women. This study was approved by the University of KwaZulu-Natal and University of Cape Town Ethics Committees, and all participants provided informed consent.

STI and BV diagnosis

A gynaecological examination was performed and two vulvovaginal swabs were collected.[20] Swabs were screened for C. trachomatis, N. gonorrhoeae, M. genitalium, Herpes simplex virus (HSV) and T. vaginalis by PCR. BV was diagnosed by Gram staining using Nugent's criteria (score ≥7 indicating BV; 4–6 indicating intermediate flora) and a subset of slides (n=172) was examined for the presence of fungal hyphae. Blood was collected for syphilis (Treponema pallidum) diagnosis and HSV-2 serology. ELISA was used to detect HSV-2 gG-2 IgG (HerpeSelect, Focus Diagnostics, USA). BD Macro-Vue Rapid Plasma Reagin (RPR) and haemagglutination tests (ImmuTrep TPHA, Omega Diagnostics, UK) were used to screen for T pallidum.

Cytokine measurements

CVLs (10 mL sterile saline) for cytokine measurements were collected, centrifuged and supernatants stored at −80°C.[25] CVLs were not collected from menstruating participants. Blood was collected by venepuncture into Acid-Citrate-Dextrose (ACD) vacutainer tubes, plasma isolated and stored at −80°C.

In an exploratory analysis, 42 cytokines were measured in CVLs from 227/242 women, and 13 cytokines were measured in plasma from 142/242 women. CVLs were prefiltered by centrifugation using 0.2 μm cellulose acetate filters (Sigma, USA). The concentrations of all 42 cytokines included in LINCOplex Human Cytokine and High Sensitivity Human Cytokine kits (LINCO Research, USA) were measured in CVLs. These included immunoregulatory cytokines (IL-10 and IL-1Ra), growth factors (vascular endothelial growth factor (VEGF), TGF-α, platelet-derived growth factor (PDGF)-AA, PDGF-AB/BB, FGF-2, epidermal growth factor (EGF)), haematopoietic cytokines (IL-9, IL-7, IL-3, GM-CSF, G-CSF, FLT3 Ligand (FLT3L)), adaptive immune mediators (sIL-2Rα, sCD40L, IL-17, IL-15, IL-13, IL-5, IL-4, IL-2, IFN-γ), pro-inflammatory cytokines and chemokines (IFN-α, RANTES/CCL5, MIP-1α/CCL3, MIP-1β/CCL4, MDC/CCL22, MCP-1/CCL2, MCP-3/CCL7, IP-10, IL-8/CXCL8, growth related oncogene (GRO) family (CXCL1-CXCL3), fractalkine, eotaxin, TNF-β, TNF-α, IL-12p70, IL-12p40, IL-6, IL-1β, IL-1α). The lower limit of detection of these kits ranged between 0.01 and 27.65 pg/mL for each of the cytokines. Data was collected using a Bio-Plex Suspension Array Reader (Bio-Rad Laboratories) and a 5 PL regression formula was used to calculate cytokine concentrations from the standard curves (BIO-Plex manager V.4). Cytokine concentrations below the lower limit of detection were reported as the mid-point between the lowest concentrations measured and zero.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, USA) and STATA V.10 (StataCorp, Texas, USA). Mann–Whitney U test was used for comparisons and Spearman Rank test for correlations. Logistic regression was used to assess associations between STIs/BVand $\log_{10}$-transformed cytokine concentrations, while adjusting for coinfections. p Values were adjusted using a false-discovery rate step-down procedure to reduce false positive results when multiple comparisons were made.[26] Confirmatory factor analysis was used to explore underlying associations between cytokines.[27] Factor scores, which are linear combinations of concentrations of each cytokine in a factor, weighted according to their factor loadings, were generated for further analysis. Ingenuity Pathway Analysis was used to explore biological functions of the cytokines (Ingenuity Systems). Cytokine concentrations >2-fold above the median in the no STI/BV group were considered upregulated.

RESULTS

A total of 227 HIV-uninfected women were included in this study to evaluate whether common STIs and BV were associated with specific changes in genital cytokine profiles in women at high risk of HIV infection (table 1).[20]

The median age of these women was 36 years (range 18–58), and most (95%) reported having more than one casual sexual partner within the last 3 months. More than half the women had BV (53%; 120/227; Nugent score ≥7; table 1), 13% (30/227) had intermediate flora (Nugent score 4–6), and 30% (68/227) had a

Table 1 Demographics, behavioural and clinical characteristics

Demographic characteristics (n=227)	n (%)
Race (number of black women)	224 (98.7)
Median age in years (range)	36 (18–58)
Behavioural characteristics	**n/total (%)**
Women with multiple partners	129/226 (57.1)
Single women	17/226 (7.5)
Married or in a stable relationship	80/226 (35.4)
Women with >1 casual sexual partner	209/221 (94.6)
Ever had anal sexual intercourse	77/225 (34.2)
Ever had oral sexual intercourse	59/226 (26.1)
Age at sexual debut (years; median (range))	17 (12–33)
Intravaginal substance use	22/226 (9.7)
Contraception	**n/total (%)**
Condoms used as contraception	133/226 (58.9)
Injectable hormonal contraception	63/226 (27.9)
Oral contraception	5/226 (2.2)
Intrauterine device	5/226 (2.2)
Clinical and laboratory findings (n=227)	**n (%)**
No STI or bacterial vaginosis	85 (37.4)
Trichomonas vaginalis (PCR positive)	47 (20.7)
Chlamydia trachomatis (PCR positive)	10 (4.4)
Neisseria gonorrhoea (PCR positive)	13 (5.7)
Mycoplasma genitalium (PCR positive)	3 (1.3)
HSV-2 IgG	198 (87.2)
HSV (PCR positive)	6 (2.6)
Treponema pallidum (RPR>1:4, TPHA positive)	3 (1.3)
Bacterial vaginosis (Nugent score >7)	120 (52.9)
Intermediate flora (Nugent score 4–6)	68 (30.0)
Any STI or bacterial vaginosis	142 (62.6)
Any STI (excluding bacterial vaginosis)	68 (30.0)
Multiple infections (including bacterial vaginosis)	49 (21.6)
Vaginal discharge	34 (15.0)
Genital ulceration	0 (0.0)

HSV-2, herpes simplex virus type 2; RPR, rapid plasma reagin; STI, sexually transmitted infection; TPHA, Treponema Pallidum hemagglutination.

STI (excluding BV and HSV-2 serology). Of those seropositive for HSV-2 (198/227), only six had detectable HSV in their genital tracts and were considered to have reactivated HSV-2 infections, none had visible ulceration. Three women had RPR titres >1:4 with a positive TPHA test, normally indicative of active syphilis but could indicate late-latent or treated syphilis. Three women were infected with M. genitalium. All women with syphilis or M. genitalium also had BV, therefore, cytokine changes in these women could not be evaluated in this study. Of women who had a STI or BV, only 27/141 (19%) had clinical signs of an infection.[20]

Genital cytokine concentrations were elevated in women with active STIs

Of the common STIs evaluated in this study, women who had chlamydia (10/227) or gonorrhoea (13/227) had the highest CVL cytokine concentrations, despite only 3/10 women with chlamydia and 2/13 women with gonorrhoea having clinical signs of infection (figure 1; see online supplementary figure S1). Concentrations of 17/42 cytokines, including proinflammatory cytokines (IL-1α, IL-1β, IL-6, IL-12p70, TNF-α), chemokines (IL-8, MIP-1β, RANTES), haematopoietic cytokines and growth factors (G-CSF, Flt3L, TGF-α), and adaptive mediators (IL-2, IL-4, IL-5, IL-13, IL-15, IL-17), were higher in CVLs from women with chlamydia compared with women who did not have a STI or BV (n=85), after adjusting for multiple comparisons (figure 1). After adjusting for coinfections, BV and injectable hormone contraceptive use using logistic regression, IL-1β, IL-6, TNF-α, IP-10, MDC, MIP-1α, MIP-1β, RANTES, G-CSF, EGF, FGF-2, PDGF-AA, PDGF-AB/BB, TGF-α, IL-4, IL-5, IL-13, IL-17 and IL-10 concentrations remained significantly associated with chlamydia infections.

Women infected with gonorrhoea displayed a largely overlapping cytokine profile to women with chlamydia, with 11/17 of the same cytokines elevated in women with chlamydia also elevated in women with gonorrhoea (IL-1α, IL-1β, IL-12p70, TNF-α, RANTES, G-CSF, Flt3L, IL-2, IL-5, IL-15 and IL-17). In addition, certain unique cytokines were upregulated in women with gonorrhoea (TNF-β, eotaxin and VEGF; figure 1). Since the majority of women with gonorrhoea also had a coinfection or BV (11/13), none of these cytokines remained significant after adjustment for coinfections, BV and injectable hormone contraceptive use. However, gonorrhoea was associated with high levels of inflammation overall, with a large cumulative β-coefficient (figure 2A).

Although women with active HSV-2 infections (6/227) tended to have elevated CVL concentrations of several proinflammatory cytokines, none was significantly higher than those found in women who did not have an infection, after adjusting for multiple comparisons and/or coinfections (figure 1). However, due to the small number of women who were shedding HSV-2, this analysis was underpowered to detect the changes in proinflammatory cytokine concentrations, particularly after accounting for multiple comparisons. Detection of HSV-2 antibodies in serum alone was not associated with changes in genital cytokine concentrations (data not shown).

Despite being clinically implicated in vaginitis, trichomoniasis (in the absence of BV or coinfections; 13/227) was the least inflammatory infection in this study, with none of the cytokines elevated relative to women with no STI/BV after adjusting for multiple comparisons (figure 1; see online supplementary figure S1). In a logistic regression analysis including all women with trichomoniasis (47/227), no cytokines were significantly associated with this infection after adjustment for coinfections and multiple comparisons (data not shown).

Mixed genital cytokine profile in women with BV

Certain proinflammatory cytokines (IL-1α, IL-1β and TNF-β) were significantly elevated in women who had BV in the absence of a coinfection (n=73), relative to women who had neither BV nor a STI (figure 1; see online supplementary figure S1). However, women with BV simultaneously had lower concentrations of several chemokines (IP-10, GRO, MDC and MIP-1α) and haematopoietic cytokines (IL-7 and GM-CSF), suggesting that cytokine changes associated with BV were complex. Cytokine concentrations in women with intermediate vaginal flora alone (n=18) did not differ from women who did not have a STI/BV (data not shown).

A subset of Gram-stained slides (n=172) was examined for the presence of fungal hyphae. Women with visible fungal hyphae (n=19) had lower genital concentrations of IFN-α, IL-9, IL-15 and IL-17, after adjusting for STIs and BV using logistic regression, with IL-9 remaining significantly lower after adjusting for multiple comparisons.

STIs did not influence cytokine concentrations in plasma

Plasma cytokine concentrations did not differ in women with BV, or active C. trachomatis, N. gonorrhoeae, T. vaginalis, or HSV-2 infections compared to women who did not have a STI/BV. None of the cytokines measured in plasma correlated with those measured in CVLs from the same women (data not shown).

Genital cytokines with similar biological functions clustered together

Confirmatory factor analysis was used to group cytokines according to their primary roles in the immune response in order to reduce the complexity of the dataset and explore the relationships between functional groups of cytokines and STIs/BV (see online supplementary table S1). Cytokines were grouped as proinflammatory (IL-1α, IL-1β, IL-6, IL-12p40, IL-12p70, TNF-α, TNF-β), chemokines (eotaxin, fractalkine, GRO, IL-8, IP-10, MCP-1, MCP-2, MIP-1α, MIP-1β, MDC, RANTES), haematopoeitic (FLT3L, G-CSF, GM-CSF, IL-3, IL-7, IL-9), growth factors (EGF, FGF-2, PDGF-AA, PDGF-AB/BB, TGF-α, VEGF) and adaptive mediators (IFN-γ, IL-2, IL-4, IL-5, IL-13, IL-15, IL-17, sCD40L, sIL-2Rα). Most of the cytokines (34/42) were significantly associated with the functional group to which they were assigned, as indicated by factor loadings >0.32 (which can be interpreted in a similar manner as correlations coefficients).[27] This indicates that the concentrations of most cytokines within each functional classification correlated with one another, and are thus likely to be regulated by common underlying influences.

All functional cytokine groups were positively associated with chlamydia after adjusting for coinfections, while all groups except the anti-inflammatory cytokine group were positively associated with gonorrhoea (figure 2B). In support of the mixed cytokine profile observed for BV (figure 1), the proinflammatory cytokine cluster was positively associated with BV, while the chemokine cluster was inversely associated, after adjusting for coinfections and multiple comparisons.

Cytokines upregulated in women with STIs promote immune cell migration, activation, proliferation and differentiation

The Ingenuity Knowledge Base (Ingenuity Systems) was used to explore the effects that cytokine changes in response to STIs and BV may have at the cellular level in the female genital tract

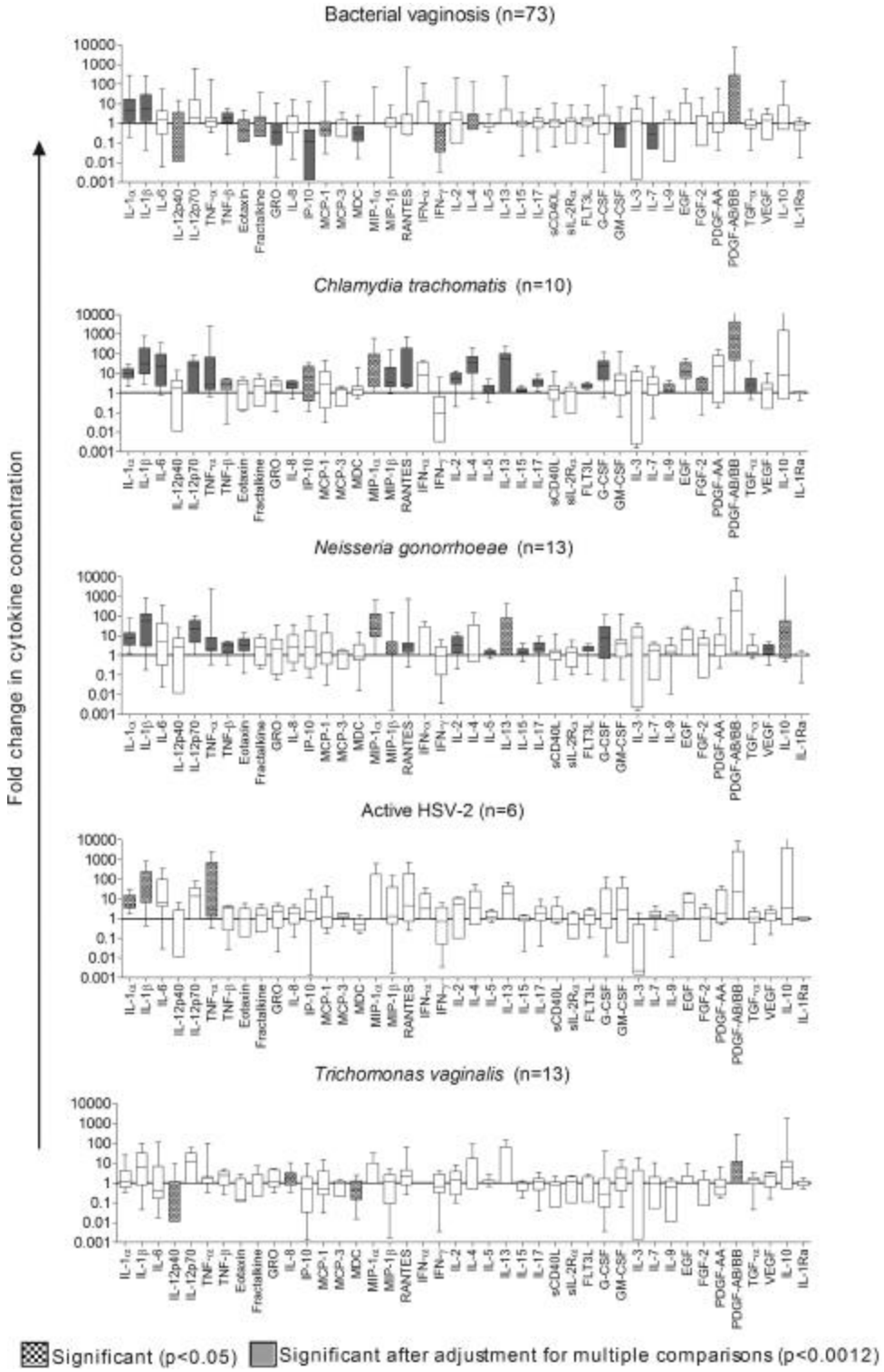

Figure 1 Fold change in cytokine concentrations in cervicovaginal lavage (CVL) from women who had a sexually transmitted infection (STI) or bacterial vaginosis (BV) relative to median concentrations in women who did not have an STI/BV. Several women who had chlamydia, gonorrhoea or herpes simplex virus type 2 (HSV-2) had other coinfections. For the analysis of BV or trichomoniasis, only women who had single infections were included. Mann–Whitney U test was used for comparisons and p values were adjusted for multiple comparisons using a false discovery rate step-down procedure. Cytokines that were significantly altered relative to women who did not have STIs or BV before adjusting for multiple comparisons are indicated by shaded bars (p<0.05). Cytokines that were upregulated or downregulated after adjustment are shown by solid grey bars (p<0.0012).

(table 2). The cytokines assessed in this study primarily play roles in pathways associated with cellular trafficking, death, survival, growth, proliferation, activation and differentiation. Chlamydia (in particular), gonorrhoea and HSV-2, were each associated with upregulation of the largest number of cytokines involved in these pathways, while BV and trichomoniasis were associated with few upregulated cytokines. Women with chlamydia, gonorrhoea or HSV-2 had upregulated concentrations of

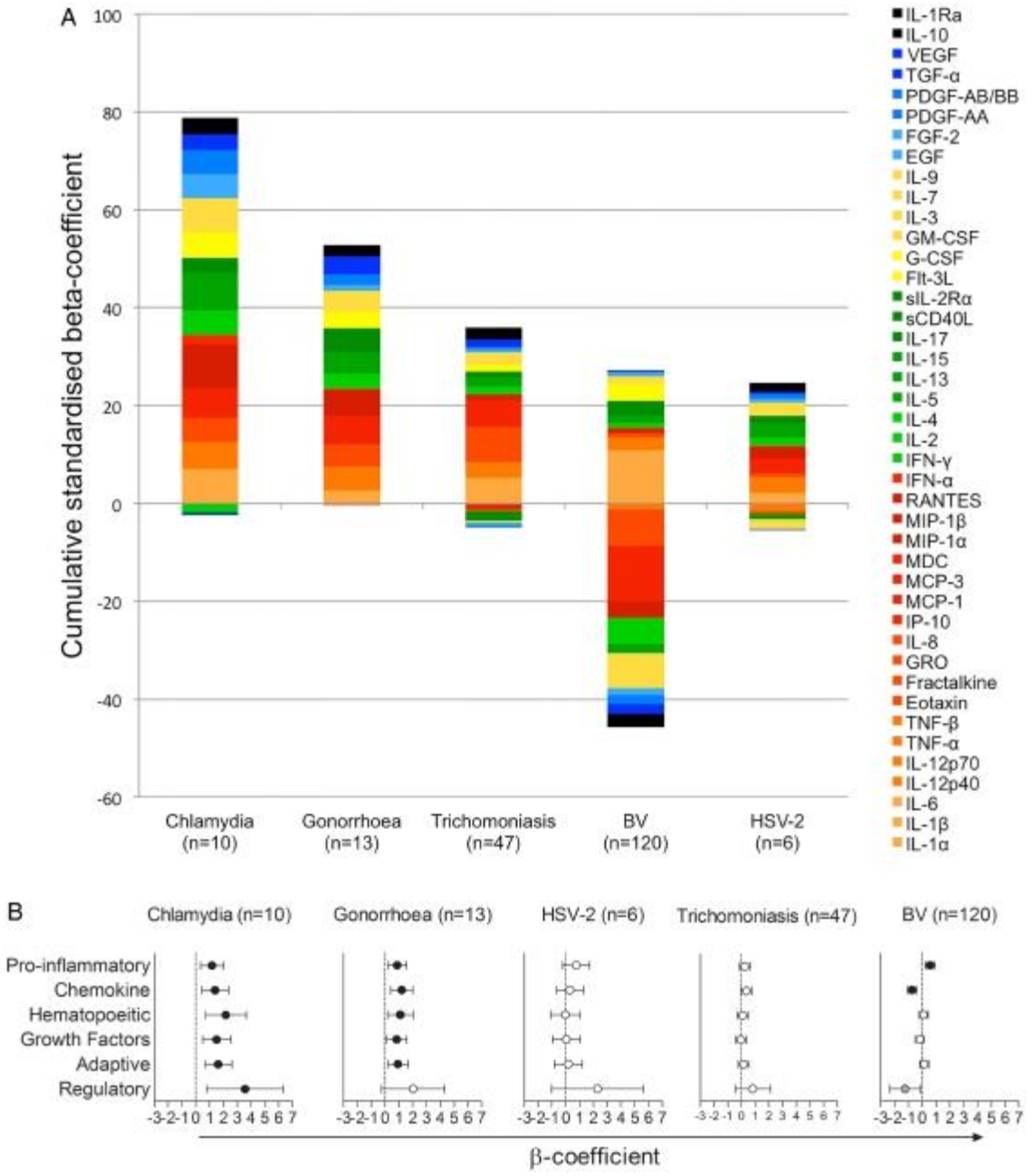

Figure 2 Relationships between cytokines and bacterial vaginosis (BV), trichomoniasis, chlamydia, gonorrhoea and active HSV-2 following adjustment for coinfections. (A) Cytokines were $\log_{10}$-transformed and standardised, and β-coefficients were calculated using logistic regression in order to adjust for coinfections. The β-coefficient, divided by the SE, for each of the 42 cytokines is represented in each stacked bar for each sexually transmitted infection (STI) and BV. A positive β-coefficient indicates that higher concentrations of the cytokine are associated with the STI or BV, while a negative β-coefficient indicates that the cytokine concentration is more likely to be lower in cervicovaginal lavage from women who had an STI or BV, relative to women who did not have an infection. Proinflammatory cytokines and chemokines are shown in shades of orange and red. Adaptive immune mediators are green, haematopoietic cytokines are yellow, growth factors are blue, and immunoregulatory IL-10 and IL-1Ra are black. (B) Associations between factor scores and each STI and BV. Factor scores for each function group were generated using confirmatory factor analysis. Dots indicate β-coefficients of logistic regression analyses, following adjustment for coinfections, error bars represent 95% CIs. Black dots indicate associations that were significant after adjusting for coinfections and multiple comparisons, grey dots indicate associations that fell away after adjusting for multiple comparisons. p Values were adjusted for multiple comparisons using a false discovery rate step-down procedure. HSV-2, Herpes simplex virus type 2.

cytokines that mediate chemotaxis of T cells, natural killer cells, phagocytes, monocytes, dendritic cells, neutrophils, eosinophils, granulocytes and endothelial cells. Cytokines involved in cellular apoptosis, viability, activation, proliferation and differentiation were upregulated in women with chlamydia, gonorrhoea or HSV-2. These findings indicate that increases in the concentrations of these cytokines in the genital tracts of women with chlamydia and, to a lesser extent, gonorrhoea and HSV-2, would likely result in the accumulation of highly activated and differentiated immune cells.

Table 2 Biological pathway analysis of cytokine functions

Biological pathway	Cell type	Cytokines assessed (n)	Number of cytokines >2-fold upregulated (n (%))				
			Chlamydia	Gonorrhoea	HSV-2	Trichomoniasis	BV
Cellular trafficking	All cell types	35	28 (80)	23 (66)	12 (34)	4 (11)	2 (6)
	Leukocyte	31	24 (77)	20 (65)	11 (35)	4 (13)	2 (6)
	T cell	12	11 (92)	8 (67)	5 (42)	2 (17)	0 (0)
	NK cell	7	6 (86)	6 (86)	3 (43)	1 (14)	0 (0)
	Phagocyte	24	20 (83)	17 (71)	14 (58)	4 (17)	2 (8)
	Monocyte	18	15 (83)	13 (72)	9 (50)	3 (17)	1 (6)
	DC	10	7 (70)	6 (60)	5 (50)	2 (20)	1 (10)
	Neutrophil	13	12 (92)	9 (69)	8 (62)	1 (8)	2 (15)
	Granulocyte	19	15 (79)	13 (68)	9 (47)	3 (16)	2 (11)
	Eosinophil	13	11 (85)	9 (69)	6 (46)	2 (15)	1 (8)
	Endothelial	11	10 (91)	7 (64)	5 (45)	1 (9)	0 (0)
Growth and proliferation	All cell types	35	29 (83)	25 (71)	15 (43)	4 (11)	2 (6)
Death and viability	All cell types	33	26 (79)	21 (64)	13 (39)	4 (12)	2 (6)
Activation	All cell types	28	22 (79)	19 (68)	14 (50)	5 (18)	1 (4)
Differentiation	All cell types	27	20 (74)	17 (63)	12 (44)	3 (11)	2 (7)

BV, bacterial vaginosis; DC, dendritic cell; HSV-2, herpes simplex virus type 2; NK, natural killer.

DISCUSSION

In women at high risk of HIV infection, chlamydia and gonorrhoea were associated with broadly elevated genital cytokine concentrations compared with women who did not have a STI or BV. Although few women were shedding HSV-2, several key cytokines with inflammatory functions were upregulated in these women compared with those with no STI or BV. By contrast, BV was associated with a mixed inflammatory profile, with upregulated proinflammatory cytokine concentrations, but downregulated concentrations of chemokines and haematopoietic cytokines. While clear inflammatory cytokine signatures were found in genital secretions of women with BV and certain STIs, they did not have similar cytokine signatures in their plasma. No significant correlations were observed between plasma and genital cytokine concentrations, suggesting that inflammatory responses in the female genital tract are independent of blood cytokine concentrations. This is likely because cytokines are locally produced in response to pathogen recognition. Although certain STIs, in particular chlamydia and gonorrhoea, were associated with high concentrations of cytokine biomarkers of genital inflammation, only 19% of women who had a STI/BV had clinical signs (3/10 and 2/13 women with chlamydia and gonorrhoea, respectively). As previously shown in this cohort, women with an STI but no clinical signs had similar cytokine profiles as women with clinical signs.[20]

The reasons for the mixed cytokine expression profile in women with BV remain unclear, although Ryckman et al[17] similarly reported, using a smaller cytokine panel, that women with BV had elevated proinflammatory IL-1α concentrations, but lower chemokine concentrations (IP-10 and MCP-1) than women with no BV. Others have demonstrated elevated genital IL-1β concentrations from women with BV compared with those with normal flora.[28 29 W1(suppl 3)] Cytokine downregulation associated with BV may reflect a state of tolerance to abnormal vaginal microbes, which may be derived from normal gut microflora, may exist as a result of coevolution of these microbes with humans.[30] These results demonstrate that BV induces changes in the genital cytokine milieu distinct from those that occur in response to STIs.

T. vaginalis infections, which are associated with vaginitis, cervicitis and PID, and increased risk of HIV acquisition,[18 W2 (suppl 3)] were prevalent in this cohort of women (20.7%). Although several cytokines were higher in CVL from women with trichomoniasis compared with women without an infection, none was significant. A previous study similarly reported that women with chlamydia or gonorrhoea had increased endocervical CD4 T cells than women with no STI, which was not observed in women with trichomoniasis.[5] These findings suggest that asymptomatic trichomoniasis, perhaps involving low pathogen loads only detectable by PCR, are not necessarily inflammatory. Trichomoniasis, identified by culture, was associated with increased IL-8 concentrations in vaginal fluid from pregnant women compared with those with no infection.[W3(suppl 3)]

Reddy et al[8] also demonstrated that IFN-γ, TNF-α, IL-10 and IL-12 were upregulated in the genital secretions of women with chlamydia relative to uninfected women. Cohen et al[W4(suppl 3)] showed that IL-10 was more often detectable in genital secretions from women with gonorrhoea, chlamydia or BV, but not trichomoniasis, compared with women with no infection. Infection of cervical and vaginal cell lines with N. gonorrhoeae resulted in upregulated IL-1, IL-6 and IL-8 production.[6] Contrary to these results, some studies have found no difference in cytokines in genital samples from women with chlamydia[W5(suppl 3)] and gonorrhoea,[W6(suppl 3)] compared with uninfected women.

In this cohort, we previously reported that gonorrhoea, chlamydia and M. genitalium infections and elevated genital concentrations of IL-1β, IL-6, IL-8 and sCD40L were associated with increased HIV risk.[20] Genital cytokine upregulation may increase HIV risk by recruiting/activating HIV target cells, reducing epithelial barrier integrity and activating NF-kB.[21 22–24] Many of the upgregulated cytokines in the CVLs from women with chlamydia, gonorrhoea or HSV-2 are involved in recruitment, activation, proliferation and differentiation of HIV target cells. Increased genital concentrations of these cytokines could result in accumulation of activated/differentiated target cells for HIV infection. Women with BV are also at increased risk of HIV acquisition.[19] Although chemokine downregulation in the genital tracts of these women may result in reduced immune cell recruitment, upregulation of proinflammatory cytokines may reduce the integrity of the epithelial barrier, activate HIV target cells that are already present, and promote HIV replication by activating NF-κB.

This study has several limitations: relatively few women had gonnorhoea and chlamydia, possibly limiting our conclusions. Other factors may influence cytokine concentrations in the genital tract. While we showed that women with microscopically evident genital fungal hyphae had lower IFN-α, IL-9, IL-15 and IL-17 concentrations, and the relationships between cytokines and STIs/BV were not influenced by hormone contraceptive use, we have no information on semen exposure, antibiotic use and menstrual cycle phase, which may influence cytokine concentrations. The study was cross-sectional, so long-term implications of these profiles could not be addressed. Finally, this study was largely exploratory and observational, and mechanisms for cytokine differences require further investigation before an intervention can come from these data.

Although chlamydia, gonorrhoea and M. genitalium infections were associated with increased risk of HIV infection in this cohort, most of the women who had one or more of these STIs did not have a clinical sign.[20] As a result, women with these infections would likely have remained untreated in settings where STIs are managed syndromically. This study highlights the urgent need for better strategies to manage asymptomatic STIs, as infections such as chlamydia, gonorrhoea and active HSV-2 were found to be highly inflammatory.

Key messages

- Chlamydia was associated with the highest genital cytokine levels, followed by gonorrhoea, herpes simplex virus type 2, trichomoniasis and bacterial vaginosis (BV).
- Although women with chlamydia or gonorrhoea had very high genital cytokine biomarkers of inflammation, potentially putting them at risk of HIV infection, most lacked clinical signs.
- Women with BV had a mixed genital cytokine profile, with upregulated proinflammatory cytokine concentrations, but also downregulated concentrations of chemokines and haematopoietic cytokines.
- Genital infections did not cause changes in plasma cytokine concentrations, and genital and plasma cytokine concentrations did not correlate.

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Contributors LM performed the laboratory work, analysed the data and prepared the manuscript; KM designed and managed the cohort, analysed the data and contributed to manuscript preparation; FL analysed the data and prepared the manuscript; NNM performed some of the laboratory work, analysed the data and contributed to manuscript preparation; KR analysed the data and prepared the manuscript; HG performed some of the laboratory work, analysed the data and contributed to manuscript preparation; CW analysed the data and prepared the manuscript; LRM analysed the data and prepared the manuscript; GW performed some of the laboratory work and contributed to manuscript preparation; QAK and SAK conceptualised the cohort and prepared the manuscript; J-ASP developed the hypothesis, analysed the data and prepared the manuscript.

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Competing interests None.

Patient consent Obtained.

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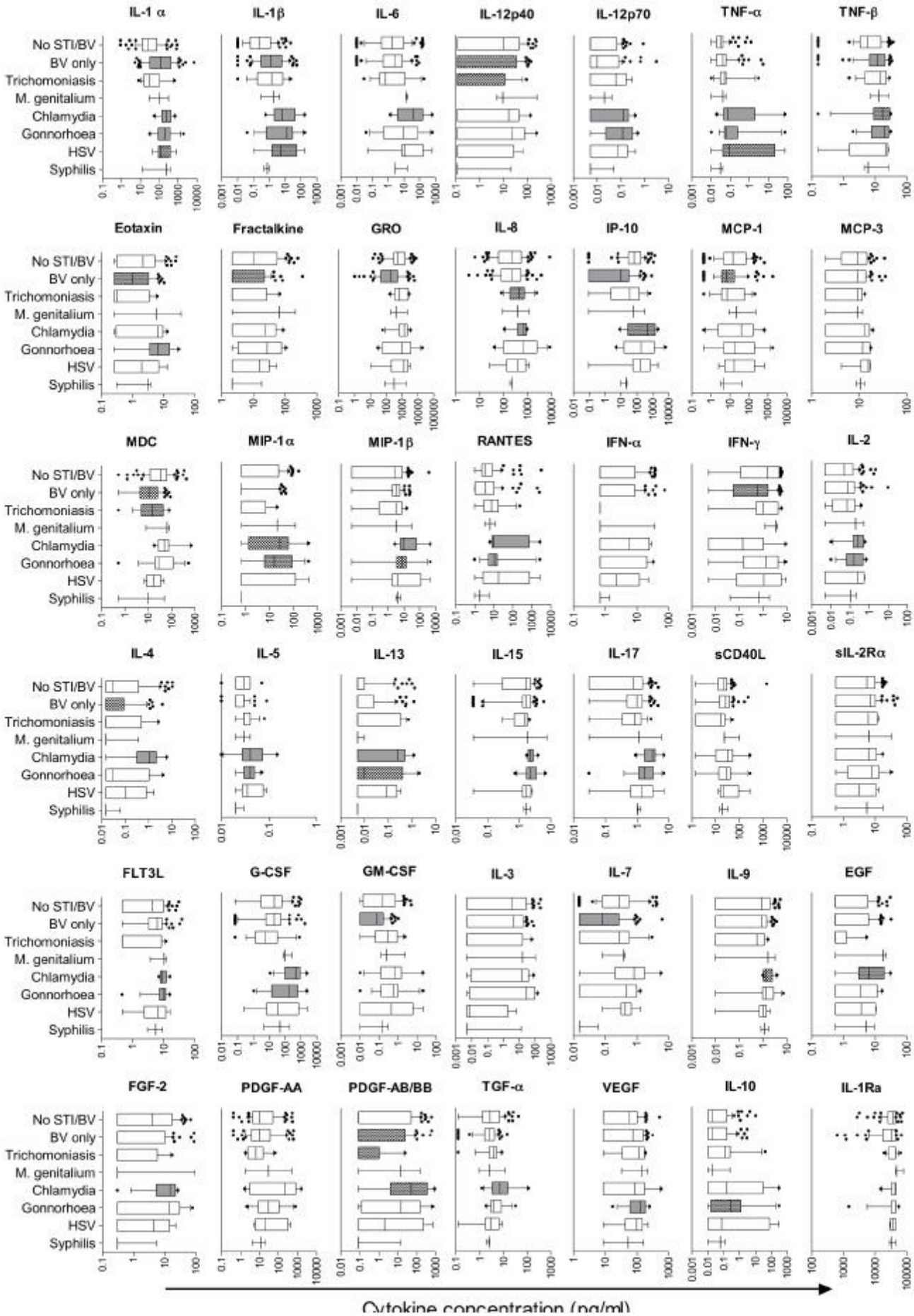
Supplementary Table 1 Factor loadings

	Biological functions (from CFA)		Oblique groupings (from EFA)	
Cytokine	Factor	Loading [a]	Factor	Loading[a]
IL-1α	Pro-inflammatory	**0.49**	Factor 3	**0.62**
IL-1β		**0.81**	Factor 3	**0.89**
IL-6		**0.70**	Factor 3	**0.57**
IL-12p40		0.16	Factor 2	**0.82**
IL-12p70		**0.66**	Factor 4	**0.68**
TNF-α		**0.72**	Factor 3	**0.60**
TNF-β		0.23	Factor 1	**0.51**
Eotaxin	Chemokine	**0.55**	Factor 2	**0.83**
Fractalkine		**0.50**	Factor 2	**0.43**
GRO		**0.72**	Factor 5	**0.73**
IL-8		**0.52**	Factor 3	**0.66**
IP-10		**0.73**	Factor 5	**0.79**
MCP-1		**0.55**	Factor 5	**0.60**
MCP-3		0.29	Factor 4	**-0.30**
MDC		**0.66**	Factor 5	**0.78**
MIP-1α		**0.63**	Factor 2	**0.78**
MIP-1β		**0.43**	Factor 1	**0.73**
RANTES		0.21	Factor 3	**0.39**
IFN-α	Innate		Factor 2	**0.62**
FLT3L	Hematopoietic	**0.72**	Factor 1	**0.71**
G-CSF		**0.55**	Factor 3	**0.43**
GM-CSF		**0.36**	Factor 4	**0.68**
IL-3		**0.53**	Factor 2	**0.56**
IL-7		0.27	Factor 4	**0.69**
IL-9		**0.71**	Factor 1	**0.91**
EGF	Growth Factor	**0.65**	Factor 2	**0.43**
FGF-2		**0.74**	Factor 2	**0.76**
PDGF-AA		**0.43**	Factor 5	**0.41**
PDGF-AB/BB		**0.71**	Factor 2	**0.68**
TGF-α		**0.54**	Factor 1	**0.60**
VEGF		**0.26**	Factor 1	**0.41**
IFN-γ	Adaptive	**-0.40**	Factor 1	**-0.57**
IL-2		**0.61**	Factor 4	**0.53**
IL-4		**0.54**	Factor 4	**0.58**
IL-5		**0.36**	Factor 4	**0.66**
IL-13		**0.52**	Factor 4	**0.40**
IL-15		**0.66**	Factor 1	**0.83**
IL-17		**0.72**	Factor 1	**0.86**
sCD40L		0.17	Factor 1	0.24
sIL-2Rα		**0.57**	Factor 4	**0.38**
IL-10	Anti-inflammatory	0.20	Factor 3	**0.52**
IL-1Ra		0.20	Factor 5	**0.39**

[a]A factor loading can be interpreted in a similar manner as a correlation coefficient. CFA: Confirmatory factor analysis.

Supplementary Figure 1



Supplementary Figure 1. Cytokine concentrations in the genital tracts of women who did not have a sexually transmitted infection (STI) or bacterial vaginosis (BV) were compared to those of women who had BV (n=73), trichomoniasis (n=13), *Mycoplasma genitalium* (n=3), chlamydia (n=10), gonorrhoea (n=13), active HSV (detection of HSV in the genital tract by PCR accompanied by a positive HSV-2 IgG test; n=6) or active syphilis (n=3). Boxes indicate 25-75th percentiles; lines indicate medians; whiskers indicate 10-90th percentiles; dots indicate outliers. Several women who had *M. genitalium*, chlamydia, gonorrhoea, active HSV or active syphilis had other co-infections. For the analysis of BV or trichomoniasis, only women who had single infections were included. P-values were adjusted for multiple comparisons using a false discovery rate step-down procedure. Cytokines that were significantly altered relative to women who did not have an STI or BV before adjusting for multiple comparisons are indicated by shaded bars ($p<0.05$). Cytokines that were up- or down-regulated after adjustment are shown by solid grey bars ($p<0.0012$).

Chapter 6:

6.1 Challenges of Diagnosing Acute HIV-1 Subtype C Infection in African Women: Performance of a Clinical Algorithm and the Need for Point-of-Care Nucleic-Acid Based Testing.

Mlisana, Koleka, Magdalena Sobieszczyk, Lise Werner, Addi Feinstein, Francois van Loggerenberg, Nivashnee Naicker, Carolyn Williamson, and Nigel Garrett. PloS one 8, no. 4 (2013): e62928.

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Challenges of Diagnosing Acute HIV-1 Subtype C Infection in African Women: Performance of a Clinical Algorithm and the Need for Point-of-Care Nucleic-Acid Based Testing

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Abstract

Background: Prompt diagnosis of acute HIV infection (AHI) benefits the individual and provides opportunities for public health intervention. The aim of this study was to describe most common signs and symptoms of AHI, correlate these with early disease progression and develop a clinical algorithm to identify acute HIV cases in resource limited setting.

Methods: 245 South African women at high-risk of HIV-1 were assessed for AHI and received monthly HIV-1 antibody and RNA testing. Signs and symptoms at first HIV-positive visit were compared to HIV-negative visits. Logistic regression identified clinical predictors of AHI. A model-based score was assigned to each predictor to create a risk score for every woman.

Results: Twenty-eight women seroconverted after a total of 390 person-years of follow-up with an HIV incidence of 7.2/100 person-years (95%CI 4.5–9.8). Fifty-seven percent reported $1 sign or symptom at the AHI visit. Factors predictive of AHI included age ≥ 25 years (OR = 3.2; 1.4–7.1), rash (OR = 6.1; 2.4–15.4), sore throat (OR = 2.7; 1.0–7.6), weight loss (OR = 4.4; 1.5–13.4), genital ulcers (OR = 8.0; 1.6–39.5) and vaginal discharge (OR = 5.4; 1.6–18.4). A risk score of 2 correctly predicted AHI in 50.0% of cases. The number of signs and symptoms correlated with higher HIV-1 RNA at diagnosis ($r = 0.63$; $p \geq 0.001$).

Conclusions: Accurate recognition of signs and symptoms of AHI is critical for early diagnosis of HIV infection. Our algorithm may assist in risk-stratifying individuals for AHI, especially in resource-limited settings where there is no routine testing for AHI. Independent validation of the algorithm on another cohort is needed to assess its utility further. Point-of-care antigen or viral load technology is required, however, to detect asymptomatic, antibody negative cases enabling early interventions and prevention of transmission.

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Introduction

The devastating toll of the HIV/AIDS epidemic on sub-Saharan Africa, with 67% of the estimated 33.3 million world-wide HIV infections in this region alone, is well recognized [1]. Individuals with acute HIV infection (AHI) have been shown to be more infectious compared to individuals with chronic infection. Data from several cohorts provide estimates of HIV transmission per coital act in sub-Saharan Africa, with dramatically higher transmission rates during acute and early, compared to latent or chronic infection [2–4]. One study modeling the impact of antiretroviral therapy (ART) on the HIV epidemic estimated that 38.4% of ongoing HIV transmissions are attributable to sexual contact with early HIV index cases [5]. This is presumably due to several factors including very high plasma and genital tract HIV viral load [6–9]. Early identification of individuals in the acute/early stage of infection would not only enable prompt intervention to prevent transmission to negative partners, but may also allow for earlier initiation of treatment. Data on the clinical benefits of ART in patients with acute HIV are conflicting [10–12]. Theoretical benefits of this intervention include potentially preserving immune function, reducing the pool of latently infected CD4 cells, thereby altering the virologic set point and delaying

disease progression. These have to be weighed against side effects and patient readiness to start antiretrovirals [3,13–17].

Making the diagnosis of acute HIV infection is challenging since the signs and symptoms of acute retroviral syndrome are non-specific. Of note, data on clinical characteristics of subtype C infection, particularly among women, are currently limited [18,19]. Days to weeks after acquisition, 40–90% of individuals develop signs and symptoms including a flu-like illness or mononucleosis-like syndrome [20]. The onset of symptoms coincides with viral replication and peak viremia [21–23]. During this window period, only HIV RNA can be detected in plasma until the appearance of p24 antigen and, subsequently, HIV antibodies. Laboratory-based fourth generation Ag/Ab combination assays improve detection of acutely-infected individuals compared to third generation antibody assays. However, in resource limited, high prevalence settings, health services rely predominantly on point-of-care testing (POCT) and, disappointingly, a recent evaluation found that the antigen component of the fourth generation DetermineH HIV-1/2 Ag/Ab Combo rapid test performed poorly in detecting acute infection [24]. Initiating RNA testing still requires clinician's high index of suspicion and availability of resources. Thus, a clinical algorithm which prioritizes nucleic acid HIV testing would be of benefit, especially in decentralized health care settings.

The study, conducted by the Centre for the AIDS Programme of Research in South Africa (CAPRISA), was a prospective cohort study to examine the pathogenesis of acute HIV subtype C infection and to describe the immunologic, virologic and clinical characteristics of acute and early infection [25]. The purpose of the current analysis was (i) to describe the signs and symptoms of AHI in order to develop a diagnostic model for identifying cases with high probability of AHI which would allow interventions like targeted HIV-1 RNA or p24 antigen testing; (ii) to calculate sensitivity and specificity of the risk score to predict presence or absence of AHI; (iii) to correlate clinical signs and symptoms of AHI with early virologic and clinical progression of HIV.

Methods

Ethics Statement

Ethics approval for the study was obtained from the University of KwaZulu-Natal and the University of Cape Town. Written informed consent was obtained from all participants.

Cohort

Between August 2004 and May 2005, a cohort of 245 HIV-uninfected women at high risk of infection was recruited in Durban, South Africa [25]. Women were considered high-risk if they reported having more than three sexual partners in the past 3 months or self-identified as sex workers.

Study Evaluations and Definition of Acute HIV Infection

Demographic and behavioural questionnaires were administered at enrollment. Women were evaluated at baseline and monthly thereafter with standardized interviews using a locally developed Clinical Evaluation Tool (CET) to screen for signs and symptoms associated with AHI. Interviews were performed by the research clinician who either spoke the local language or was assisted by a nurse who acted as an interpreter. Signs and symptoms screened for included: fever, fatigue, lethargy, night sweats, rash, headache, swollen lymph glands, sore throat, myalgia, any swollen joints, morning stiffness (joints), nausea, vomiting, diarrhoea, mucocutaneous ulceration, gingivitis, loss of appetite, reported weight loss, confusion, photophobia, neck stiffness, retro-orbital pain and vaginal discharge. Women also underwent a physical examination to determine the presence or absence of fever ($\geq$ 38^{o}C), measured weight loss, evidence of rash, lymphadenopathy, pharyngitis, thrush, mucocutaneous ulceration, conjunctivitis, hepatomegaly and splenomegaly. Whilst sexually transmitted infections (STIs) were managed syndromically, six monthly laboratory screening for STIs was performed as well.

The CET was administered before the HIV status was established. HIV testing algorithm consisted of two rapid HIV-1 antibody tests: the Determine HIV-1 test (Abbott Diagnostics, Johannesburg, South Africa) and the Capillus HIV-1/HIV-2 test (Trinity Biotech, USA). Discordant rapid antibody results were confirmed by HIV enzyme immunoassay (EIA) (BEP 2000; Dade Behring, Marburg, Germany). A pooled plasma HIV-1 RNA (AMPLISCREEN^{TM} HIV-1 Tests, v1.5– Roche Diagnostics) was performed at enrollment and each visit subsequently, irrespective of antibody result. A primary pool containing 24 samples was used and if positive disaggregated into smaller secondary pools. Diagnosis of AHI was made if a woman had detectable plasma HIV RNA level (using $\leq$ 400 copies/mL lower limit of detection assay) and/or a positive HIV antibody test following a previously documented negative test. Women presenting for unscheduled visits also completed a CET and were tested for HIV following the same algorithm. Participants underwent risk reduction counseling and were provided with male and female condoms at every visit.

Data Analysis

Signs and symptoms reported on the CET at all HIV-negative visits and the AHI visit were used in the analysis. Prevalence of signs and symptoms at both the AHI and non-AHI visits was assessed. Weight before and after seroconversion was compared using a Wilcoxon signed rank test. A Pearson's correlation coefficient was calculated to assess the strength of the linear relationship between the number of signs and symptoms and viral load, as well as CD4 count, measured at seroconversion, 6 and 12 months post infection. Signs/symptoms at the AHI visit were compared to those captured on the CET at HIV-negative visits from both seroconverters and those who remained HIV negative. To determine which signs/symptoms were associated with AHI, symptoms in women who seroconverted were compared with symptoms in women who remained HIV negative. Odds ratios (OR) for each of the signs/symptoms were calculated using a generalized estimating equation model, using a binomial distribution, accounting for multiple visits for the same participant. Two adjusted models were fitted to assess clinical factors predictive of AHI. Variables which were associated with AHI at $p \leq 0.15$ in each of the models were then included in the final model, which was used to develop a model-based score to predict AHI. In addition to applying a risk score model to predict AHI, the absolute number of signs/symptoms was also assessed to determine association with AHI. Sensitivity, specificity and positive likelihood ratios (with 95% confidence intervals) for detecting AHI were calculated for different numbers of signs and symptoms. This was also repeated excluding POCT negative, RNA positive participants reflecting current clinical practice in South Africa where RNA testing is not part of the algorithm for HIV diagnosis. All data analysis was performed using SAS version 9.2 (SAS Institute Inc., Cary).

Results

Demographic Characteristics of the Cohort

Between August 2004 and May 2005, 775 high risk women were screened and 245 HIV uninfected women were enrolled

and followed monthly for two years. The demographic and behavioural characteristics of the 245 women have been described in detail elsewhere [25] and are summarized in Table 1. Briefly, the median age of the participants was 36 (interquartile range (IQR): 24–42) years, 41.2% had at least 11 years of education, 58.8% reported using a condom at last sexual encounter, 13.9% and 35.0% indicated that they were never able to insist on condom use with casual and steady partners, respectively [26]. Most women (85.4%) reported having 2–5 casual partners in the 3 months prior to enrollment and 78.8% were self-identified commercial sex workers. Of note, the prevalence of HIV among women screening for enrollment into the HIV negative cohort was 59.6% [25].

Signs and Symptoms in Acute HIV Infection

The 245 HIV negative women had 4845 HIV negative visits, scheduled monthly, with a missed visit rate of 3.9%. The median time between visits was 28 days (IQR 28 - 34 days). Twenty-eight women seroconverted after a total of 390 person-years of follow-up with an HIV incidence of 7.2/100 person-years (95%CI: 4.5–9.8).

The frequency of signs/symptoms reported at AHI visit was higher than that reported at the HIV-negative visits (Table 2). The most prevalent signs/symptoms reported at AHI visit were loss of appetite (28.6% vs. 2.1% in HIV uninfected, $p < 0.001$), headache (25.0% vs. 5.7%, $p < 0.001$), rash (25.0% vs. 2.9%, $p < 0.001$), sore throat (21.4% vs. 2.1%, $p < 0.001$), fever (17.9% vs. 2.9%, $p < 0.001$), arthralgia (17.9% vs. 2.9%, $p < 0.001$), and reported weight loss (21.4% vs. 1.3%, $p < 0.001$). Of note, of the 28 acute infections, 60.7% (n = 17) had confirmed (measured) weight loss in the preceding two weeks, with a median reduction of 2.0 kg (IQR 1.3–2.6 kg). The median weight loss during seroconversion for all women with acute infection was 1.3 kg (IQR 0.0–2.2 kg; $p = 0.025$).

Fifty-seven percent of participants with AHI reported at least one sign or symptom and the specificity and likelihood ratios increased with an increasing number of reported symptoms (Table 3). Participants with one to three signs/symptoms were more likely to have AHI compared to women with no signs/symptoms (OR = 3.9; 95% CI: 1.6–9.2; p = 0.002); and presence of $4 signs/symptoms was strongly associated with AHI (OR = 17.3; 95% CI: 6.7–44.7; $p < 0.001$). When excluding all POCT Ab negative, RNA positive AHI cases, the prevalence of signs/symptoms increased to 76.5% and the association with AHI was even stronger (1–3 symptoms: OR = 10.3; 95% CI: 3.1–34.5, $4 symptoms: OR = 36.9. 95% CI: 9.8–140). When comparing AHI and non-AHI visits among women who seroconverted, loss of appetite, fatigue, headache, rash and sore throat remained the most commonly reported symptoms.

Table 1. Baseline demographic and behavioural characteristics of South African women at high risk of HIV acquisition stratified by HIV status [25].

Characteristic	All % (N) (N = 245)	HIV negative % (N) (N = 217)	AHI[#] cases % (N) (N = 28)
Age in years			
$25	73.5% (180)	75.6% (164)	57.1% (16)
< 25	26.5% (65)	24.4% (53)	42.9% (12)
Relationship status			
No partner (single, widowed, divorced)	7.4% (18/244)	7.9% (17/216)	3.6% (1)
One partner (stable or married)	35.7% (87/244)	37.5% (81/216)	21.4% (6)
Many partners	57.0% (139/244)	54.6% (118/216)	75.0% (21)
Number of casual partners in the last 3 months (baseline)			
0–1	5.0% (12/240)	4.7% (10/212)	7.1% (2)
2–5	85.4% (205/240)	85.9% (182/212)	82.1% (23)
> 5	9.6% (23/240)	9.4% (20/212)	10.7% (3)
Proportion of commercial sex workers	78.8% (193)	79.7% (173)	71.4% (20)
Highest level of school completed			
Grade < 8	24.5% (60)	26.3% (57)	10.7% (3)
Grade 8–10	34.3% (84)	32.3% (70)	50.0% (14)
Grade 11–12	41.2% (101)	41.5% (90)	39.3% (11)
Mean years sexually active (SD)	17.2 (10.38)	17.7 (10.35)	13.5 (10.08)
Age at sexual debut			
< 16	28.2% (69)	26.7% (58)	39.3% (11)
$16	71.8% (176)	73.3% (159)	60.7% (17)
Partner type at last sexual act (baseline)			
Steady Partner	75.9% (186)	77.9% (169)	60.7% (17)
Casual Partner	24.1% (59)	22.1% (48)	39.3% (11)
Proportion with condom use at last sex act	58.8% (144)	58.1% (126)	64.3% (18)

[#] Acute HIV Infection.

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Table 2. Signs and symptoms associated with acute HIV infection.

Signs or symptoms	Total cohort HIV negative visits N (%) (N = 4845)		AHI cases N (%) (N = 28)		*Unadjusted Odds Ratio (95% CI)	p-value	Sensitivity (%)	Specificity (%)
Headache	277	(5.7)	7	(25.0)	5.5 (2.3–13.0)	≤0.001	25.0	94.3
Fever	140	(2.9)	5	(17.9)	7.3 (2.7–19.5)	≤0.001	17.9	97.1
Sore throat	100	(2.1)	6	(21.4)	12.9 (5.1–32.6)	≤0.001	21.4	97.9
Night sweats	119	(2.5)	3	(10.7)	4.8 (1.4–16.0)	0.012	10.7	97.5
Fatigue	133	(2.7)	7	(25.0)	11.8 (4.9–28.3)	≤0.001	25.0	97.3
Swollen glands	280	(5.8)	3	(10.7)	2.0 (0.6–6.5)	0.275	10.7	94.2
Rash	138	(2.8)	7	(25.0)	11.4 (4.8–27.2)	≤0.001	25.0	97.2
Loss of appetite	103	(2.1)	8	(28.6)	18.4 (7.9–42.8)	≤0.001	28.6	97.9
Weight loss	63	(1.3)	6	(21.4)	20.7 (8.1–52.8)	≤0.001	21.4	98.7
Nausea	80	(1.7)	5	(17.9)	12.9 (4.8–34.9)	≤0.001	17.9	98.3
Diarrhoea	102	(2.1)	4	(14.3)	7.8 (2.6–22.7)	≤0.001	14.3	97.9
Arthralgia	139	(2.9)	5	(17.9)	7.4 (2.8–19.6)	≤0.001	17.9	97.1
Myalgia	47	(1.0)	3	(10.7)	12.3 (3.6–42.0)	≤0.001	10.7	99.0
Lethargy	49	(1.0)	3	(10.7)	11.7 (3.4–40.2)	≤0.001	10.7	99.0
Oral ulcers	48	(1.0)	2	(7.1)	7.7 (1.8–33.3)	0.006	7.1	99.0
Genital ulcers	8	(0.2)	2	(7.1)	46.5 (9.4–229.6)	≤0.001	7.1	99.8
Vaginal discharge	91	(1.9)	3	(10.7)	6.3 (1.9–21.1)	0.003	10.7	98.1

*Odds Ratio adjusted for repeated measures.

Note: Signs and symptoms reported with ≤5% frequency in the AHI cases or p≥0.15 were omitted. These included splenomegaly, anal ulcers, swollen joints, gingivitis, observed fever, aseptic meningitis, peripheral neuropathy, conjunctivitis, hepatomegaly, photophobia, parasthaesia, retro-orbital headache and neck stiffness.

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Factors Predictive of Acute HIV Infection

In an adjusted model, six predictors of AHI were identified: age ≤25 years (OR = 3.2, 95% CI: 1.4–7.1), rash (OR 6.1, 95% CI: 2.4–15.4), sore throat (OR 2.7, 95% CI: 1.0–7.6), loss of appetite (OR 4.7, 95% CI: 1.4–15.6), weight loss (OR 4.4, 95% CI: 1.5–13.4), vaginal discharge (OR: 5.4, 95% CI: 1.6–18.4) and genital ulcer (OR 8.0, 95% CI: 1.6–39.5) (Table 4). In this cohort, behavioural factors, including number of partners, condom use, whether the partner was casual or steady, and educational level were not associated with AHI in the final model (data not shown).

Table 3. Sensitivity and Specificity for different number of signs and symptoms.

Number of symptoms	Sensitivity (%)	Specificity (%)	+LR (95% CI)
All AHI cases included (N = 28)			
≥1	57.1%	81.5%	3.09 (2.23–4.28)
≥2	42.9%	91.4%	4.98 (3.22–7.71)
≥3	32.1%	95.3%	6.86 (3.95–11.93)
≥4	25.0%	97.3%	9.11 (4.69–17.68)
≥5	25.0%	98.1%	13.31 (6.79–26.09)
Excluding POCT* negative, RNA positive cases (N = 17)			
≥1	76.5%	81.5%	4.13 (3.15–5.42)
≥2	58.8%	91.4%	6.82 (4.54–10.27)
≥3	41.2%	95.3%	8.76 (4.89–15.69)
≥4	29.4%	97.2%	10.65 (5.00–22.68)
≥5	29.4%	98.1%	15.68 (7.30–33.69)

*POCT: point of care test.

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Signs and Symptoms According to HIV Diagnostic Test

All participants had two POCTs and a viral load RNA performed at each visit. Positive results were followed up with a laboratory-based third generation ELISA test. Of the 28 women with AHI, 17 (60.7%) had positive POCTs including one participant with discordant POCTs, while all were HIV positive on RNA testing (Table 5). Three-quarters of women (13/17; 76.5%) with positive or discordant POCTs were symptomatic compared to only one quarter with two negative POCTs at AHI (3/11; 27.3%, p = 0.019) who would have been identified with our clinical algorithm. However, it is important to note that, 8/28 women (28.6%) were POCT negative and experienced no signs or symptoms at the AHI visit and would therefore not have been identified by POCT testing or any clinical evaluation of AHI symptoms.

Risk-score Model Predicting Acute HIV Infection

The variables significantly associated with AHI in the adjusted analysis were used to construct a predictor risk score for AHI using the regression coefficients from the final adjusted model. The signs/symptoms in the model were weighted by the regression coefficients, rounded off to the nearest integer. Hence, the risk score algorithm was calculated as follows:

Risk Score ~ 2 if rash + 1 if sore throat + 2 if loss of appetite + 1 if weight loss + 2 if vaginal discharge + 2 if genital ulcer + 1 if age ≤ 25 years

Table 4. Unadjusted and adjusted analysis of signs and symptoms associated with acute HIV Infection.

Predictor	Unadjusted Odds Ratio (95% CI)	Adjusted* Odds Ratio (95% CI)	Risk score model**: Adjusted Odds Ratio (95%CI)
Demographics			
Age			
$25	1.0	1.0	1.0
,25	2.77 (1.30–5.86)	2.60 (1.23–5.50)	3.15 (1.41–7.07)
Symptoms and Signs			
Headache	5.50 (2.32–13.04)	–	–
Fever	7.31 (2.74–19.50)	–	–
Sore Throat	12.94 (5.14–32.61)	3.16 (1.01–9.83)	2.73 (0.99–7.56)
Night Sweats	4.77 (1.42–16.00)	–	–
Fatigue	11.81 (4.93–28.26)	–	–
Swollen Glands	1.96 (0.59–6.52)	–	–
Rash	11.37 (4.75–27.19)	5.42 (2.10–13.99)	6.07 (2.39–15.43)
Loss of Appetite	18.42 (7.93–42.78)	4.91 (1.40–17.29)	4.72 (1.42–15.63)
Weight Loss	20.70 (8.12–52.80)	3.50 (1.17–10.41)	4.43 (1.46–13.43)
Nausea	12.95 (4.80–34.91)	–	–
Diarrhoea	7.75 (2.64–22.74)	–	–
Athralgia	7.36 (2.76–19.64)	–	–
Myalgia	12.25 (3.58–41.98)	–	–
Lethargy	11.74 (3.43–40.19)	–	–
Oral Ulcers	7.69 (1.77–33.30)	–	–
Genital Ulcers	46.51 (9.42–229.60)	8.11 (1.97–33.49)	7.95 (1.60–39.49)
Vaginal Discharge	6.27 (1.86–21.13)	6.19 (1.73–22.15)	5.38 (1.57–18.40)

*Model for behavioural and demographic factors adjusted for age in years, relationship status, education and age at sexual debut (data not shown), while model for symptoms and signs adjusted for sore throat, rash, loss of appetite, weight loss, genital ulcers and vaginal discharge.
**Risk score model adjusting for age, sore throat, rash, loss of appetite, weight loss, genital ulcers and vaginal discharge.
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A risk score was calculated for each visit. The sensitivity and specificity of different cut-off scores were determined by comparing the risk score with the HIV status (HIV-1 RNA and/or antibody positive) of the participant at every visit. Simply RNA PCR testing every person with at least one symptom would yield a sensitivity of 57.1%. A risk score of at least 1 occurred at 28.0% of participant visits. Having a score of at least 1, that is, presenting with any of the risk score symptoms or being under 25 years of age, would identify 67.9% cases of AHI and positive predictive value (PPV) of the test would be low at 1.4%. Using this scoring system, a score of 2 could identify 50.0% of AHI cases by testing only 7.3% of participants, with a PPV of 3.9%. At a cut-off of 3, only 3.0% of participants would be tested to identify 39.3% of all AHI, with a PPV of 7.5%.

Correlation of Number of Signs and Symptoms and Markers of HIV Disease Progression

There was a significant positive correlation between the number of signs/symptoms and the HIV-1 plasma viral load at the AHI visit (correlation coefficient of 0.63, $p = ,0.001$) with participants who had a high viral load at seroconversion having a greater number of signs/symptoms. However, this trend did not persist, and no correlation was observed between the initial number of signs/symptoms and HIV-1 plasma viral loads at 6 or 12 months. There was no statistically significant correlation between the number of signs/symptoms and CD4 count at seroconversion (correlation coefficient of 0.16, $p = 0.41$). There was also no significant difference in the duration of signs/symptoms and self-reported severity of diarrhoea, skin rash, lethargy and pharyngitis (mild to severe) between women with AHI and HIV-negative women (data not shown).

Discussion

The period of acute HIV infection contributes to a considerable proportion of transmission events and, therefore, identifying those who are acutely infected is of paramount importance in curbing the epidemic [20]. Accurate diagnosis of acute HIV infection includes use of molecular techniques like HIV RNA PCR detection which may either be expensive or not readily available in some settings.

In this prospective cohort study of 245 high-risk women we identify clinical signs and symptoms predictive of acute subtype C infection. We present a screening algorithm which can be used to enhance detection of acute HIV infection in a resource-limited setting. Importantly, we report that younger age, rash, sore throat, loss of appetite, weight loss and vaginal discharge or genital ulcers were associated with AHI. A similar constellation of symptoms has been reported in other studies [27–32], although in our cohort none of the signs or symptoms had prevalence above 28.6%. In fact, fever, commonly reported in other studies, was found in only 17.9% of our participants [27–32]. Most available data, however, pertains to predominantly male cohorts with subtype B infection.

Data in the sub-Saharan African context is more limited [18,19,33], particularly among women with subtype C infection.

Table 5. Signs and symptoms according to HIV diagnostic test.

HIV diagnostic testing	N (%) of AHI cases (N = 28)	N (%) with no symptoms	N (%) with any symptoms	p-value*
POCT antibody positive or discordant	17 (60.7%)	4/17 (23.5%)	13/17 (76.5%)	0.0189
POCT antibody negative	11 (39.3%)	8/11 (72.7%)	3/11 (27.3%)	–

*Fisher's Exact test.

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Available literature on acute infection reports higher prevalence of symptoms than what was noted in our cohort. For example, in a prospective cohort of female sex workers in Kenya, 81% of women had at least one symptom, in comparison to only 57.1% in our cohort [18]. More recently, in a cross-sectional cohort of predominantly male adults presenting with fever in Mozambique, among those identified with acute infection, all had reported sore throat and 67% complained of a mononucleosis-like syndrome. The limitation of this particular study, however, was a small number of individuals with acute HIV infection and that the administration of the questionnaire a week after the diagnosis visit may have influenced the findings [34].

Our AHI cohort included individuals who were diagnosed by RNA testing alone potentially before a possible seroconversion illness could have manifested itself. Excluding these cases, the number of women who reported signs/symptoms was 76.5% similar to what has been previously reported [18,19]. Other contributing reasons for the observed difference may have been cultural or gender-specific characteristics in reporting signs/symptoms, or differences in how HIV subtypes manifest disease [35]. In addition, the fact that our participants were not being evaluated in an STI clinic or referred for a particular complaint, meant that they were probably less likely to report signs/symptoms. It is therefore possible that if the clinical algorithm was applied to a primary health care facility or STI clinic, the sensitivity and specificity of the algorithm would improve, as well as its utility as part of an HIV screening initiative in these settings.

This study demonstrates that it is possible to create a model predictive of AHI in this population. RNA-PCR testing everyone with a risk cut-off of $1 (28.0% of participant visits), would allow for the identification of 67.9% of acutely-infected individuals. In contexts of limited resources, the cut-off score could be increased, thus reducing the number of tests done in order to identify acute infection. In our cohort, testing only women with a risk score of $2 would result in RNA-PCR testing of only 7.3% of participant visits, and half (50.0%) of all acute infections would be identified. The costs of these additional tests could be offset by the benefit of being able to intervene early in acutely-infected individuals, to initiate care and help prevent HIV transmission during this high-risk period.

Our algorithm did not yield as high a predictive value or sensitivity as the algorithm created from a cross-sectional assessment of individuals presenting at an STI clinic in Malawi. Powers et al, who included discordant POCT results in their model, were able to identify 95% of AHI cases by performing RNA or p24 testing in 40% of their population [19]. Apart from a difference in testing methodologies between the two studies, it is possible that the models were affected by differences in the population with higher overall prevalence of signs/symptoms such as genital ulcer disease in individuals presenting for diagnosis and treatment at an STI clinic in the Malawi study.

Many demographic factors such as number of sexual partners, age at sexual debut, education level and condom use were not significantly associated with AHI in our cohort. A possible explanation is that behavioural risk assessment was performed only at enrollment and thus, because of ongoing risk reduction counseling, it is possible that women modified their risk behaviour over the course of the study resulting in a decrease of HIV incidence over time [25]. Only age ≥ 25 years was strongly predictive of acute HIV infection (OR 3.2). This finding reflects what has been reported in other studies that indicate a higher incidence of HIV in younger women [36].

Availability of longitudinal follow-up data from the immediate post-seroconversion period, allowed us to demonstrate a correlation between the number of signs/symptoms and early HIV RNA levels. While in our study this association did not persist beyond the early HIV infection period, previous studies found an association between the number, severity and duration of symptoms and higher viral load set-points in patients infected with subtype B virus [37–39]. For example, one cohort study with over 600 individuals found that HIV RNA levels remained persistently higher in symptomatic compared to asymptomatic patients highlighting the need to identify those individuals and ensure access to care [40]. Data from sub-Saharan Africa is limited but our findings differ from what has been reported in a cohort of women in Kenya where higher set-point viral load and more severe illness at acute HIV infection predicted faster disease progression [41,42].

Our study demonstrates that a considerable proportion of individuals (28.6%) neither test positive on conventional point-of-care testing nor report any symptoms when presenting to testing facilities with acute HIV infection. This population provides a true challenge for the scientific community to develop reliable fourth generation Ag/Ab combination POCTs or low-cost point-of-care viral load technology. Although the DetermineH HIV-1/2 Ag/Ab Combo rapid test performed poorly in detecting acute infection in a recent trial [24] we hope that this is not the end of POCT Ag technology. Furthermore, several POCT viral load assays are currently being evaluated including the SAMBA (simple amplification based assay; Diagnostics Development Unit University of Cambridge) a semi quantitative test for using isothermal amplification and visual detection by dipstick, the Liat TM Analyser, manufactured by IQuum (Marlborough, MA), is a real-time, battery operated, small, portable PCR kit providing quantitative results and the Alere NAT system (Alere Technologies, Stirling, UK). The hope would be that the implementation of this technology, primarily developed in order to monitor HIV positive patients on ART, will also allow health care providers in less developed settings to test for AHI.

The strengths of our study rest in the fact that this is a longitudinal cohort composed of women with subtype C infection which was conducted in a non-STI clinic setting and therefore may be more representative of the general population. The cohort design included clinical assessment at every visit which allowed comparison of signs/symptoms pre-seroconversion to AHI visit. Given the poor specificity of signs/symptoms and concurrent

background of other illnesses, this systematic clinical assessment is an advantage of this study. In contrast to previous studies, all participants underwent antibody and RNA PCR testing at every visit allowing a detailed analysis of the characteristics of these tests and correlation with symptoms.

Our study highlights the challenges associated with recognizing and diagnosing acute HIV infection in a cohort of women at high risk of infection. Although signs/symptoms of AHI are non-specific and highly variable, it is possible to identify a large proportion of individuals who have acute or early HIV infection if there is a high index of suspicion to consider the diagnosis and initiate appropriate testing. More advanced laboratory diagnostic tools offer promise of earlier detection of incident infections [21], but even then clinical judgement guiding prioritization of testing will remain critical, especially in resource-limited settings. Our risk score model based on several signs/symptoms most predictive of AHI may allow identification of women likely to be acutely infected with HIV and prioritize testing with RNA or newer generation assays, ultimately reducing cost of AHI diagnosis. Further independent validation of the model in different settings and larger sero-incidence cohorts would be important to assess the utility of the model in practice. In a setting where nucleic acid testing is not yet readily available, the ability to develop a checklist of signs/symptoms that would increase clinical suspicion of AHI would be of benefit for triaging of high-risk women and may lead to earlier detection of infection.

A recent study by Powers and colleagues concluded that in order to have a large impact on decreasing HIV prevalence, it is critical to identify and engage in care individuals in acute and early stages of HIV infection [5]. Thus, rapid detection of incident infections is an important component of test-and-treat and other prevention strategies. Screening for AHI is, however, associated with substantial costs of using newer generation HIV diagnostics and adds operational challenges [43]. Until rapid and cheap point-of-care assays which reliably detect HIV RNA or p24 antigen are commercially available and deployable in resource-limited settings, detecting AHI will remain a challenge. In the meantime, a clinically relevant risk algorithm which would decrease the number of individuals needed to screen with laboratory tests to detect AHI, is critical. The window of opportunity to intervene is narrow but the potential to benefit the individual and prevent onward transmission is considerable.

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Author Contributions

Conceived and designed the experiments: KM MS FvL CW. Performed the experiments: KM FvL NN. Analyzed the data: KM LW AF NG. Wrote the paper: KM LW AF NG.

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Chapter 7:

7.1 Genital Tract Inflammation During Early HIV-1 Infection Predicts Higher Plasma Viral Load Set Point in Women.

Roberts, Lindi, Jo-Ann S. Passmore, Koleka Mlisana, Carolyn Williamson, Francesca Little, Lisa M. Bebell, Gerhard Walzl et al. Journal of Infectious Diseases 205, no. 2 (2012): 194-203.

MAJOR ARTICLE

Genital Tract Inflammation During Early HIV-1 Infection Predicts Higher Plasma Viral Load Set Point in Women

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Background. The biggest challenge in human immunodeficiency virus type 1 (HIV-1) prevention in Africa is the high HIV-1 burden in young women. In macaques, proinflammatory cytokine production in the genital tract is necessary for target cell recruitment and establishment of simian immunodeficiency virus (SIV) infection following vaginal inoculation. The purpose of this study was to assess if genital inflammation during early HIV-1 infection predisposes women to rapid disease progression.

Methods. Inflammatory cytokine concentrations were measured in cervicovaginal lavage (CVL) from 49 women 6, 17, 30, and 55 weeks after HIV-1 infection and from 22 of these women before infection. Associations between genital inflammation and viral load set point and blood CD4 cell counts 12 months after infection were investigated.

Results. Elevated genital cytokine concentrations 6 and 17 weeks after HIV-1 infection were associated with higher viral load set points and, to a lesser extent, with CD4 depletion. CVL cytokine concentrations during early infection did not differ relative to preinfection but were elevated in women who had vaginal discharge, detectable HIV-1 RNA in their genital tracts, and lower blood CD4 counts.

Conclusion. Genital inflammation during early HIV-1 infection was associated with higher viral load set point and CD4 depletion, which are markers of rapid disease progression. Strategies aimed at reducing genital inflammation during early HIV-1 infection may slow disease progression.

In sub-Saharan Africa, which has the highest prevalence of human immunodeficiency virus type 1 (HIV-1) worldwide, most new infections occur by sexual transmission to women [1]. The genital mucosa is the initial site of viral replication following vaginal transmission of HIV-1 in women and simian immunodeficiency virus (SIV) in rhesus macaques [2, 3]. In macaques, vaginal inoculation with SIV is followed by proinflammatory cytokine production and recruitment of $CD4^{1}$ T cells that are necessary for local viral expansion and dissemination to the systemic compartment [4–6]. Proinflammatory cytokine expression in the genital mucosa correlates with viral replication and approaches baseline as peak SIV viremia declines [6]. HIV-1 infection may likewise be accompanied by an early inflammatory cascade in the genital tract that is associated with viral replication in this compartment. HIV-1 has been shown to directly induce inflammatory cytokine production via Toll-like receptor 7 and 8 activation [7]. Elevated concentrations of inflammatory cytokines in turn may upregulate HIV-1 replication by recruiting and activating target cells and through NF-jB activation [4, 8–11].

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Several studies have shown that cervicovaginal proinflammatory cytokines are upregulated in women with early or chronic HIV-1 infection compared with HIV-uninfected women [10, 12–16]. However this upregulation may be attributed to the high frequency of sexually transmitted infections (STIs) or bacterial vaginosis (BV) in these individuals rather than HIV-1 itself [17]. For example, BV was associated with increased concentrations of proinflammatory interleukin (IL)–1b, whereas chronic HIV-1 infection was not [18]. HIV-1 shedding, which is associated with STIs [19], may induce further inflammatory cytokine production.

Plasma cytokine concentrations during early HIV-1 infection are predictive of plasma viral load set points and CD4 depletion [20], and treatment with cytokines such as IL-12p70 and IL-15 during acute SIV infection is associated with altered disease course in macaques [21–23]. Several studies have suggested that cytokine responses in the genital tract during the early stages of HIV-1 infection may likewise be associated with disease progression. In macaques, induction of inflammatory cytokines and immune cell influx into the genital tract prior to vaginal SIV inoculation was associated with increased plasma viral load set points [24]. This suggests that preexisting genital inflammation in humans may similarly influence HIV-1 disease progression. Zara et al [14] demonstrated that upregulation of IL-1b in the genital tracts of HIV-1–infected women was associated with increased plasma viral loads. Recently, we reported that elevated proinflammatory cytokines in cervicovaginal lavage (CVL) correlated with lower blood $CD4^1$ T-cell counts during early HIV-1 infection [15].

In this study, the relationships between genital cytokine concentrations during early HIV-1 infection and plasma viral load set point and blood $CD4^1$ T-cell counts 12 months postinfection were investigated.

MATERIALS AND METHODS

Study Participants

Forty-nine South African women recently infected with HIV-1 subtype C were recruited as part of the CAPRISA Acute Infection Study [25]. Each woman provided informed consent and then attended regular evaluations of HIV-1 disease status. Time of infection was defined as the midpoint between the last HIV-1 antibody negative test result and the first HIV-1 antibody positive test result, or as 14 days prior to a positive RNA polymerase chain reaction (PCR) assay on the same day as a negative HIV-1 enzyme immunoassay. This study was approved by the University of Kwazulu-Natal and University of Cape Town ethics committees.

Cytokine Measurements

CVL samples were collected, as described elsewhere [15], at 5 time points: 22 women were assessed preinfection (36 weeks preinfection, range, 2–92), 39 were assessed 6 weeks postinfection (range, 1–13), 32 were assessed at 17 weeks postinfection (range, 14–23), 39 were assessed at 30 weeks postinfection (range, 24–36), and 40 were assessed at 55 weeks postinfection (range, 50–62). Eighteen of the 22 women who were assessed preinfection had matching 6-week postinfection CVL samples available. CVLs were prefiltered using 0.2 Im Costar Spin-X cellulose acetate filters (Sigma) and the supernatant was stored at 280°C. Concentrations of IL-1a, IL-1b, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-15, interferon (IFN)–a, eotaxin, fractalkine, granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage (GM)–CSF, monocyte chemotactic protein 1, macrophage inflammatory protein (MIP)–1a, MIP-1b, MIP-3a, RANTES, soluble CD40 ligand (sCD40L), and tumor necrosis factor (TNF)–a were measured using Human Cytokine LINCOplex premixed kits (LINCO Research), Milliplex kits (Millipore), and enzyme-linked immunosorbent assay (R&D Systems). The sensitivity of these kits ranged between 0.05 and 18.33 pg/mL. Multiplex data were collected using a Bio-Plex Suspension Array Reader (Bio-Rad Laboratories), and a 5 PL regression formula was used to calculate cytokine concentrations from the standard curves (Bio-Plex Manager software, version 4). Cytokine concentrations below the lower limit of detection were reported as the midpoint between the lowest concentration measured for each cytokine and zero.

Clinical Characteristics

Blood was collected by venipuncture into acetate citrate dextran vacutainer tubes. Absolute blood $CD4^1$ T-cell counts were measured using a FACSCalibur flow cytometer. Plasma HIV-1 RNA concentrations were quantified using the COBAS AMPLICOR HIV-1 Monitor v1.5 or COBAS Ampliprep/COBAS TaqMan 48 Analyzer (Roche Diagnostics). CVL viral loads were determined using Nuclisens Easyq HIV-1 (version 1.2). As viral load set point after 3 months postinfection is predictive of time to AIDS [26], the average viral load measurement of 3 consecutive visits overlying 12 months postinfection (range, 37–69) was used to assess disease progression. Additionally, associations between cytokines and (1) average $CD4^1$ T-cell measurements of the same 3 visits and (2) $CD4^1$ T-cell loss between preinfection and 12 months postinfection were investigated.

Participants were screened for Chlamydia trachomatis, Neisseria gonorrhoeae, and herpes simplex virus type 2 (HSV-2) in cervical swab samples by PCR and Trichomonas vaginalis by Diamond's culture and PCR. Agents of BV were assessed by Gram staining.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism software, version 5 (GraphPad) and Stata, version 10 (StataCorp). Distribution of variables was assessed by Shapiro-Wilk and Shapiro-Francia tests. The v^2 test was used to compare proportions. Mann-Whitney U and Wilcoxon signed-rank tests

were used for unmatched and matched comparisons, respectively. Spearman rank test was used for correlations. The relationships between cytokine concentrations and markers of disease progression were evaluated using multivariate linear regression. Variables were log-transformed, and cytokines were standardized to allow for direct comparison of b-coefficients. Mixed-effects logistic regression was used to assess the change in HIV-1 shedding over time. P values , .05 were considered significant. P values were adjusted using false discovery rate step-down procedure in order to reduce false-positive results when multiple comparisons were made [27].

RESULTS

Forty-nine women recently infected with HIV-1 were recruited and followed longitudinally. Their median age was 24 years (range, 18–59). Most women (98%) were unmarried, 20% had multiple partners, and 32% reported using injectable hormonal contraceptives. The median CD4^1 T-cell count and viral load measurements at 6 weeks postinfection were 524 cells/lL and 56 500 copies/mL, respectively (Table 1). The median CD4^1 T-cell loss during the first 12 months of HIV-1 infection was 486 cells/lL, while the median set-point viral load was 39 783 copies/mL. STIs and BV were prevalent in this cohort, both pre– and post HIV-1 infection.

Cytokine Concentrations in the Genital Tract of Women Recently Infected With HIV-1 Were Not Significantly Elevated Compared With Preinfection

The concentrations of 22 cytokines in CVL from women prior to HIV-1 infection (median, 36 weeks preinfection) were compared with those of the same women during early HIV-1 infection (median, 6 weeks postinfection) for whom samples at both time points were available (n 5 18). In matched samples, TNF-a and eotaxin concentrations were higher in CVLs from early HIV-1 infection compared with preinfection, whereas MIP-3a concentrations were lower (P , .05; Figure 1A). However, these changes were not significant after adjustment for multiple comparisons. Furthermore, in an unmatched analysis including all women who had either preinfection samples (n 5 22) or 6-week postinfection samples available (n 5 39), only MIP-3a concentrations were lower in postinfection CVLs, before adjusting for multiple comparisons. These data indicate that, in contrast to previous reports that have compared genital cytokine concentrations in HIV-1 infected women to unmatched HIV-uninfected women [10, 12–16], the women in this study did not have significantly altered CVL cytokine concentrations during early HIV-1 infection relative to preinfection samples from women who later became infected with HIV-1.

Interestingly, the concentrations of 7 of 22 cytokines measured in CVLs correlated between pre– and post HIV-1 infection (Figure 1B). MIP-1a and sCD40L remained significantly correlated after adjusting for multiple comparisons. Following evaluation of cytokine concentrations in longitudinal samples (17, 30, and 55 weeks postinfection), it was found that 11 of 20 cytokines correlated between at least 3 of the 5 time points. After adjusting for multiple comparisons, IL-1a, IL-b, IL-6, IL-8, MIP-1a, G-CSF, and GM-CSF were significantly correlated across $3 time points. These findings suggest that the relative degrees of cervicovaginal inflammation in individual women remained relatively constant during the study period.

Table 1. Clinical Characteristics of Study Participants

CD4^1 T-Cell Counts (Cells/lL)	Cells/lL, Median (IQR)	No.
Preinfection CD4^1 T-cell count	975 (860–1149)	22
Six-week postinfection CD4^1 T-cell count	524 (379–685)	48
CD4^1 T-cell count set point (average of 3 visits overlying 12 months postinfection)	408 (339–551)	46
CD4^1 T-cell loss (preinfection minus 12-month postinfection CD4^1 T-cell count)	486 (254–653)	22
Plasma viral loads (copies/mL)	Copies/mL, Median (IQR)	No.
Six-week postinfection plasma viral load	56 500 (14 200–370 500)	49
Plasma viral load set point (average of 3 visits overlying 12-months postinfection)	39 783 (7248–102 208)	46
Sexually transmitted infections	Preinfection, No./Total (%)	Early infection, No./Total (%)
Prevalence of active STIs (women with laboratory-diagnosed STI[a])	8/22 (36.3)	17/44 (38.6)
Bacterial vaginosis (women with Gram stain positive for BV)	15/22 (68.2)	34/44 (77.4)
Vaginal discharge (women with visible discharge)	3/22 (13.7)	8/49 (16.3)
Genital ulcer (women with visible genital ulcer[s])	0/22 (0)	6/49 (12.2)
Multiple HIV-1 variant transmission	Dual, No./Total (%)	Heterogenous, No./Total (%)
Participants with multiple transmitted variants	4/45 (8.9)	7/45 (15.6)

Abbreviations: BV, bacterial vaginosis; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; STI, sexually transmitted infection.

[a] Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, herpes simplex virus type 2.

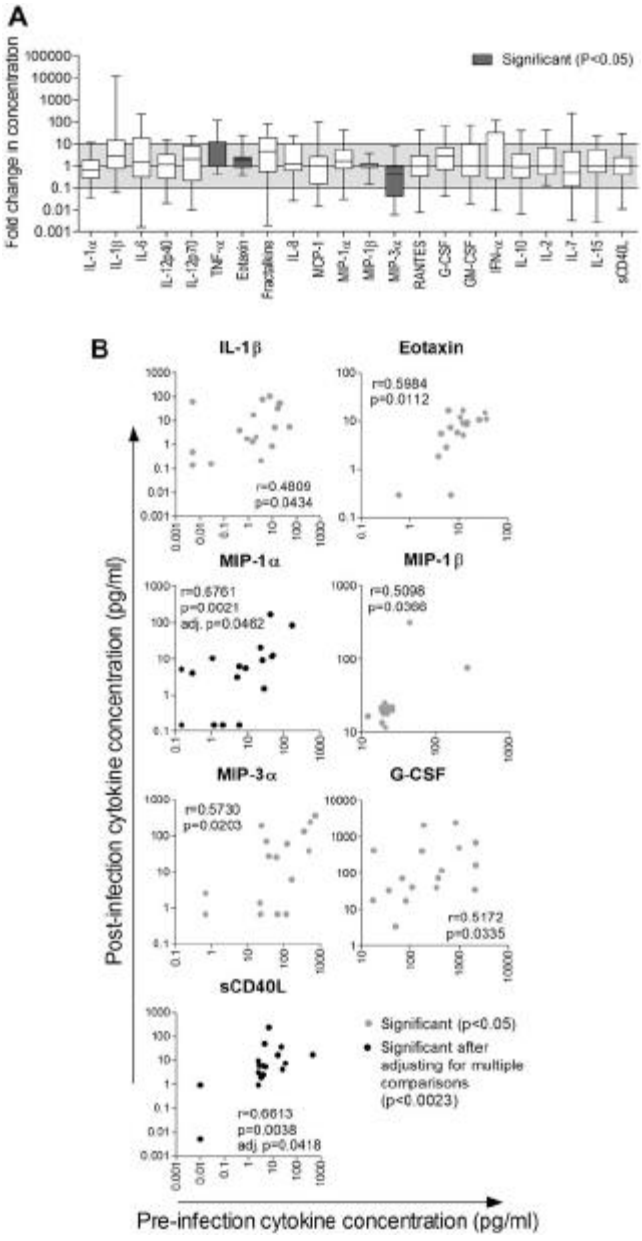

Figure 1. Comparison of cervicovaginal lavage (CVL) cytokine concentrations in women (n 5 18) before infection (median, 36 weeks preinfection) and during early human immunodeficiency virus type 1 (HIV-1) infection (median, 6 weeks postinfection). A, Fold changes in cytokine concentrations following HIV-1 infection are shown as box-and-whisker plots; error bars indicate the range. Wilcoxon signed-rank test was used to compare cytokine concentrations in CVL from the same women pre– and post HIV-1-infection. Changes in cytokine concentrations that were significant before adjusting for multiple comparisons and are indicated by gray bars ($P < .05$). B, Spearman correlations between cytokine concentrations measured in pre- and postinfection CVL. MIP-1b was no longer correlated between time points following exclusion of outliers. Gray dots indicate cytokines that correlated significantly before adjusting for multiple comparisons ($P < .05$). Black dots indicate cytokines that remained significantly correlated after adjustment ($P < .0023$). G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN, interferon; IL, interleukin; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; sCD40L, soluble CD40 ligand; TNF, tumor necrosis factor.

Genital Tract Inflammation During Early HIV-1 Infection Was Not Associated With Multivariant HIV-1 Transmission

Abrahams et al [29] have reported that in this cohort of women, 22% were infected with ≥2 HIV-1 genetic variant. To investigate the relationship between genital tract inflammation and transmission of multiple HIV-1 variants, cytokine concentrations at 6 weeks postinfection were compared in women with multiple (n 5 10) or single (n 5 25) transmitted variants. No differences in cervicovaginal cytokines were found between women infected with multiple or single variants (data not shown), suggesting that genital inflammation was not associated with the break in HIV-1 transmission bottleneck in this cohort.

Cervicovaginal Inflammation During Early HIV-1 Infection Was Associated With STIs

Cytokine concentrations in CVL from women with early HIV-1 infection (6 weeks postinfection) who tested positive for ≥1 STI or had symptoms of STIs were compared with those of women who did not have an STI or BV (Figure 2). Women who had ≥1 active STI (C. trachomatis, N. gonorrhoeae, T. vaginalis, or HSV-2; n 5 14) tended to have elevated cytokine concentrations; however, these changes were not significant after adjusting for multiple comparisons. Abnormal vaginal discharge, which may be caused by other factors in addition to STIs, including BV, Candida albicans, allergic reaction, irritation, or physiologic changes [28], was associated with elevated concentrations of proinflammatory IL-1a, IL-1b, IL-12p40, eotaxin, MIP-1a, and T-cell homeostatic IL-15, after adjustment for multiple comparisons.

Genital Tract Inflammation During HIV-1 Infection Was Associated With HIV-1 Shedding

HIV-1 viral loads were measured in CVL samples collected at 6, 17, 30, and 55 weeks postinfection as an indicator of HIV-1 shedding. CVL viral loads and proportions of women with detectable HIV-1 RNA did not differ significantly between time points (Figure 3). CVL viral loads did not correlate between time points, indicating that different women were shedding HIV-1 at different times. Cytokine concentrations in CVL correlated with CVL viral loads at all time points, and women who had detectable HIV-1 RNA in their genital tracts had higher cytokine concentrations than did women who were not shedding HIV-1 (Table 2). Preinfection cytokine concentrations did not differ between women who were shedding and women who were not shedding HIV-1 6 weeks postinfection. Additionally, lower blood CD4 counts and/or higher plasma viral loads were associated with CVL viral loads at each time point. The prevalence of STIs did not differ between women with detectable HIV-1 RNA levels in their genital tracts [3 of 7 (42.9%)] and women who were not shedding [11 of 31 (35.5%)]. Furthermore, there were no significant differences in occurrence of BV, vaginal discharge, and genital ulcers in women shedding HIV-1 compared with women who were not shedding.

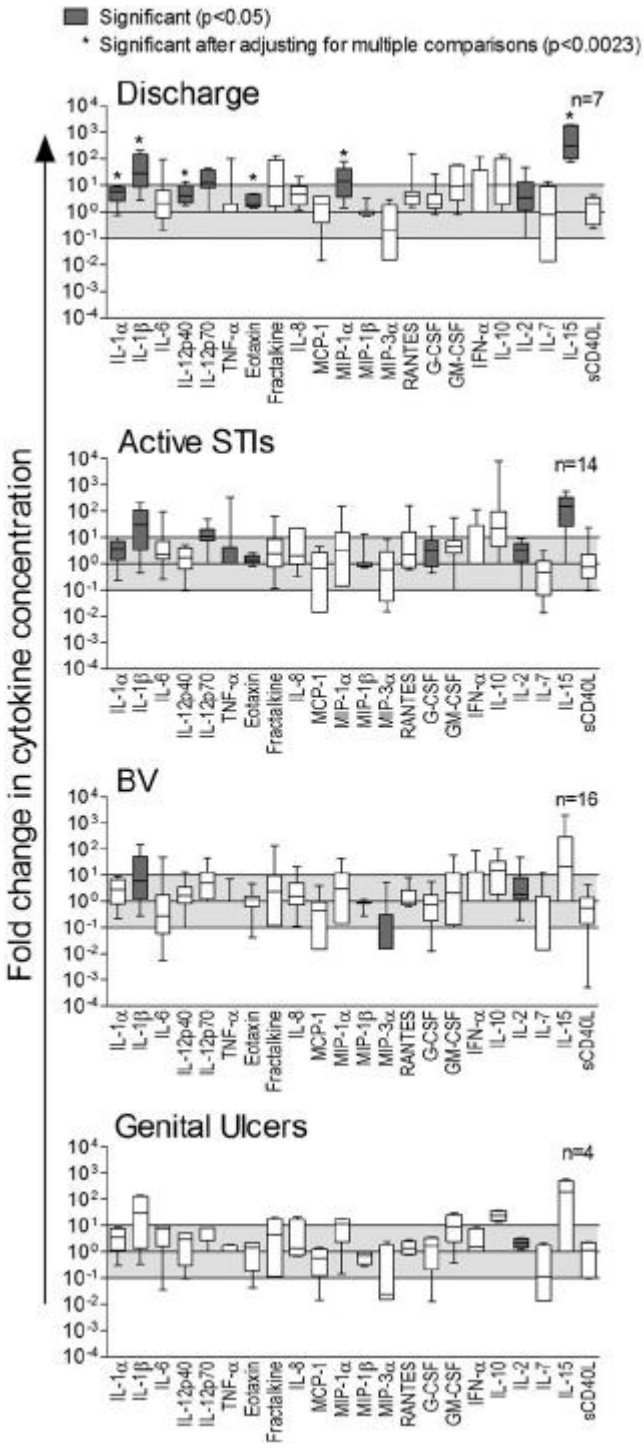

Figure 2. Fold change in cytokine concentrations in cervicovaginal lavage (CVL) samples from women who had ≥1 sexually transmitted infection (STI) or bacterial vaginosis (BV) compared with the median concentrations in women who did not have an STI. Cytokine concentrations were measured in CVL samples from women infected with human immunodeficiency virus type 1 (HIV-1) (median, 6 weeks postinfection) who had no STI (n = 7), women who had visible vaginal discharge (n = 7) or genital ulceration (n = 4), women who had ≥1 laboratory-diagnosed active STIs (Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, or herpes simplex virus type 2 [HSV-2]; n = 14) and women who had BV (n = 16). Mann-Whitney U test was used to compare cytokine concentrations in CVL from women with STIs, BV, or symptoms to women with no STIs and/or BV. Fold changes in cytokine concentrations are shown as box-and-whisker plots; error bars indicate the range. Gray bars indicate cytokines that were significantly different before adjusting for multiple comparisons ($P < .05$). Stars indicate cytokines that remained significant after adjustment ($P < .0023$).

Elevated Genital Tract Cytokine Concentrations During Early HIV-1 Infection Correlated With Lower Blood CD4+ T-Cell Counts

We have previously reported that cervicovaginal IL-1b, IL-6, and IL-8 concentrations during early HIV-1 infection were associated with lower blood CD4+ T-cell counts at these early time points [15]. In this study, it was similarly found that higher concentrations of IL-6 (q = 20.455, adjusted P = .030), TNF-a (q = 20.498, adjusted P = .017), RANTES (q = 20.460, adjusted P = .026), and IL-10 (q = 20.503, adjusted P = .027) in CVL correlated with lower blood CD4 counts during early HIV-1 infection. Only weak associations between 17-, 30-, and 55-week cytokine concentrations and concurrent CD4 cell counts were found, and these were not significant after adjusting for multiple comparisons, indicating that the relationship was restricted to early HIV-1 infection.

Despite finding a significant correlation between (1) plasma viral load and HIV-1 shedding and (2) genital inflammation and HIV-1 shedding, few associations were found between genital cytokine concentrations and plasma viral loads measured at the same time points. This suggests that changes in inflammatory cytokine production in the genital tract during early HIV-1 infection are largely independent of systemic viral load.

Cervicovaginal Inflammation During Early HIV-1 Infection Was Associated With CD4+ T-Cell Depletion During the First Year of Infection

To determine whether genital cytokine concentrations during HIV-1 infection were associated with disease progression, associations between cytokines and (1) average blood CD4 counts measured at 3 consecutive visits overlying 12 months postinfection and (2) CD4 loss between preinfection and 12 months postinfection were investigated. Although higher concentrations of several cytokines at multiple time points were associated with lower CD4 counts 12 months postinfection before adjusting for multiple comparisons, only GM-CSF concentrations 6 weeks postinfection remained significantly associated after adjustment (Figure 4A). No significant relationship between preinfection cytokine concentrations and CD4 counts 12 months postinfection was found, although relatively few CVL samples (22 of 49) from preinfection time points were available for this analysis.

Blood CD4 counts and plasma viral loads during early HIV-1 infection were also associated with 12 month postinfection CD4 counts (b = .70 [95% CI, .46 to .95] and b = −2.07 [95%

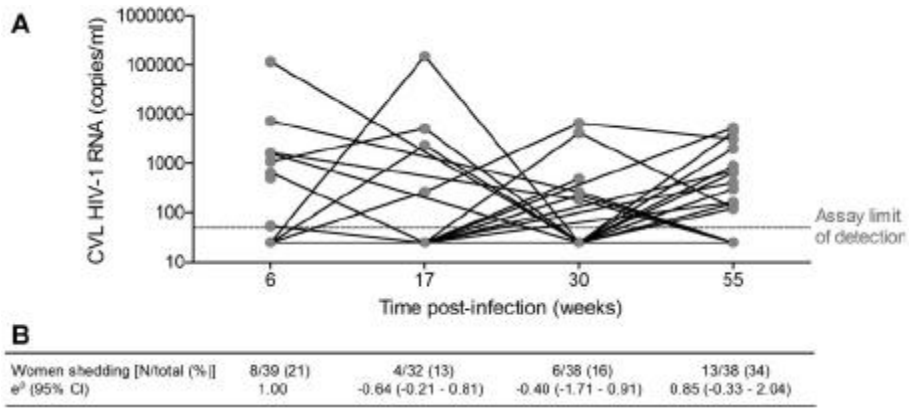

Figure 3. Human immunodeficiency virus type (HIV-1) RNA concentrations in cervicovaginal lavage (CVL) at 6, 17, 30, and 55 weeks postinfection. A, HIV-1 viral loads in CVL from each woman are indicated by gray dots. Lines connect RNA concentrations in CVL from the same women at different time points. No significant Spearman correlations were found between HIV-1 RNA levels at each time point, indicating that different women were shedding at each time point. B, Proportion of women who had detectable HIV-1 RNA at each time point. Mixed-effects logistic regression was used to determine whether the proportion of women shedding HIV-1 at 17, 30, and 55 weeks postinfection differed relative to 6 weeks postinfection; e^b indicates the change in number of women shedding at each time point relative to 6 weeks postinfection (reference time point). No significant differences were found.

CI, −.12 to −.02], respectively). HIV-1 disease status could predispose individuals to higher incidence of STIs and genital shedding of HIV-1 [30, 31], thereby exacerbating genital inflammation, which would therefore be indirectly rather than directly associated with disease progression. Multivariate regression was thus used to adjust for CD4 counts and viral loads measured at the same time points as cytokine concentrations. Again, there was a strong trend toward an association between higher cytokine concentrations and lower 12-month CD4 counts.

Finally, associations between preinfection (n = 22) and 6-week postinfection (n = 18) cytokine levels and blood $CD4^+$ T-cell loss during the first 12 months of HIV-1 infection were investigated in a subset of women for whom preinfection CD4 count measurements were available. CD4 depletion during the first year of infection was associated with higher preinfection cervicovaginal concentrations of RANTES (q = 0.46, P = .03) and 6-week postinfection concentrations of IL-1b (q = 0.53, P = .02) and GM-CSF (q = 0.62, P = .006). However, these associations were not upheld after adjusting for multiple comparisons, which may be due to the relatively small sample sizes available for this analysis.

Genital Inflammation During Early HIV-1 Infection Was Associated With Higher Viral Load Set Point

It was found that the concentrations of several inflammatory and T-cell homeostatic cytokines in CVL during early HIV-1 infection (6 and 17 weeks postinfection) were associated with higher plasma viral load set point (Figure 4B), even though these cytokines did not correlate with viral loads measured at the same time points as cytokine measurements. No associations between cytokine concentrations at later time points postinfection (30 and 55 weeks postinfection) and viral load set point were significant after adjusting for multiple comparisons. Additionally, although cytokine concentrations did not differ between preinfection and early infection time points, no significant associations between preinfection cytokine concentrations and viral load set point were found. Elevated GM-CSF, IL-1b, IL-12p70, and IL-15 concentrations at 6 weeks postinfection and GM-CSF, IL-1b, IL-10, IL-6, RANTES, MIP-1b, and IL-2 concentrations 17 weeks postinfection were associated with higher viral load set point. Following adjustment for CD4 counts and viral loads measured at the same time points as cytokine measurements, elevated GM-CSF concentrations 6 weeks postinfection remained significantly associated with higher viral load set point. This suggests that the relationship between this inflammatory cytokine and disease progression is at least partly independent of early infection systemic disease state.

DISCUSSION

Previous studies have shown that women with early and chronic HIV-1 infection have elevated genital inflammatory cytokine concentrations relative to unmatched HIV-uninfected women [10, 12–16]. This study is the first to compare cytokine concentrations in cervicovaginal samples from the same women preinfection and during early HIV-1 infection. It was found that inflammatory cytokine levels preinfection were correlated with those postinfection and in fact were not significantly elevated shortly following HIV-1 infection (6 weeks postinfection). However, genital cytokine concentrations were higher in women who had vaginal discharge and in women who were shedding HIV-1 in their genital secretions. In addition, cytokine concentrations were elevated in women who had lower blood

Table 2. Spearman Rank Correlations Between CVL HIV-1 RNA Concentrations and Blood CD4 Counts, Plasma Viral Loads, and CVL Cytokine Concentrations

	6 Weeks Postinfection		17 Weeks Postinfection		30 Weeks Postinfection		55 Weeks Postinfection	
	q (P Value)		q (P Value)		q (P Value)		q (P Value)	
Blood CD4 ^{1}T-cell count	−0.485	(.002)	−0.305	(.090)	−0.428	(.007)	−0.047	(.782)
Plasma viral load	0.430	(.006)	0.533	(.002)	0.314	(.055)	0.371	(.022)
IL-1a	0.236	(.147)	0.154	(.401)	0.260	(.114)	0.337	(.039)[a]
IL-1b	0.355	(.026)[a]	0.478	(.006)[b]	0.207	(.212)	0.513	(.001)[b]
IL-6	0.427	(.007)[a]	0.446	(.011)[b]	0.261	(.113)	0.338	(.038)[a]
IL-12p40	0.159	(.333)	0.211	(.247)	0.294	(.073)	0.175	(.293)
IL-12p70	0.325	(.044)	0.204	(.263)	0.177	(.287)	0.027	(.873)
TNF-a	0.388	(.015)[a]	0.382	(.031)[a]	0.415	(.010)[a]	0.309	(.059)
Eotaxin	0.216	(.186)	0.227	(.211)	−0.038	(.821)	0.473	(.003)[b]
Fractalkine	0.163	(.321)	0.405	(.022)[a]	−0.186	(.264)	0.331	(.042)[a]
IL-8	0.323	(.045)	0.279	(.122)	0.393	(.015)[a]	0.437	(.006)[b]
MCP-1	0.419	(.008)[a]	0.487	(.005)[b]	0.099	(.555)	0.432	(.007)[b]
MIP-1a	0.155	(.345)	0.385	(.030)[a]	0.059	(.726)	0.128	(.445)
MIP-1b	0.186	(.258)	0.570	(.001)[b]	0.087	(.604)	0.314	(.055)
RANTES	0.490	(.002)[a]	0.531	(.002)[b]	0.267	(.106)	0.549	(.0004)[b]
G-CSF	0.310	(.055)	0.457	(.009)[b]	0.198	(.234)	0.379	(.019)[a]
GM-CSF	0.090	(.585)	0.537	(.002)[b]	0.315	(.054)	0.077	(.645)
MIP-3a	0.226	(.207)	ND		ND		ND	
IFN-a	0.212	(.201)	ND		ND		ND	
IL-10	0.378	(.018)[a]	0.247	(.173)	0.038	(.821)	−0.160	(.336)
IL-2	0.385	(.015)[a]	0.534	(.002)[b]	0.137	(.413)	−0.134	(.421)
IL-7	0.260	(.110)	0.119	(.517)	0.126	(.450)	−0.057	(.736)
IL-15	0.382	(.016)[a]	0.220	(.226)	0.375	(.020)[a]	−0.013	(.939)
sCD40L	0.331	(.040)[a]	0.309	(.085)	0.218	(.188)	0.123	(.462)

Significant associations are shown in bold (P < .05). Associations that were significant after adjusting for multiple comparisons are underlined.

Abbreviations: CVL, cervicovaginal lavage; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HIV -1, human immunodeficiency virus type 1; IFN, interferon; IL, interleukin; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; ND, not done; sCD40L, soluble CD40 ligand; TNF, tumor necrosis factor.

[a] Cytokine concentration significantly higher in women who had detectable HIV-1 RNA in their genital tracts compared with women who had undetectable HIV-1 RNA concentrations (P < .05).

[b] Cytokine concentration significantly higher in women who had detectable HIV-1 RNA after adjusting for multiple comparisons.

CD4^{1} T-cell counts 6 weeks postinfection [15]. Elevated cervicovaginal cytokines during early HIV-1 infection (6 and 17 weeks postinfection) were associated with higher plasma viral load set point, which is predictive of time to AIDS [26]. Additionally, higher GM-CSF concentrations 6 weeks postinfection were associated with lower CD4 counts 12 months postinfection.

When genital cytokine concentrations were compared in the same women preinfection and at several time points during the first year of HIV-1 infection, some women maintained higher levels of genital inflammation over time, while others had consistently low cytokine concentrations. STI prevalence did not differ between pre– and post HIV-1 infection; thus, the cause of sustained inflammation may be STI recurrence. Mitchell et al [18] demonstrated that genital inflammatory cytokines were not elevated in chronically HIV-1 infected women relative to uninfected women but rather were associated with BV. The higher frequency of STIs in HIV-1 infected women compared with uninfected women [17] may account for previous findings that genital inflammatory cytokines are higher in women with early HIV-1 infection. It is possible that elevated genital inflammatory cytokine responses, similar to those reported shortly after SIV infection of macaques [6], may have subsided by 6 weeks postinfection. Alternatively, cervicovaginal inflammatory cytokine concentrations may increase over time during HIV-1 infection and may thus be higher in chronically infected women relative to those with early infection [12].

Genital HIV-1 RNA concentrations correlated not only with higher levels of genital inflammation but also with lower blood CD4 counts and higher plasma viral loads at the same time points. Although these findings may suggest that high systemic viral loads drive HIV-1 shedding, which in turn induces genital

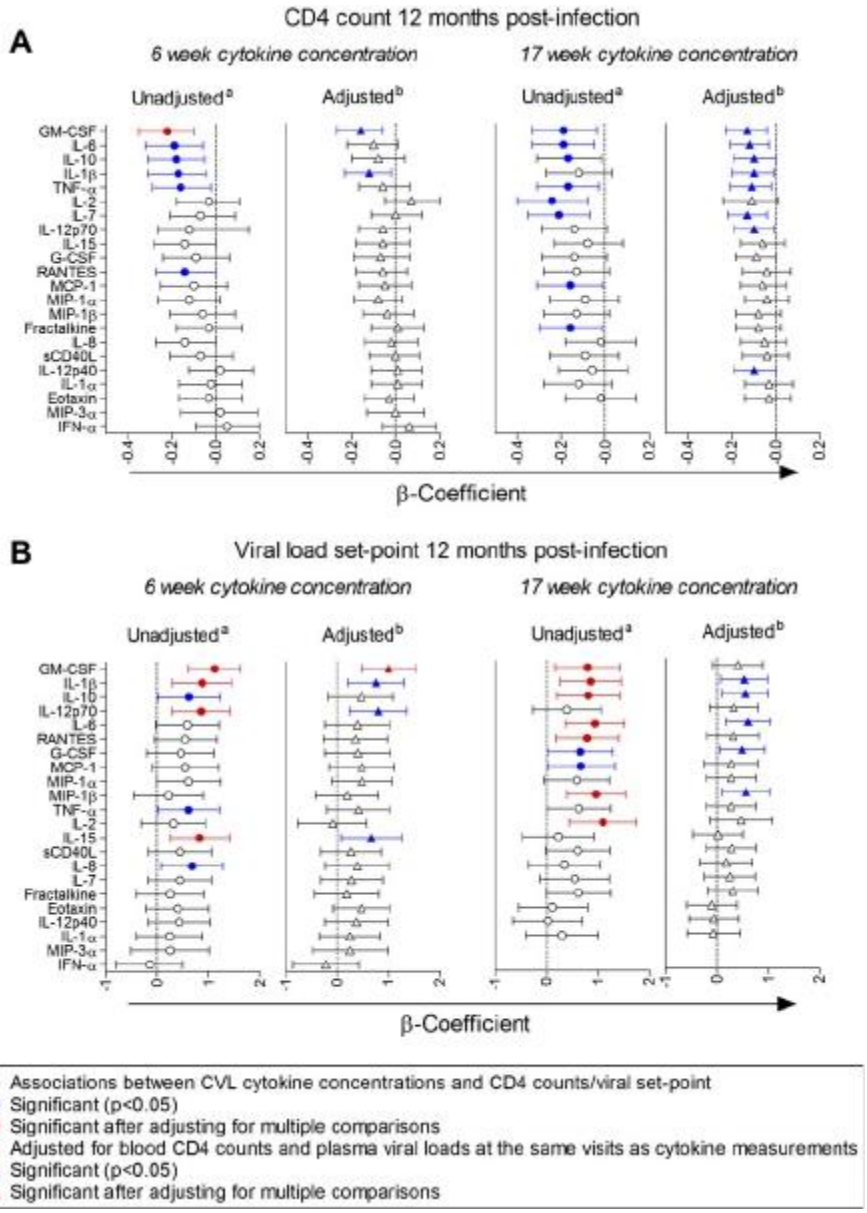

Figure 4. Linear regression was used to assess relationships between cytokine concentrations in cervicovaginal lavage (CVL) from women 6 weeks (n 5 37) and 17 (n 5 31) weeks postinfection and average blood CD4 counts of 3 consecutive visits overlying the 12-month postinfection time point (A) and viral load set points (B). Viral loads, CD4 counts, and cytokine concentrations were log-transformed, and cytokine concentrations were standardized to allow for direct comparison between b-coefficients. b-Coefficients that were generated by univariate regression are indicated by circles and show the relationship between each cytokine and 12-month CD4 counts or viral load set points. b-Coefficients that indicate the relationships between cytokine concentrations and 12-month CD4 counts or viral load set points, following adjustment for CD4 counts and viral loads at the same visits as cytokine measurements using multivariate regression, are represented by triangles. Error bars indicate 95% confidence intervals. Cytokines were ranked according to the strength of their associations with 12-month CD4 counts or viral load set point. Significant associations are shown in blue (P , .05). Associations that were significant after adjusting for multiple comparisons are shown in red. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN, interferon; IL, interleukin; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; sCD40L, soluble CD40 ligand; TNF, tumor necrosis factor.

inflammation, which is thus indirectly associated with disease progression, shedding during early HIV-1 infection was not associated with 12-month CD4 counts or viral load set point. Additionally, genital inflammation during early HIV-1 infection was only weakly associated with concurrent plasma viral load before adjusting for multiple comparisons but was strongly associated with plasma viral loads at subsequent time points. It was also found that, although CVL cytokine levels

correlated positively over time, different women were shedding at different time points during HIV-1 infection. Therefore, shedding was not the cause of sustained cervicovaginal inflammation that was observed in this cohort, and genital inflammation may rather facilitate HIV-1 shedding in individuals who have high plasma viral loads by recruiting HIV-1-infected cells to the genital tract and by promoting viral replication.

It was found that elevated CVL cytokine concentrations 6 weeks postinfection were associated with lower blood CD4 counts at the same time point. Blood CD4 depletion may reflect CD4 depletion in the genital tract. This would contribute to genital inflammation by inducing T-cell homeostatic cytokine production [32], which in turn induces inflammatory cytokine production [33, 34]. In support, T-cell homeostatic cytokines IL-2 and IL-15 were inversely associated with blood CD4 counts before adjusting for multiple comparisons. Therefore, STIs and other factors that are associated with vaginal discharge are likely to be major contributors to cervicovaginal inflammation during early HIV-1 infection, with inflammation exacerbated by HIV-1 replication in the genital tract and the homeostatic response to CD4 depletion. A recent macaque study demonstrated the importance of proinflammatory cytokine production in the genital tract in establishment of productive SIV infection following vaginal inoculation [4]. Wang et al [24] further showed that induction of inflammatory cytokine responses in the genital tracts of macaques prior to vaginal inoculation with SIV was associated with increased plasma viral load set point, suggesting that genital cytokine concentrations at the time of infection may influence disease progression. Similar to macaque studies, it was found in this study that higher levels of cervicovaginal proinflammatory and T-cell homeostatic cytokines during early HIV-1 infection were associated with more rapid HIV-1 disease progression. Additionally, the findings of this study suggest that the association between genital inflammation during early infection and disease progression is partly independent of blood CD4 counts and plasma viral loads measured at the same time points as cytokine concentrations. Although the earliest time point included in this study (6 weeks postinfection) was past viral dissemination to blood, preinfection genital cytokine concentrations correlated with those measured during the first year of HIV-1 infection. Therefore, genital inflammation 6 weeks postinfection may reflect inflammation at the time of infection. Although preinfection genital inflammation may be associated with HIV-1 disease progression, this could not be investigated here as the number of women for whom preinfection samples were available was small (22 of 49 women). Cytokine concentrations measured 6 weeks postinfection (range, 1–13) may, however, be a closer representation of the level of genital inflammation present at the time of HIV-1 infection than were preinfection cytokine concentrations that were measured 36 weeks prior to infection (range, 2–92). Higher concentrations of cervicovaginal inflammatory cytokines at the time of HIV-1 transmission may favor disease progression by recruiting and activating $CD4^1$ T cells for HIV-1 infection and directly promoting viral replication [4, 9–11]. These findings suggest that the inflammatory environment in the genital tracts of women who become infected with HIV-1 through sexual transmission may influence disease outcome and that strategies to reduce genital inflammation may slow disease progression.

Notes

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Chapter 8:

8.1 Rapid Disease Progression in HIV-1 Subtype C Infected South African Women. *Mlisana, Koleka, Lise Werner, Nigel J. Garrett, Lyle R. McKinnon, Francois van Loggerenberg, Jo-Ann S. Passmore, Clive M. Gray, Lynn Morris, Carolyn Williamson, and Salim S. Abdool Karim. Clinical Infectious Diseases (2014): ciu573.*

Rapid Disease Progression in HIV-1 Subtype C–Infected South African Women

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Background. Whereas human immunodeficiency virus (HIV) subtype B–infected individuals generally progress to AIDS within 8–10 years, limited data exist for other clades, especially from Africa. We investigated rates of HIV disease progression of clade C–infected South African women.

Methods. Prospective seroincidence cohorts in KwaZulu-Natal were assessed for acute HIV infection monthly (n = 245) or every 3 months (n = 594) for up to 4 years. Rapid disease progression was defined as CD4 decline to <350 cells/μL by 2 years postinfection. Serial clinical and laboratory assessments were compared using survival analysis and logistic regression models.

Results. Sixty-two women were identified at a median of 42 days postinfection (interquartile range, 34–59), contributing 282 person-years of follow-up. Mean CD4 count dropped by 39.6% at 3 months and 46.7% at 6 months postinfection in women with preinfection measurements. CD4 decline to <350 cells/μL occurred in 31%, 44%, and 55% of women at 1, 2, and 3 years postinfection, respectively, and to <500 cells/μL in 69%, 79%, and 81% at equivalent timepoints. Predictors of rapid progression were CD4 count at 3 months postinfection (hazard ratio [HR], 2.07; 95% confidence interval [CI], 1.31–3.28; P = .002), setpoint viral load (HR, 3.82; 95% CI, 1.51–9.67; P = .005), and hepatitis B coinfection (HR, 4.54; 95% CI, 1.31–15.69; P = .017). Conversely, presence of any of HLAB*1302, B*27, B*57, B*5801, or B*8101 alleles predicted non–rapid progression (HR, 0.19; 95% CI, .05–.74; P = .016).

Conclusions. Nearly half of subtype C–infected women progressed to a CD4 count <350 cells/μL within 2 years of infection. Implementing 2013 World Health Organization treatment guidelines (CD4 count <500 cells/μL) would require most individuals to start antiretroviral therapy within 1 year of HIV infection.

Keywords. HIV disease progression; acute HIV infection; subtype C; viral load; women.

Human immunodeficiency virus (HIV) disease progression is highly variable between individuals and populations and is determined by genetic, immunologic, virologic, and environmental factors [1–3]. $CD4^+$ T-cell decline has been recognized as one of the major markers of the rate of HIV disease progression. Once CD4 counts drop below 200 cells/μL, the risk of opportunistic infections and death increases dramatically. In addition to CD4 decline, disease progression can be defined by time to antiretroviral therapy (ART) initiation, diagnosis of AIDS-defining illnesses, or death. Whereas the majority of HIV-infected individuals (70%–80%) fall into an intermediate category, rapid progression and long-term nonprogression represent extreme phenotypes with respect to CD4 decline [4]. In addition, the infecting subtype can have a significant impact on the rate of CD4 decline [5, 6]. This has been best defined for clades A and D in East Africa,

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where studies demonstrated a shorter median time to AIDS in subtype D–infected individuals than in subtype A–infected individuals [7–9]. Studies from sub-Saharan Africa suggest faster disease progression in subtype C–infected individuals [10, 11], despite observations that subtype C may replicate less efficiently than subtype B [12].

A major immunologic predictor of progression is the level of host immune activation, which predicts rates of CD4 decline more accurately than plasma viral load (VL) [13]. African populations have reportedly higher levels of immune activation than US populations, possibly linked to a higher burden of comorbidities and micronutrient deficiencies [14, 15]. Indirect immune activation caused by microbial translocation and loss of gut-associated CD4 cells has also been demonstrated in HIV rapid progressors [16]. Another major host predictor of disease progression is the human leukocyte antigen (HLA) background. HLA-B35, B8, B45, and B53 have been associated with rapid progression, whereas HLA-B57 and B27 are more common in slow progressors [11, 17–19].

Results from mostly subtype B–infected cohorts suggest that rapid progression is uncommon, with only about 5% of individuals progressing from acute HIV infection to AIDS within 3 years [20, 21]. However, there are limited data on disease progression in other subtypes, especially from prospective African cohorts infected with subtype C. This study describes HIV type 1 (HIV-1) disease progression in South African women and identifies host and viral factors associated with progression.

METHODS

Study Population

Between August 2004 and May 2005, a cohort of HIV-uninfected women at high risk of HIV acquisition was enrolled into the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 002 Acute Infection Study, recruited at 2 sites within the province of KwaZulu-Natal in South Africa—an urban site in Durban and a rural site in Vulindlela. The aim of the study was to describe immunologic, virologic, and clinical characteristics of HIV-1 subtype C acute infection and investigate the natural history of HIV-1 subtype C infection [22]. Women aged ≥18 years who self-reported as sex workers or having >3 sexual partners within the last 3 months were screened. Those testing HIV negative were offered enrollment and followed for up to 4 years, with monthly testing to identify acute HIV infections [23]. In addition, women were also recruited from a rural family planning and sexually transmitted infection (STI) clinics in Durban [24] with quarterly HIV testing and follow-up. Written informed consent was obtained from all participants, and ethical approval for the study was granted by the University of KwaZulu-Natal (E013/04), University of Cape Town (025/2004), and University of the Witwatersrand (M040202).

HIV Testing and Time of Infection

HIV diagnosis was based on antibody and/or nucleic acid detection. Two point-of-care antibody tests were used, the third-generation Determine (Abbott Laboratories, Abbott Park, Illinois) and Capillus (Trinity Biotech, Jamestown, New York). An HIV enzyme-linked immunosorbent assay was performed to confirm HIV diagnosis using the HIV enzyme immunoassay BEP 2000 (Dade Behring, Marburg, Germany). HIV reverse transcription polymerase chain reaction (RT-PCR) testing was performed at every testing with antibody-negative results. COBAS Amplicor version 1.5 RT-PCR testing (Roche Diagnostics, Rotkreuz, Switzerland) was performed using a pooling strategy of 24 samples per pool [25, 26]. Time of infection was estimated as the midpoint between last antibody-negative and first antibody-positive test or 14 days before a positive RT-PCR result for those diagnosed preseroconversion.

Once HIV diagnosis was confirmed, women were enrolled into the acute HIV infection cohort. The visit structure was divided into 3 phases: weekly to fortnightly visits up to 3 months postinfection (acute infection), monthly visits from 3 to 12 months (early infection), and quarterly visits thereafter (established infection) until ART initiation. Samples for immunologic, virologic, and clinical parameters were collected, and VL and CD4 counts (FACSCalibur Flow Cytometer, BD Biosciences, San Jose, California) were measured at each visit. Screening for STIs with multiplex PCR testing and Gram stain for bacterial vaginosis were performed at enrollment and every 6 months thereafter [27]. Women with positive results were recalled for treatment or referred to the nearest clinic. Any other STIs were treated syndromically on presentation according to South African National Guidelines.

High-resolution HLA typing was performed on all participants. DNA was extracted from either peripheral blood mononuclear cells or granulocytes using the Pel-Freez DNA Isolation kit. HLA-A, -B, and -C typing was performed by sequencing of exons 2, 3, and 4 using Atria AlleleSeqr kits (Abbott), and data were analyzed using Assign-SBT 3.5 (Conexio Genomics). Any ambiguities resulting from either polymorphisms outside the sequenced exons or identical heterozygote combinations were resolved using sequence-specific primers.

Statistical Analysis

Basic descriptive statistics were used to summarize frequencies and timing of the first occurrences of clinical signs and symptoms. Linear mixed models were used to estimate the CD4 slope within 12 months of HIV infection. Kaplan–Meier analyses were carried out using estimated time of infection to endpoints of CD4 counts of <200, <350, and <500 cells/μL. Stratified analyses using 3- and 6-month postinfection VL (<5 and ≥5 log copies/mL) were also performed. Whereas rapid disease progression is currently defined as ≥2 CD4 measurements <350 cells/μL within 3 years of seroconversion [28], we

Table 1. Demographic and Behavioral Characteristics of the Acute HIV Infection Cohort

Characteristic	All (N = 62)	Rapid Progressors (n = 27)	Non–Rapid Progressors (n = 35)	P Value
Days postinfection at enrollment ,[a]median (IQR)	42 (34–59)	42 (30–59)	46 (34–60)	.804
Age, y, median (IQR)	25 (21–33)	27 (23–36)	24 (21–33)	.163
Urban	74.2% (46)	85.2% (23)	65.7% (23)	.142
Completed high school	29.0% (18)	29.6% (8)	28.6% (10)	1.000
Marital status				
Married or stable partner	71.0% (44)	59.3% (16)	80.0% (28)	.240
Many partners	16.1% (10)	22.2% (6)	11.4% (4)	
No partners	12.9% (8)	18.5% (5)	8.6% (3)	
No. of sexual partners in last 3 mo prior to HIV infection[a]				
0–1	68.3% (41)	55.6% (15)	78.8% (26)	.093
≥2	31.7% (19)	44.4% (12)	21.2% (7)	
Commercial sex workers	32.3% (20)	33.3% (9)	31.4% (11)	1.000
Condom used at last sex act	56.5% (35)	59.3% (16)	54.3% (19)	.798
Ever had anal sex	14.5% (9)	22.2% (6)	8.6% (3)	.160
History of tuberculosis	8.1% (5)	3.7% (1)	11.4% (4)	.376
BMI[a], median (IQR)	26.6 (23.5–31.8)	26.8 (23.9–32.1)	26.4 (22.7–31.3)	.287
CD4 count[a], mean (SD)	520 (193.61)	413 (128.95)	603 (195.88)	<.001
Log viral load[a], median (SD)	4.62 (0.92)	4.96 (0.84)	4.36 (0.90)	.009
Bacterial vaginosis[a]	73.3% (44/60)	66.7% (18/27)	78.8% (26/33)	.382
Any STI[a,b]	44.3% (27/61)	40.7% (11/27)	47.1% (16/34)	.796
HSV-2 Ab positive[a]	91.9% (57)	92.6% (25)	91.4% (32)	1.000
HBcAb positive[a]	50.0% (31)	59.3% (16)	42.9% (15)	.306

Data are presented as % (No.) unless otherwise specified.

Abbreviations: Ab, antibody; BMI, body mass index; HBcAb, hepatitis B core antibody; HIV, human immunodeficiency virus; HSV-2, herpes simplex virus type 2; IQR, interquartile range; SD, standard deviation; STI, sexually transmitted infection.

[a] Measured at enrollment into acute HIV infection cohort.

[b] Any sexually transmitted disease, defined as testing positive for Treponema pallidum, Trichomonas vaginalis, Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, or HSV-2 polymerase chain reaction.

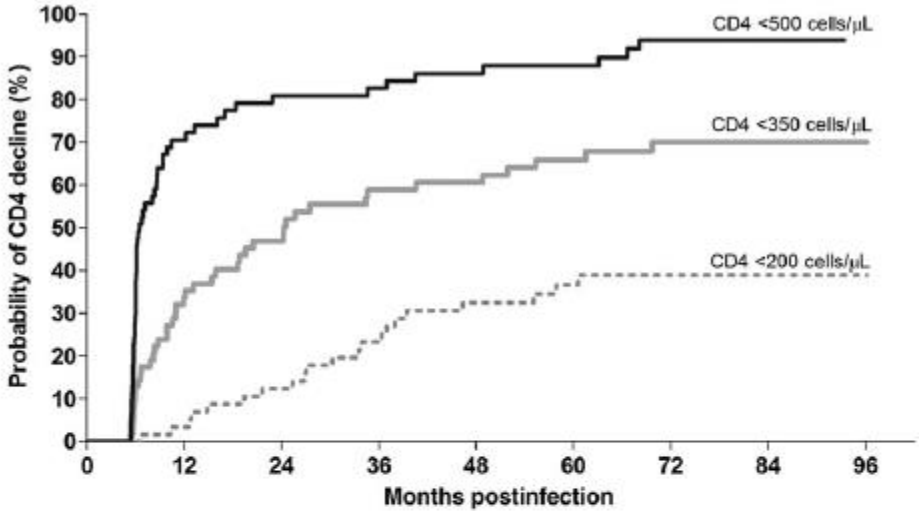
conservatively defined it as 2 consecutive CD4 counts <350 cells/μL between 6 months (to exclude seroconversion decline) and 24 months postinfection. Non–rapid progressors (NRPs) were those maintaining a CD4 count >350 cells/μL beyond 24 months. Baseline differences between rapid progressors (RPs) and NRPs were assessed by Wilcoxon rank-sum and Fisher exact tests. Cox proportional hazards regression modeling was used to assess predictors of rapid progression. Participants who did not progress rapidly were censored at their last follow-up visit or 24 months postinfection, whichever came first. Predictors in the unadjusted model with P values <.20, or deemed important, were included in the adjusted model. Statistical analysis was performed using SAS software, version 9.3 (SAS Institute Inc., Cary, North Carolina); graphs were prepared using Graph Pad Prism 5.

RESULTS

Baseline Characteristics of the Cohort

Of the 245 HIV-negative women followed up monthly for 4 years, 35 (14.3%) acquired HIV. The 594 HIV-negative women followed quarterly from seroincidence cohorts yielded 39 HIV seroconversions; 27 of these women consented to further follow-up. HIV incidence rates were similar between cohorts (6.5/100 person-years [95% confidence interval {CI}, 4.6–8.9] for the N = 594 women and 7.2/100 person-years [95% CI, 4.5–9.8] for the N = 245 women) [29], and the cohorts had minimal influence on progression rates (hazard ratio [HR], 0.61 [95% CI, .29–1.32]; P = .209). Of the 62 HIV infections, 17 (27%) were diagnosed before seroconversion. The median time from estimated date of infection to first HIV-positive sample was 42 days (interquartile range [IQR], 34–59 days).

Demographic, behavioral, and clinical characteristics of these women are summarized in Table 1. The median age at HIV infection was 25 years (IQR, 21–33 years), and most women (71.0%) were either married or had a stable partner. At enrollment into the acute HIV infection cohort, STIs were common (44.3%), and most women (91.9%) were seropositive for herpes simplex virus type 2 (HSV-2). The overall mean CD4 count at this first postseroconversion timepoint was 520 cells/μL. This was significantly lower than the preinfection CD4 counts



Months postinfection	12	24	36	48	60	72	84	96
Event: CD4 <200 cells/μL								
# reaching CD4 <200 (%)	2 (3.2%)	7 (11.3%)	13 (21.0%)	18 (29.0%)	20 (32.2%)	21 (33.8%)	21 (33.8%)	21 (33.8%)
Cumulative PY	59.9	110.6	155.4	193.6	226.1	250.1	263.8	268.4
Cumulative IR (95% CI)	3.3 (.4-12.1)	6.3 (2.6-13.0)	8.4 (4.5-14.3)	9.3 (5.5-14.7)	8.8 (5.4-13.7)	8.4 (5.2-12.8)	8.0 (4.9-12.2)	7.8 (4.8-12.0)
Event: CD4 <350 cells/μL								
# reaching CD4 <350 (%)	19 (30.6%)	27 (43.5%)	34 (54.8%)	35 (56.5%)	38 (61.3%)	40 (64.5%)	40 (64.5%)	40 (64.5%)
Cumulative PY	54.6	89.7	116	139.3	159	172.8	180.1	181.5
Cumulative IR (95% CI)	34.8 (21.0-54.4)	30.1 (19.9-43.8)	29.3 (20.3-41.0)	25.1 (17.5-34.9)	23.9 (16.9-32.8)	23.2 (16.5-31.5)	22.2 (15.9-30.3)	22.0 (15.8-30.0)
Event: CD4 <500 cells/μL								
# reaching CD4 <500 (%)	43 (69.4%)	49 (79.0%)	50 (80.6%)	52 (83.9%)	53 (85.5%)	56 (90.3%)	56 (90.3%)	56 (90.3%)
Cumulative PY	42	55.4	66.3	74.7	81.1	85.5	87.6	88.9
Cumulative IR (95% CI)	102.3 (74.1-137.8)	88.5 (65.5-117.0)	75.5 (56.0-99.5)	69.6 (52.0-91.2)	65.3 (48.9-85.5)	65.4 (49.4-85.0)	63.9 (48.3-83.0)	63.0 (47.6-81.8)

Figure 1. Superimposed Kaplan–Meier graphs of time to CD4 count <200, <350, and <500 cells/μL since estimated date of infection. Abbreviations: CI, confidence interval; IR, incidence rate; PY, person-years.

(mean CD4 count, 993 cells/μL available for 25 of 62 women; P < .001). The CD4 counts differed significantly between RPs and NRPs (413 vs 603 cells/μL; P < .001; Table 1) at first post-seroconversion with a mean log VL of 4.62 log copies/mL (SD = 0.92) and higher levels in RPs than NRPs (4.96 vs 4.36 log copies/mL; P = .009).

Prospective Analysis

A total of 282 person-years of ART-naive follow-up amongst participants not yet initiated on ART were observed, including a median of 33 CD4 count or VL measurements (range, 3–45) per participant. In the first 2 years of HIV infection, the median number of CD4 count measures for RP and NRP participants was 19 (IQR, 16–20) and 19 (IQR, 18–20), respectively. At the time of analysis, 17 of 62 women remained ART-naive and were an estimated 6.4 years postinfection (IQR, 5.9–7.1 years) at their last follow-up visit. Of the remainder, 37 initiated ART, 5 died (2 HIV-related deaths), 2 were lost to follow-up (prior to ART initiation), and 1 declined study participation. Almost one-third (30.6%) reached a CD4 count of <350 cells/μL within 6–12 months of infection, 43.5% within 24 months, and 54.8% within 36 months postinfection (Figure 1). Increasing the treatment threshold to CD4 count <500 cells/μL, in keeping with the 2013 consolidated World Health Organization (WHO) guidelines [30], would have resulted in 69.4% of women qualifying for treatment within 6–12 months post-infection, 79.0% within 24 months, and 80.6% within 36 months.

Rapid progressors lost 11.5 $CD4^+$ cells/μL per month in the first year of infection, compared with NRPs who lost 6.1 $CD4^+$ cells/μL (P = .019). For the 25 women with available preinfection CD4 counts (mean, 993 cells/μL), this count dropped by 39.6% (SD, 20.25%) at 3 months and 46.7% (SD, 22.04%) at 6 months postinfection. Rapid progressors experienced a larger CD4 drop during acute infection (48.2% vs 30.2% from preinfection to seroconversion; P = .023). There were no significant differences in

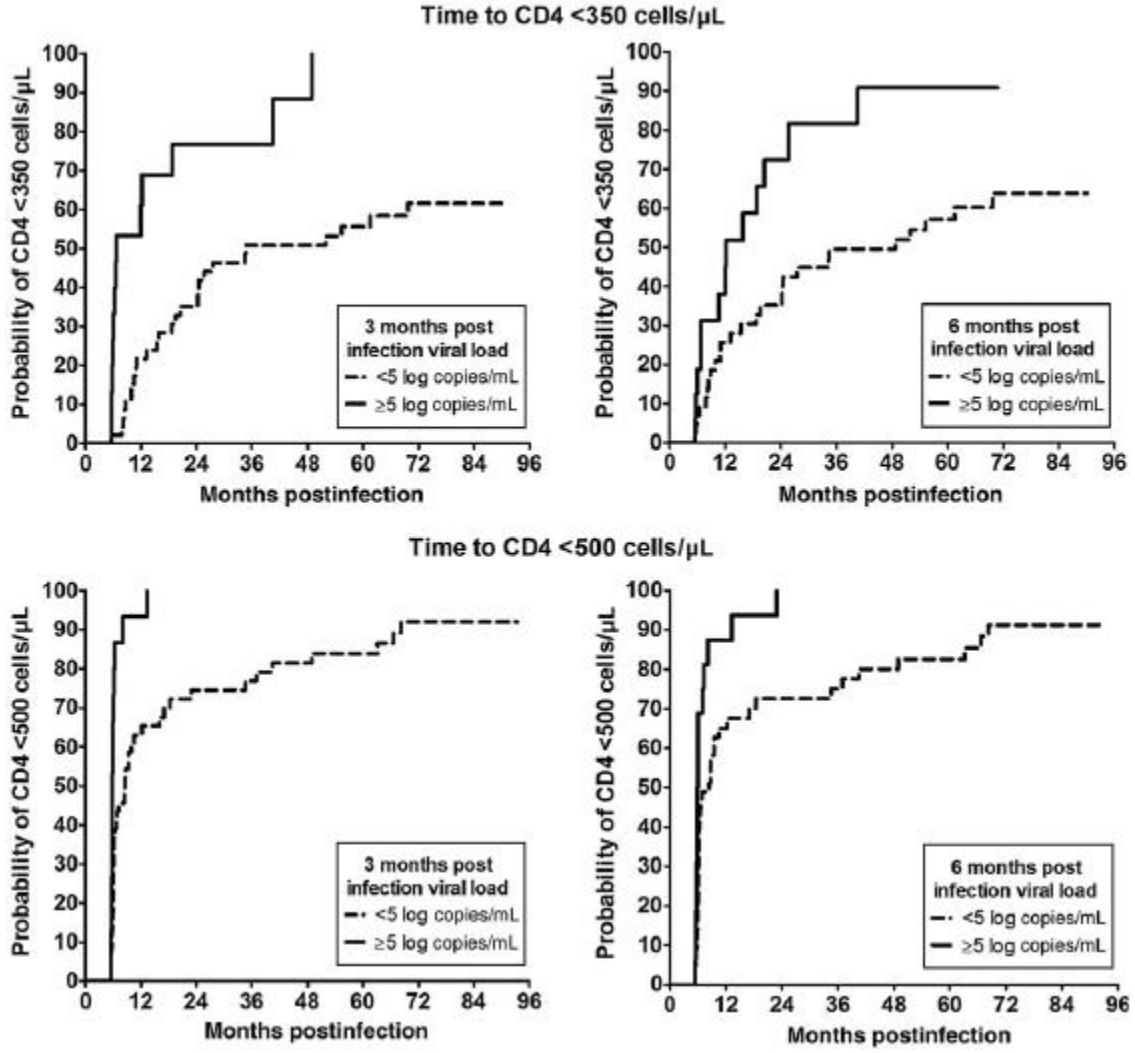


Figure 2. Kaplan–Meier graphs depicting time to CD4 <350 cells/μL and CD4 <500 cells/μL stratified by viral load at 3 and 6 months postinfection.

the preinfection CD4 counts between RPs and NRPs (mean CD4 count, 960 cells/μL and 1029 cells/μL, respectively; P = .649) (Supplementary Figure 1). These data suggest that severe depletion of CD4 T cells in early infection is highly predictive of subsequent disease progression.

Plasma Viral Loads as a Predictor of Rapid Disease Progression

The mean of the first available VL in this cohort (including the available preseroconversion RT-PCR results) was 4.83 log copies/mL (SD, 1.10), at a median of 31 days postinfection (IQR, 14–58 days), likely representing the decline following peak viremia. Mean VL dropped to 4.26 (SD, 0.91) and 4.20 (SD, 0.95) log copies/mL at 6 and 12 months, respectively, representing setpoint viremia. Viral loads were significantly higher in RPs than in NRPs, with a mean VL at 3 months postinfection of 4.86 log copies/mL (SD, 0.62) compared with 4.13 log copies/mL (SD, 0.86; P < .001).

To further determine the impact of VL on disease progression, we compared CD4 decline in participants with high VL, using a cutoff of 5 log copies/mL. Participants with higher VL at 3 months postinfection were more likely to reach a CD4 count <350 cells/μL sooner (log-rank P < .001), compared with women with lower VL. At 24 months postinfection, 77% of those who had high VL at 3 months reached the endpoint, compared with 35% of the lower VL group. The median time to endpoint for those with high VL was 6.7 months from infection compared with 34.6 months with a low VL (Figure 2). Similar results were obtained for VL at 6 months postinfection. The data for a CD4 count endpoint of <500 cells/μL were even more dramatic, with the probability of reaching the endpoint by 2

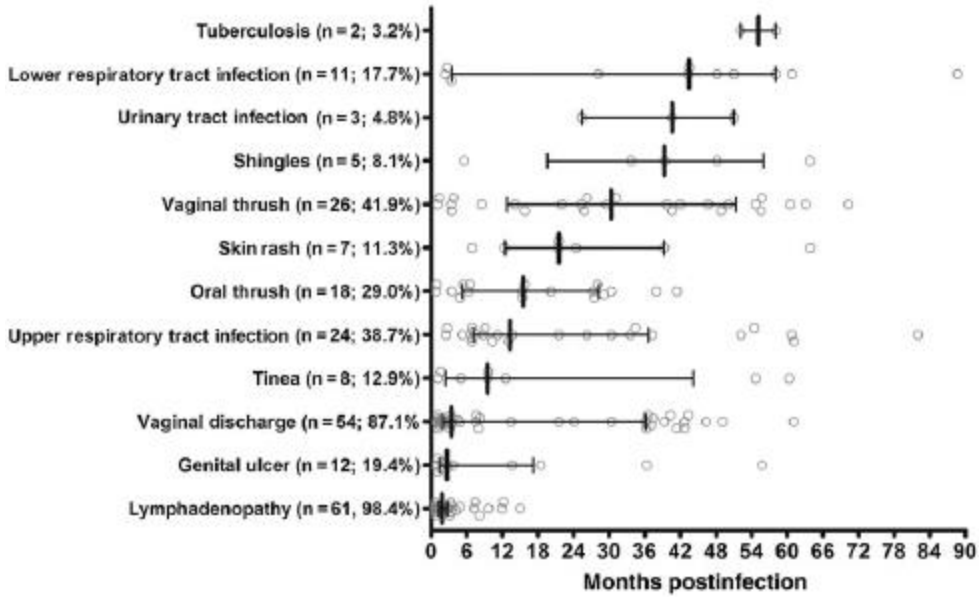


Figure 3. Time to first episode of clinical conditions from acute human immunodeficiency virus infection experienced by study participants. Circles represent participants and when they experienced the first episode of the corresponding clinical condition.

years postinfection for those with high and low VL, reaching 0.93 and 0.75, respectively.

Timing of Clinical Conditions From Acute Infection

The timing of the first presentation of clinical events during the acute phase and subsequent course of HIV infection is shown in Figure 3. Almost all participants experienced lymphadenopathy (98.4%), and many presented with upper respiratory tract infection (38.7%), vaginal discharge (87.1%), and genital ulcer disease (19.4%) during acute HIV infection. Whereas women presented with fungal infection and nonspecific skin rashes throughout the disease course, other infections such as shingles, lower respiratory tract infections, and tuberculosis were diagnosed later during HIV infection. Aside from 2 participants who died of HIV-related diseases (tuberculosis and cryptococcal meningitis), there were no other AIDS-defining diagnoses in the cohort. Of 41 participants, who became eligible and were referred for ART during the study, 37 have so far initiated ART.

Predictors of Rapid HIV Disease Progression

In the unadjusted analysis, associations were noted between rapid progression and not having a regular partner (HR, 3.03 [95% CI, 1.07–8.53]; P = .036), having ≥2 sexual partners in the 3 months prior to infection (HR, 2.06 [95% CI, .96–4.41]; P = .063), and being enrolled at the urban site (HR, 2.47 [95% CI, .85–7.14]; P = .096). CD4 count decline from pre–HIV infection (HR, 1.56 [95% CI, 1.12–2.17 per 10% decrease]; P = .008) and incident hepatitis B infection during the first 6 months (HR, 8.98 [95% CI, 2.21–36.47]; P = .002) were also predictive of rapid disease progression. Notably, in the 3 cases of incident hepatitis B infection identified between HIV acquisition and 6 months postinfection, all rapidly progressed to a CD4 count <350 cells/μL. No associations were observed for age, education, sex worker status, or clinical data including a history of tuberculosis, body mass index, an abnormal full blood count result, or STIs at enrollment into the acute HIV infection cohort (Table 2).

Overall, the best independent predictors of CD4 decline and rapid disease progression were CD4 count at 3 months (HR, 2.07 [95% CI, 1.31–3.28 per 100 cells/μL decrease]; P = .002), VL during early infection (HR, 3.82 [95% CI, 1.51–9.67 per 1-log increase]; P = .005), and hepatitis B infection at the time of HIV infection (HR, 4.54 [95% CI, 1.31–15.69]; P = .017). Conversely, the best predictors of non–rapid progression were any of the previously described protective HLA genotypes (B*1302, B*27, B*57, B*5801, B*8101), with an HR of 0.19 (95% CI, .05–.74; P = .016).

DISCUSSION

The rate of HIV disease progression is highly variable, with important implications for both clinical disease management and program planning. Previous studies have estimated that rapid HIV progression is relatively uncommon, using definitions of CD4 decline within 3 years [31]. Here, we show that nearly half of the clade C–infected women met our conservative definition of rapid disease progression (CD4 decline to <350 cells/

Table 2. Predictors of Rapid Disease Progression Defined as CD4 Count <350 Cells/μL Within 2 Years of Infection

Variable	Level	No. With Rapid Progression/ No. Total	Unadjusted Analysis		Adjusted Analysis[a]	
			HR (95% CI)	P Value	HR (95% CI)	P Value
Demographic and behavioral characteristics						
Age (per 5 y increase)			1.03 (.85–1.24)	.761	0.82 (.55–1.21)	.317
Completed high school	No	19/44	1.0 (Ref)			
	Yes	8/18	1.09 (.48–2.49)	.840		
Marital status	Married or stable partner	16/44	1.0 (Ref)		1.0 (Ref)	
	Many partners	6/10	2.05 (.80–5.25)	.135	0.19 (.03–1.42)	.105
	No partner	5/8	3.03 (1.07–8.53)	.036	0.22 (.03–1.64)	.139
Site	Rural	4/16	1.0 (Ref)		1.0 (Ref)	
	Urban	23/46	2.47 (.85–7.14)	.096	2.09 (.41–10.63)	.374
No. of sexual partners in last 3 m	0–1	15/41	1.0 (Ref)		1.0 (Ref)	
	≥2	12/19	2.06 (.96–4.41)	.063	4.21 (.70–25.35)	.117
Condom at last sex act	Yes	16/35	1.0 (Ref)			
	No	11/27	0.84 (.39–1.82)	.666		
Sex worker	No	18/42	1.0 (Ref)			
	Yes	9/20	1.07 (.48–2.39)	.864		
Ever had anal sex	No	21/53	1.0 (Ref)		1.0 (Ref)	
	Yes	6/9	1.93 (.78–4.79)	.157	0.91 (.23–3.57)	.894
Routine clinical and laboratory assessment						
Personal or family history of hypertension or diabetes	No	15/34	1.0 (Ref)			
	Yes	12/28	0.93 (.44–1.99)	.858		
Creatinine count at 3 mo postinfection			0.96 (.91–1.01)	.087	0.97 (.91–1.04)	.369
History of tuberculosis	No	26/57	1.0 (Ref)			
	Yes	1/5	0.40 (.05–2.97)	.373		
BMI at enrollment, kg/m^2			0.99 (.94–1.05)	.823		
Hemoglobin at 3 mo, g/dL	≥12	15/31	1.0 (Ref)			
	<12	12/31	0.72 (.33–1.53)	.387		
Neutrophil count at 3 mo, ×10^9/L	>2.5	13/29	1.0 (Ref)			
	<2.5	14/32	1.03 (.49–2.20)	.932		
Platelets at 3 mo, ×10^9/L			1.00 (.99–1.00)	.692		
Elevated liver function tests at 3 mo[b]	No	22/51	1.0 (Ref)			
	Yes	5/11	1.00 (.38–2.65)	.993		
CD4$^+$ count decline from baseline (by 10% decrease)			1.56 (1.12–2.17)	.008		
CD4$^+$ count at 3 mo (by 100 cells/μL decrease)			1.98 (1.43–2.74)	<.001	2.07 (1.31–3.28)	.002
Log viral load at 3 mo (by 1 log increase)			2.77 (1.63–4.70)	<.001	3.82 (1.51–9.67)	.005
Any STI[c] at enrollment	No	16/34	1.0 (Ref)			
	Yes	11/27	0.58 (.38–1.78)	.621		
HBcAb status at enrollment	Negative	11/31	1.0 (Ref)		1.0 (Ref)	
	Positive	16/31	1.52 (.70–3.27)	.287	4.54 (1.31–15.69)	.017
HBcAb status at 6 mo	Remained negative	7/24	1.0 (Ref)			
	Baseline positive	14/27	2.10 (.85–5.22)	.109		
	New infection	3/3	8.98 (2.21–36.47)	.002		

Table 2 continued.

Variable	Level	No. With Rapid Progression/ No. Total	Unadjusted Analysis HR (95% CI)	Unadjusted Analysis P Value	Adjusted Analysis[a] HR (95% CI)	Adjusted Analysis[a] P Value
HLA types						
Protective HLA-B types[d]	No	24/47	1.0 (Ref)		1.0 (Ref)	
	Yes	3/15	0.30 (.09–.99)	.048	0.19 (.05–.74)	.016
Harmful HLA-B types[d]	No	18/43	1.0 (Ref)			
	Yes	9/19	1.04 (.47–2.32)	.922		

Abbreviations: BMI, body mass index; CI, confidence interval; HBcAb, hepatitis B core antibody; HLA, human leukocyte antigen; HR, hazard ratio; Ref, reference; STI, sexually transmitted infection.

[a] Due to missing data, the adjusted analysis was performed on a total of 55 participants, with 26 events of rapid disease progression.

[b] Elevated liver function tests defined as having alanine aminotransferase level >35 IU/L and/or aspartate aminotransferase level >35 IU/L.

[c] Any sexually transmitted disease, defined as testing positive for Treponema pallidum, Trichomonas vaginalis, Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, or herpes simplex virus type 2 polymerase chain reaction.

[d] Protective HLA-B types are B*1302, B*27, B*57, B*5801, B*8101. Harmful HLA-B types are B*1801, B*35, B*5802.

μL within 2 years of HIV-1 infection). Similar findings have been reported from Argentina where 26% of untreated acute HIV infections showed clinical or immunological progression within 12 months of infection [32]. The high proportion of women experiencing rapid disease progression suggests that this may be the dominant phenotype in the natural history of HIV-1 disease in our setting. This finding supports the argument of starting ART early, especially in settings where the risk of coinfections such as tuberculosis is high. The 2013 WHO guidelines suggest starting ART at a CD4 count of <500 cells/μL [30]. Here, we showed that nearly three-quarters of HIV-infected individuals will reach this threshold within 1 year of infection.

Preinfection CD4 counts in this population were similar to those in developed countries, with no association between preinfection CD4 counts and disease progression rate. A striking finding in this study is the magnitude of CD4 cell depletion during acute HIV infection, with nearly half of preinfection CD4 cells lost within the first 6 months of HIV infection. We observed very little CD4 cell rebound after acute infection, in contrast to the modest and transient loss described in most published studies. The rate of CD4 cell loss in the rapid progressors is higher than that observed in other HIV-1 subtype C–infected cohorts [5].

We have previously shown in this cohort that the magnitude or breadth of T-cell recognition across the expressed genome at 3 months had no association with viral setpoint at 12 months [33] and that recognition of more conserved epitopes in Gag tended to associate with slow disease progression in the first year of infection. This was in parallel with the accumulation of less differentiated Gag-specific $CD8^+$ T memory cells by 6–9 months postinfection in individuals with lower viral setpoints [34]. It appeared that the higher setpoint induced a greater level of immune activation, which resulted in high T-cell turnover being directly proportional to the level of viremia [13, 35]. This study confirms the direct association of high VL with rapid $CD4^+$ cell loss, which, in turn, is related to levels of T-cell activation [36]. Collectively, these data suggest that recognition of class I HLA–restricted variant epitopes and the late stage of $CD8^+$ memory differentiation are concentrated in women with rapid disease progression. We have previously shown that the frequency of conserved HLA-B restricted epitopes is more abundant in non–rapid progressors [37].

The long-term follow-up of this cohort enabled us to describe the trajectory of clinical signs and symptoms occurring in these women. STIs (genital ulcers and discharge) were common during early infection, whereas other infections such as varicella and tuberculosis occurred later. This is likely because the former are co-transmitted, or are the result of reduced mucosal barrier function as a result of CD4 loss, whereas the latter require more advanced immunodeficiency. Although this cohort showed rapid immunologic progression, there was little evidence of severe clinical disease or early AIDS-defining illnesses within the first 3 years of infection [32], compared with what has been observed in other cohorts. The most likely reason for this was the close follow-up of participants and prompt ART initiation prior to onset of AIDS.

A limitation of this acute HIV-infected cohort is the relatively small sample size, albeit comparable to similar cohorts, and our findings may therefore not be generalizable outside our setting. Because this was a female cohort, caution would need to be exercised when extrapolating these results on disease progression to the male population.

The rapid progression observed in this cohort provides additional motivation to implement earlier ART initiation.

Although this may present economic and operational challenges, the fact that half of individuals need ART in the first year of HIV infection provides compelling data for continued treatment roll-out. Long-term ART use will bring challenges of adherence and potential development of drug resistance, and require more health system strengthening to manage HIV as a chronic illness. Despite the challenges, this study suggests that earlier treatment initiation carries many benefits, including the potential for major impact on individual health by increasing survival and on public health by preventing transmission. The concept of "test and treat" might even be more pertinent in KwaZulu-Natal in South Africa, where we show that rapid disease progression in women is disturbingly common. Given the new WHO guidelines of ART initiation at a CD4 count of ≤500 cells/μL, more effort should be placed into diagnosing acute HIV infection to ensure that the large proportion of women requiring treatment within the first year of infection are not missed.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. K. M., L. W., F. v. L., C. W., C. M. G., L. M., and S. S. A. K. were involved in study design, protocol writing, data collection, and analysis and interpretation of data. N. J. G., L. R. M., and J.-A. S. P. contributed to data analysis and interpretation. K. M. wrote the first draft of the manuscript. All the authors participated in the writing, reviewing, and finalization of the manuscript.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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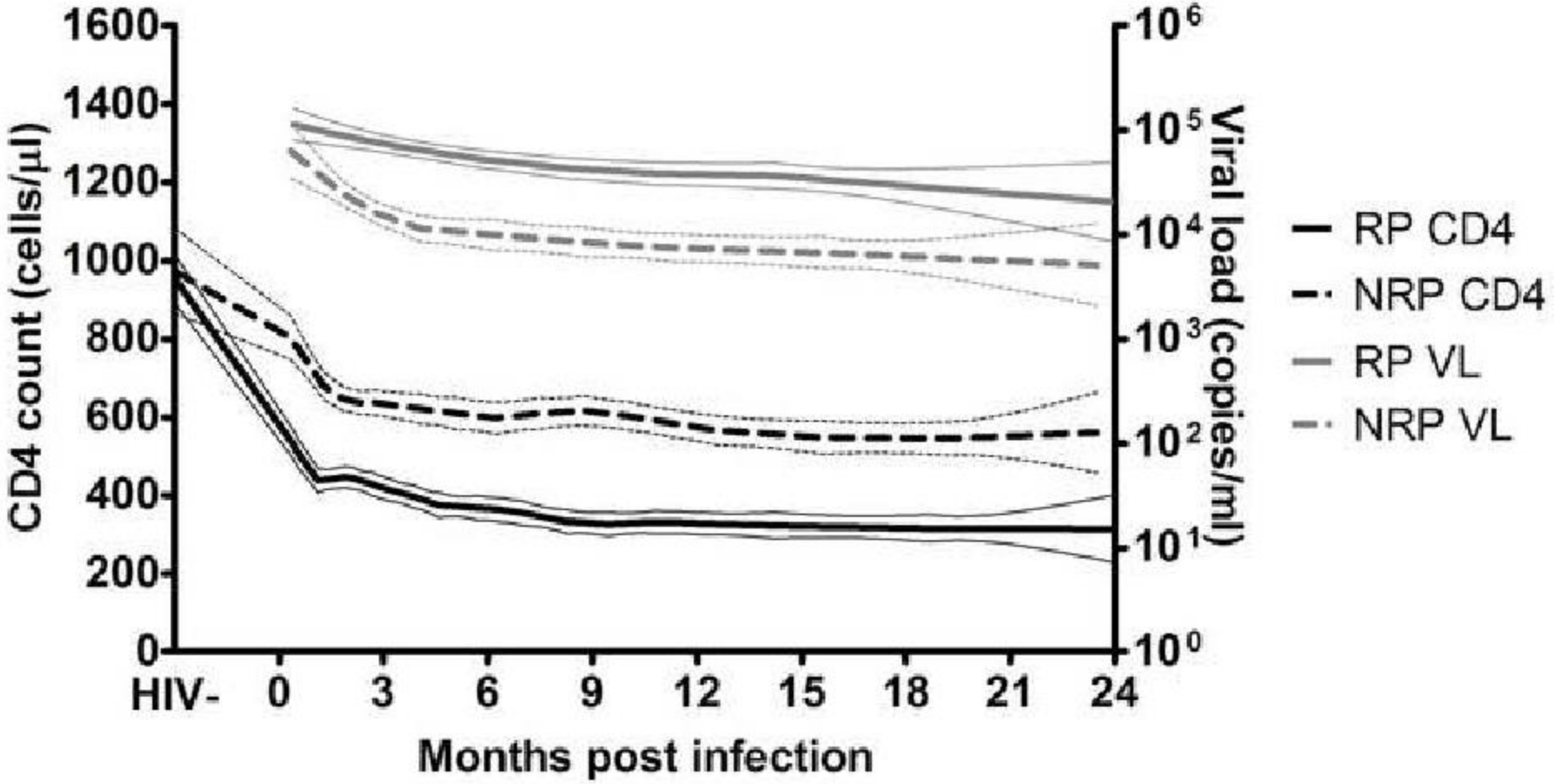
Supplementary Figure 1: Loess smoothing graphs and 95% confidence intervals of viral load and CD4 count in the first 24 months of HIV infection in rapid and non-rapid progressors.



Chapter 9:

Conclusions

Chapter 9: Conclusions

This dissertation described clinical, mucosal and systemic features associated with HIV infection and their relationship to disease progression in a population of women at high-risk for HIV infection. STIs and associated genital tract inflammatory cytokines were found to increase risk for HIV infection and only 12.4% of women who had a laboratory diagnosed STI presented with symptoms or had any clinical signs detected by the clinician. . Elevated cytokine signatures of genital inflammation were observed in both the symptomatic and asymptomatic women, signifying a similar risk for HIV infection. Of the STIs evaluated, the presence of *Neisseria gonorrhoea* in this cohort was the strongest predictor of subsequently becoming HIV infected.

The HIV incidence in this cohort was 7.2/100 person-years (95%CI 4.5–9.8). In those women who became infected with HIV, 57% presented with at least one sign or symptom at seroconversion and the specificity and likelihood ratios increased with increasing number of reported symptoms. The most prevalent signs and symptoms reported at acute HIV infection visit were loss of appetite, headache, rash, sore throat, fever, arthralgia and reported weight loss. A risk score algorithm to enhance diagnosis of acute HIV infection was developed, using the variables significantly associated with AHI (rash, sore throat, weight loss, genital ulcers, vaginal discharge and age <25 years).

Genital tract inflammation around the time of acute HIV infection, CD4+ T cell count at 3 months post-infection ($p = 0.002$), viral load set-point ($p = 0.005$) and

hepatitis B infection (p = 0.017) were found to influence the rate of disease progression . Most importantly, this study showed that almost a third of women who became infected, progressed to CD4+ T cell counts below 350 cells/ul within 6 months of infection and over half were eligible for HAART within 2 years of infection. These findings of high rates of rapid disease progression in South African women highlights the importance of active identification of acute HIV infected individuals and subsequent care and early treatment initiation. The economic implications as well as the operational challenges associated with addressing these findings, especially in the current WHO recommendation of ART initiation at 500 cells/ul, demand appropriate strategies for individual and public health benefits.

9.1 Women at high-risk for HIV infection: A prospective cohort study

A cohort of HIV-uninfected women was established in Durban, South Africa, in order to investigate clinical, immunological and virological factors that may play a role in HIV acquisition and disease progression in this population of high HIV prevalence (van Loggerenberg, Mlisana et al., 2008; 2012). This cohort comprised 245 women who were 18 years or older (median age of 34 years), of whom more than 75% self-identified as sex workers, had a high level of HIV/AIDS knowledge, and more than half reported regular condom use. It was interesting to note that choice to use condoms at their last sexual act varied depending on whether they were having sex with steady versus casual partners (57.0% versus 64.4%, p = 0.36), although their perceived ability to choose to use condoms was significantly lower with steady partners compared to casual partners (van Loggerenberg, Mlisana et al., 2012). In this cohort of high-risk HIV-uninfected women, the retention rate after 2 years of monthly follow-up visits was an acceptable 86.1%, with 28 new HIV-1 infections being

observed during 390 person-years of follow-up, resulting in an HIV incidence rate of 7.2 (95% CI 4.5-9.8) per 100 person-years (van Loggerenberg, Mlisana et al., 2008). Having such intensive clinical follow-up from pre-infection time points, through to acute HIV infection and establishment of chronic phase of infection and determining viral set-point in the same individuals enabled very detailed laboratory studies in subsequent chapters on factors associated with increased risk for HIV infection, the natural history of HIV-1 subtype C infection across the spectrum ranging from acute to chronic phases of infection, and the high rate of rapid disease progression.

9.2 Poor performance of syndromic management of STIs in settings of high risk for HIV infection

Women with a laboratory-diagnosed STIs were at 3-fold increased risk of HIV infection. Chlamydia, gonorrhoea and *M. genitalium*, were each associated with increased risk of HIV acquisition, with gonorrhoea associated with highest risk (almost 5-fold). These findings confirm those of previous studies that have demonstrated associations between these STIs and HIV acquisition risk [1-3]. Although several studies have shown BV to be associated with increased risk of HIV infection [4], it was interesting that no increased association was observed in this cohort study. It is possible that the sample size in this study and the high rates of co-infections with other inflammatory STIs may have masked this phenomenon. The emerging microbiome research studies to identify and characterize the human vaginal microbial populations will hopefully aid in further determining the relationship of BV and HIV acquisition. Additionally, although genital ulcerative STIs have been found to be associated with increased HIV acquisition [5, 6], we found that women with clinical signs of STIs, including abnormal vaginal discharge and genital ulcers, were

not more susceptible to HIV-infection, although only a small proportion of women presented with ulcers in this study.

Of the STIs assessed, chlamydia and gonorrhoea (which were both associated with increased HIV acquisition risk) were found to be the most inflammatory in this study, as indicated by up-regulated concentrations of multiple pro-inflammatory cytokines and chemokines. Trichomoniasis and HSV-2 reactivation were less inflammatory and BV was associated with up-regulated pro-inflammatory cytokines, but down-regulated chemokines. Elevated genital inflammatory cytokines, including IL-1β, IL-6, IL-8 and sCD40L, were also associated with increased risk of HIV acquisition. It has been suggested that inflammatory cytokine and chemokine up-regulation in the female genital tract may increase HIV acquisition risk by recruiting and activating HIV target cells, reducing epithelial barrier integrity and activating NF-kB.[[7-9] Although this suggests that underlying genital tract inflammation may be a biological mechanism for HIV transmission in women with STIs, it is also possible that the relationship between elevated inflammation and risk of HIV infection observed in this study was indirect.

Despite the fact that some of the STIs were associated with high concentrations of inflammatory markers, most women with these infections did not have clinical signs. Importantly, genital tract inflammatory cytokine concentrations were similarly elevated in women who had symptomatic and asymptomatic laboratory-diagnosed STIs, relative to women who had no STIs, bacterial vaginosis, or symptoms. Although the prevalence and incidence of laboratory-diagnosed STIs in this cohort were high, only 12.3% of women who tested positive for at least one STI had visible clinical signs. As a result, 87.7% of STIs in this cohort of women, who were at high risk of HIV acquisition, would have been left untreated in a syndromic management

setting such as South Africa. The findings that clinically evident and syndromically managed STIs declined during follow-up while the prevalence of laboratory-diagnosed STI remained relatively high confirms that syndromic management does not address subclinical STI infections, which may contribute to genital inflammation and risk of HIV infection.

This study highlights the urgent need for better strategies to manage women with asymptomatic STIs, as infections such as chlamydia and gonorrhoea were found to have significantly elevated laboratory markers of genital inflammation, irrespective of absence of symptoms. This has important implications not only for HIV prevention strategies, but also for complications that are associated with untreated inflammatory STIs, including pelvic inflammatory diseases, ectopic pregnancy, and infertility [10]. The burden of asymptomatic STIs highlights the need for laboratory screening of, at least high risk populations; which would include men who have sex with men (MSMs), female sex-workers (FSWs), young adults in high HIV prevalence communities. Howell et al, has described enormous cost saving estimates and prevention of related morbidities associated with STI screening of young women in the USA, using the CDC criteria [11]. In South Africa, syndromic management of STIs alone, has been estimated to have attributed a 6.6% (3.3 – 10.3%) reduction of adult HIV incidence between 1994 and 2004 when syndromic management coverage was about 52% [3]. The public health gains that would be realised with national screening of high risk populations in SA would therefore far outweigh the cost thereof.

Healthcare systems should include regular screening for STIs by means of laboratory testing in these groups rather than relying on symptoms only. The increased diagnostic power of PCR technology and the related potential to impact the risk of

HIV acquisition could outweigh its high costs. This strategy would lead to treatment of greater number of individuals with STIs, reducing prevalence and incidence. Another diagnostic approach in under-resourced areas would be to explore point-of-care STI testing as an alternative to laboratory testing. Some of these point-of-care tests have proven to be sensitive diagnostic tools in clinical trials and would offer significant benefits in poorly resource settings with absent or limited access to larger STI diagnostic laboratories [12, 13]. Mathematical modelling of point of care testing for *C. trachomatis* and *N. gonorrhoeae* amongst just sex-workers in Sub-Saharan Africa, China and South-East Asia estimates that >16.5 mil new gonococcal and chlamydia infections as well as >212 000 HIV infections could be prevented over a 4 year period by availability of such diagnostic tests and more than 4 million disability-adjusted life years could be saved. There is a definite need to ensure that STIs are diagnosed in a timely fashion and managed comprehensively by educating individuals at risk for transmission and acquisition on how to reduce the risk; early recognition of signs and symptoms of infections; improving health seeking behaviour in asymptomatic and symptomatic individuals; effective management of those symptomatic individuals seeking care and effective management of sexual partners to include education and treatment.

9.3 Challenges to diagnosis of acute HIV infection

Being able to diagnosis acute HIV infection rapidly has broad benefits to both the individual and community so defining an algorithm to identify acute HIV infection (AHI) cases in resource limited settings would be enormously useful. Accurate recognition of signs and symptoms of acute HIV infection is critical for enhancing early diagnosis of HIV infection. Chapter 6 reported that 57% of all women reported at least one sign or symptom of AHI at the study visit post HIV infection. The factors

that were strongly predictive (from highest to lowest) of AHI were genital ulceration and vaginal discharge; skin rash, losing weight, younger age, or having a sore throat. These variables significantly predicted AHI and were used to construct a predictor risk score for AHI the regression coefficients from the final adjusted model (Mlisana et al., 2013). The signs and or symptoms in the model were weighted by the regression coefficients with the final risk score algorithm calculated as follows:

Risk Score = 2 [skin rash] + 1 [sore throat] + 2 [loss of appetite] + 1 [weight] loss] + 2 [vaginal discharge] + 2 [genital ulcer] + 1 [age <25 years]

A proposed risk score of 2 correctly predicted AHI in 57% of cases, with the number of signs and symptoms correlating with higher plasma HIV-1 RNA at the time of diagnosis. This algorithm enabled risk-stratifying of individuals for AHI especially in resource-limited settings where no or limited testing for AHI exists. Although this panel of clinical symptoms or signs has been associated with AHI in several studies [14-16], HIV incidence in those studies was far less than 7.2/100 person-years seen in our cohort. Our study was the first to focus on HIV -1 subtype C infection in women, in South Africa. Whether these findings or such a scoring algorithm can be generalised to men presenting with AHI needs further exploring.

9.4 Factors influencing rate of disease progression

Sixty-two women were identified at a median of 42 days post-infection (interquartile range, 34–59), contributing 282 person-years of follow-up. Mean CD4 count dropped by 39.6% at 3 months and 46.7% at 6 months post-infection in women with pre-infection measurements. CD4 decline to <350 cells/μl occurred in 31%, 44%, and

55% of women at 1, 2, and 3 years post-infection, respectively, and to <500 cells/μl in 69%, 79%, and 81% at equivalent time points. Predictors of rapid progression were CD4 count at 3 months post infection, set point viral load, and hepatitis B co-infection. Conversely, presence of any of HLAB*1302, B*27, B*57, B*5801, or B*8101 alleles predicted non–rapid progression. Nearly half of subtype C–infected women progressed to a CD4 count <350 cells/μl within 2 years of infection. Implementing 2013 World Health Organization treatment guidelines (CD4 count <500 cells/μl) would require most individuals to start antiretroviral therapy within 1 year of HIV infection. A meta-analysis of trends in prognostic markers of HIV disease progression confirmed an increase in virulence of the virus over the epidemic course [17]. This analysis showed that a CD4+ T-cell trend of −4.93 cells/μl per year and viral RNA of 0.013 log10 copies/ml per year reflect a loss of CD4+ T-cells of 148 cells/μl and an increase of 0.39 log10 copies/ml RNA over the 30-year period of the HIV epidemic[17].

9.5 Limitations and Strengths of this study

The small sample size of the cohort is the main limitation of this study and this was conducted in women and so limits the generalizability of the conclusions to other populations. However, it is still one of the first acute HIV infection cohorts in a subtype C HIV-1 infected population. The socio-behavioural data collection relied on self-report and was therefore subject to reporting and recall bias. However, in an attempt to minimize reporting bias, the questions were constructed to be neutral and participants were assured of confidentiality. An independent validation of the acute HIV infection diagnostic algorithm on another cohort would be needed to further assess its utility. Point-of-care antigen or viral load technology is required to detect

asymptomatic, antibody negative cases enabling early interventions and prevention of transmission.

This study presented the first prospective cohort to be assembled in southern Africa for a comprehensive analysis of the behavioural, clinical and immunological characteristics associated with acute HIV infection. The longitudinal design allowed for long follow up and therefore understanding of the natural history of HIV infection. This has led to a better understanding of risk factors for HIV acquisition; subtype-C pathogenesis and disease progression. It is one of the few cohorts with pre-HIV infection, seroconversion as well as post-infection data on a significant percentage of the studied population.

9.6 Final conclusion

This work highlights key points with regards to HIV-1 subtype C infections in South African women.

- The feasibility of establishing cohorts of high risk HIV-1 uninfected women with high retention rates and intensive follow-up could be established, notwithstanding the challenges with screening and recruitment in high prevalence settings. A major contribution of this cohort was the follow-up of HIV uninfected to AHI to established infection. Such cohorts are critical for understanding host and viral dynamics for HIV prevention, treatment and vaccine research.
- Sexually transmitted infections, whether symptomatic or not, increase the risk of HIV acquisition and transmission. The burden of asymptomatic STIs needs to be urgently addressed by screening high risk populations as well as

identifying simple and affordable point-of-care tests especially for gonococcal and chlamydial infections.

- Genital inflammation in females, occurs in asymptomatic sexually transmitted infections as well, with similar consequences of HIV risk. *Chlamydia trachomatis* infection was associated with the highest genital cytokine levels, followed by *Neisseria gonorrhoea*, HSV-2, *Trichomonas vaginalis* and bacterial vaginosis.
- Acute HIV seroconversion syndrome is seen in a significant percentage of individuals in resource limited countries, and therefore, clinicians need to have an increased index of suspicion to identify these. Further studies are needed to evaluate the proposed clinical scoring algorithm for use in poorly resourced regions with high prevalence of HIV to identify acute infections.
- Genital inflammation during early HIV-1 infection was associated with higher viral load set-point and CD4 depletion. CD4 counts at 3 months post-infection, viral load set-point and Hepatitis B co-infection were predictors of rapid progression in this cohort. Nearly half of these subtype C infected women progressed to a CD4 count <350 within 2 years of infection and with the imminent increase of ART initiation at <500 cells/µL, following WHO recommendations, will see the majority of South African women requiring ART within 1 year of HIV infection.

Additional important questions that are raised by these findings, that provide avenues for future research to include:

- The role of the genital microbiome in shaping inflammatory cytokine signatures and contributing to HIV acquisition risk;

- A clearer understanding of why certain genital tract infections are associated with specific cytokine signatures and how such signatures relate to susceptibility to HIV acquisition;
- Whether these cytokine signatures could be used in developing alternative rapid diagnostic assays for STI diagnosis, particularly in high risk asymptomatic patients.
- It is important that future research should determine the impact of improved STI diagnosis and treatment on HIV acquisition and transmission as well as the impact of early antiretroviral therapy on clinical outcomes in HIV subtype C infected women.

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Appendix 1

Publications emanating from the Acute Infection Study [1-48]

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