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HIV-1, HIV-2, and dual infection with HIV-1 and HIV-2 are associated with increased risk for human papillomavirus (HPV) and high grade squamous intraepithelial lesions (HSIL) in Senegal, West Africa

Stephen Edward Hawes

A dissertation submitted in partial fulfillment of the

requirements for the degree of

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Department of Epidemiology

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Abstract

HIV-1, HIV-2, and dual infection with HIV-1 and HIV-2 are associated with increased risk for human papillomavirus (HPV) and high grade squamous intraepithelial lesions (HSIL) in Senegal, West Africa

Stephen Edward Hawes

Chair of the Supervisory Committee:
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We conducted a cross-sectional screening study of HIV-1 and HIV-2, human papillomavirus (HPV), and cervical intraepithelial lesions (SIL) in previously unscreened women in an outpatient infectious disease clinic in Dakar, Senegal. In addition, we conducted a prospective study of development of HSIL among women with HIV-1 and/or HIV-2 and HPV infection seen in that clinic and a sexually transmitted disease clinic serving commercial sex workers. Women infected with HIV-1 alone (OR=7.3, 95% CI=5.5-9.7), HIV-2 alone (OR=4.7, 95% CI=2.7-8.3), and coinfection with HIV-1 and HIV-2 (OR=10.2, 95% CI=4.3-24.4) were at significantly increased risk for prevalent infection with high risk HPV compared to women without HIV infection. Similarly,

women infected with HIV-1 alone (OR=3.7, 95% CI=1.9-7.4), HIV-2 alone (OR=7.1, 95% CI=2.9-17.4), and coinfection with HIV-1 and HIV-2 (OR=14.4, 95% CI=4.2-50) were at increased risk for prevalent high grade squamous intraepithelial lesions (HSIL) compared to women without HIV infection. In the absence of high risk HPV, women with HIV infection were not at increased risk of HSIL. In women with HIV-1 and/or HIV-2 infection, increased HIV plasma RNA loads and decreased CD4 levels were significantly associated with high risk HPV detection and cervical abnormalities. HIV-positive women (with HIV-1, HIV-2 or HIV coinfection) were ten times as likely to have invasive cervical cancer identified by cytology or biopsy. In the longitudinal study, we followed 630 women over a mean of 2.2 years, during which 73 (11%) developed HSIL as detected by cytology. Among women without oncogenic HPV types detected, HIV positive and negative women were at similar risk for development of HSIL. Persistent infection with oncogenic HPV types (RR=10.4, 95% CI=4.1-26.3) was most strongly associated with HSIL risk. Among HIV positive women, those with low CD4 counts and high HIV plasma levels were at greater risk of HSIL, through persistence of their HPV infections. After adjustment for CD4 counts and HPV infection, women with HIV-1 and HIV-2 had similar risks for HSIL. In conclusion, HPV infected women with HIV-1 and/or HIV-2, especially those who are severely immunosuppressed, are at significantly increased risk for HSIL and invasive cancer.

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DEDICATION

To my wonderful wife, Lynn, and my children Nicholas, Emma, and Sarah.

CHAPTER 1 – Human papillomavirus, high grade squamous intraepithelial lesions, and invasive cervical cancer in previously unscreened women with and without HIV-1 and HIV-2 infection

INTRODUCTION

Cancer of the uterine cervix remains a major cause of morbidity and mortality among women. Recent estimates indicate that cervical cancer is the third most common cancer among women worldwide, and accounts for the highest cancer mortality and the second highest cancer incidence among women in developing countries [Parkin, 1999; Pisani, 1999]. It is now well demonstrated that specific types of sexually transmitted human papillomaviruses (HPV) play a central role in the development of pre-invasive cervical lesions [Koutsky, 1992; Remmink, 1995; Ho, 1998] and invasive cervical cancer (ICC) [Bosch, 1995; Cuzick, 1998].

ICC was named an AIDS defining disease in women in 1993 [CDC Update, 1993]. Our previous studies and those of others have shown that infection with HIV-1 is associated with increased risk of HPV infection and cervical intraepithelial neoplasia (CIN), a precursor to invasive cervical cancer [Feingold, 1990; Vermund, 1991; Laga, 1992; Klein, 1994; Coll-Seck, 1994; Wright, 1994; Miotti, 1996; Langley, 1996; La Ruche, 1998; Six, 1998; Vernon, 1999; Massad, 1999; Ellerbrock, 2000]. However, most of these studies have focused on low grade squamous intraepithelial lesions (LSIL), generally thought to be self-limited morphological manifestations of HPV infection, rather than high grade squamous intraepithelial lesions (HSIL, lesions thought to be the

immediate precursors to ICC) or invasive cancer. Further, very few studies have been conducted in Africa, where the disproportionate share of HIV infections occur and routine cervical cancer screening is not available. Even fewer have investigated a possible role for HIV-2. We therefore conducted a cross-sectional study in Dakar, Senegal, West Africa to assess the risk of HPV infection, HSIL, and ICC associated with infection with HIV-1 or HIV-2, dual infection with both HIV-1 and HIV-2, HIV plasma RNA load, and HIV-induced immunosuppression as measured by CD4 count.

MATERIALS AND METHODS

Study Population

Between October 1994 and January 1998, all women older than 15 years presenting to the University of Dakar outpatient infectious disease clinic were offered cervical HPV DNA and Pap smear screening, and HIV-1 and HIV-2 serologic testing. Women present to this clinic for reasons including family planning (24%), gynecologic problem or exam (35%), infertility (7%), respiratory symptoms (4%), HIV infection in the patient (5%) or spouse (2%), and other specified (14%) or unspecified (9%) reasons. The study was conducted according to procedures approved by the institutional review boards of both the University of Washington and the University of Dakar, and persons found to be positive for HIV infection were counseled according to locally established guidelines.

Collection of Specimens and Study Procedures

At the screening visit, blood was collected for HIV-1 and HIV-2 serologic testing, and cervical cellular samples were obtained by cytological screening. Subjects underwent a

general physical examination and completed a short standardized interview, including questions concerning reason for visit, age, marital status, country of birth, ethnicity, religion, profession, years of formal education, number of births, current contraceptive method, prior Pap smear history, cigarette and alcohol use, sexual behavior and medical history. If the subject was found to be HIV seropositive, additional blood was obtained for HIV-1 and HIV-2 qualitative and quantitative RNA assays, and for determination of CD4 and CD8 counts. CD4 and CD8 counts were also performed on a randomly selected subset of HIV negative women.

Cytology Screening

Pap smears judged sufficient for diagnosis were interpreted and classified according to the Bethesda System [NCI Workshop, 1989] as negative, atypical cells of uncertain significance (ASCUS), LSIL, HSIL, or ICC. All Pap smears were initially read by a pathologist in Senegal, and later sent to Seattle for a second reading, if the slide was available. All slides classified as LSIL or worse by either pathologist were subsequently re-stained, re-covered and re-read by the pathologist in Seattle. For analysis purposes, the final cytology diagnosis was that made by the Seattle pathologist; however if the slide was not re-read or re-classified in Seattle, the diagnosis made by the Senegalese pathologist was used. Overall, 3,236 (79%) of 4,123 screening Pap smears were re-read in Seattle.

Histologic Methods

By study design, colposcopically-directed biopsy specimens of the uterine cervix were to be obtained from women with HSIL or invasive cervical cancer detected by cytology. Representative hemotoxylin and eosin-stained slides were prepared from paraffin-embedded biopsies, and reviewed by the pathologist without knowledge of other clinical or laboratory data. Standard gynecologic pathology criteria and terminology were used to classify all intraepithelial lesions and invasive cervical cancers.

HPV DNA Detection and Typing

Polymerase chain reaction (PCR) assays for the detection of HPV DNA were performed using HPV L1 consensus primers and HPV type-specific oligonucleotide probes to HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52 and 56 and a generic probe as previously described [Kuypers, 1993]. Initially, screening for high risk HPV types was done with a 10 probe mix, testing for DNA from HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, or 56 by PCR using consensus primers MY09 and MY11 to a highly conserved region in the L1 open reading frame. Subsequently, samples were re-amplified in order to assess presence of specific HPV types. First, samples were tested with a generic probe to detect the presence of any HPV DNA. Positive samples were then re-dotted for 12 HPV types in primer groups for HPV 6/11 ('low risk' HPV types), 16, 18, 31/33/35/39, 45/56, and 51/52 (the latter being 'high risk' HPV types). Samples positive by the generic probe but negative by the type-specific probes were called positive, but untyped. Samples positive by the initial 10 probe screening mix, but not positive by the type-specific probes were classified as 'high risk' HPV types.

HIV Serology and Lymphocyte Subset Analysis

Initial screening of serum samples for HIV-1 or HIV-2 antibodies was performed using a microwell plate enzyme immunoassay that detects antibodies to both HIV types in one well (HIV 1/2 EIA, Sanofi Diagnostics Pasteur, Redmond, WA). Positive samples were confirmed using a rapid, HIV peptide-based membrane immunoassay that distinguishes between antibodies to HIV-1 and HIV-2 (Multispot, Genetic Systems, Redmond, WA). Whole blood collected in EDTA tubes was analyzed using the FACSCount analyzer (Becton Dickinson Biosciences, San Jose, CA) to determine the number of CD4, CD8, and CD3 cells per microliter of blood. Cell counts were performed on the majority of samples from HIV-infected women, as well as on approximately 10% of samples from HIV-negative subjects.

Quantitation of HIV-1 and HIV-2 Plasma RNA

Quantitative and qualitative assays for HIV-1 RNA were performed as follows. RNA was extracted and quantified from EDTA-anticoagulated blood as described for the AMPLICOR™ Monitor HIV-1 Test (Roche Molecular Systems, Pleasanton, CA), with the exception that two additional primers were added to the mastermix. These two primers, SK145 and SK 151, are identical in length to the first generation primers (SK462 and SK431) and hybridize to the identical regions. The added primers were designed to have fewer mismatches with variant non-subtype B isolates. Whereas SK462 and SK431 inefficiently amplify some divergent isolates, these additional primers provide accurate quantification of HIV-1 RNA in plasma samples from individuals infected with different genetic subtypes [Aleus, 1999; Triques, 1999]. For qualitative detection of HIV RNA,

plasma samples were prepared as described for the quantitative assay, except that plasma samples were suspended in 200 µl of specimen diluent. RNA amplifications were performed as described for the quantitative assays but with an increased number of cycles (N=35). The amplified products were analyzed undiluted in microwell plates coated with the respective HIV and quantitation standard (QS) probes. A positive reaction was determined using a cut-off absorbance value at 450 nm of $\geq$0.350. This quantitative RNA assay can detect as few as 80 copies of HIVRNA/ml, and has a reproducible sensitivity of 400 HIV RNA copies/mL. The qualitative assay was performed on all available samples that were negative with the quantitative assay. The RNA assay can detect as few as 40 copies of HIVRNA/ml, and has a reproducible sensitivity of 200 HIV RNA copies/mL.

The HIV-2 assays use primers RAR 1000 (5'GCTGGCAGATTGAGCCCTGGGAGGTTCTCT 3') and RARO4 (5'GAATGACCAGGCGGCGACTAGGAGAGAT 3') to amplify a 201 base pair fragment of the LTR region. These primers hybridize to a region of the HIV-2 LTR that is highly conserved among the known HIV-2 and SIV isolates; the upstream primer has no more than a two base mismatch while the downstream primer has no mismatches with the sequenced isolates. As with the HIV-1 assays, an internal quantitation standard is used to monitor reaction variability. The QS harbors the HIV-2 primer binding sites but has different probe sequences to allow differentiation from the true target. The RT-PCR reactions were amplified using the following thermal cycling parameters: (a) 1 cycle at 60°C for 30 min for reverse transcription, (b) 5 cycles at 94°C for 20 sec, 60°C for 20 sec, and 72°C for 10 sec, (c) 35 cycles at 90°C for 10 sec, 65°C for 20 sec and 72°C for 20 sec,

(d) hold at 72°C. The HIV-2 quantitative assay is comparable to the HIV-1 RNA test in sensitivity, accuracy, linearity, and reproducibility and can detect as few as 40 copies of HIV-2 RNA/mL and has a reproducible sensitivity of 200 HIV-2 RNA copies/mL. For qualitative detection of HIV-2 RNA, plasma samples were prepared as described above for the HIV-1 qualitative RNA assays. This qualitative HIV-2 RNA assay can detect as few as 20 copies of HIVRNA/ml, and has a reproducible sensitivity of 100 HIV RNA copies/mL.

Statistical Methods

For most analyses, HIV-1 and HIV-2 were included as two separate, independent, dichotomous variables (infected with HIV-1 versus not infected with HIV-1, and infected with HIV-2 versus not infected with HIV-2). In other analyses, HIV antibody status was considered a categorical variable with four levels (HIV-negative, HIV-1 only, HIV-2 only, both HIV-1 and HIV-2), or a dichotomous variable (HIV-negative versus HIV-positive). Presence of HPV DNA in cervical samples initially was coded as a four level hierarchical variable: high risk HPV type (any of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, or 56 regardless of any other types detected), low risk HPV type (HPV 6/11 detected using the generic probe in the absence of detection of any high-risk HPV types), untyped HPV (HPV detected by the generic probe but not detected by the type-specific probes) and HPV not detected by either the screening probe mix, the generic probe or any of the type-specific probes (HPV negative). Cytology results from the Papanicolaou smear were classified as negative, ASCUS, LSIL, HSIL, or invasive cancer, with subjects

having unsatisfactory Pap smears excluded from analyses of factors associated with cervical dysplasia.

Demographic and behavioral variables were considered both risk factors for detection of HPV DNA or cervical dysplasia (the outcomes of interest), or potential confounders of the relationship between HIV serostatus and these outcomes.

Two-sided Mantel-Haenszel chi-square tests or Fisher's exact tests were performed to assess univariate (unadjusted) associations of HIV-1, HIV-2, and HIV-1 and HIV-2 co-infection with detection of high risk HPV types, LSIL, and HSIL. Associations with ordered categorical factors were tested using Mantel-Haenszel tests for trend in order to identify differing risks associated with increasing levels of the factor of interest. Univariate relationships for continuous factors were assessed using Student's t-test or analysis of variance (ANOVA) as appropriate.

Multivariable logistic regression analyses were performed to evaluate the independent effects of HIV status and HPV detection on risk of HSIL, after adjusting for possible confounding factors. A variable was considered to be a confounder if the difference between the adjusted and unadjusted coefficients exceeded 10%, although cytology reader (Senegal versus Seattle) and age were included as covariates in all multivariable analyses. Data analyses were conducted using SAS 8.0 for Windows (Cary, NC).

RESULTS

Demographic Characteristics of the Study Population

Between October 1994 and January 1998, 4,119 women gave informed consent and were screened for HIV, HPV, and cervical squamous intraepithelial lesions at the University of Dakar outpatient infectious disease clinic at the Fann Hospital in Dakar, Senegal. The mean age of women screened was 31.9 years (range 15 – 56). The majority of women were born in Senegal (93.8%), were married (75.7%), did not use contraception (59.2%), had multiple children (74.8%), had no formal education (61.3%), and did not smoke cigarettes (95.5%) or drink alcohol (98.0%). Overall, 433 (10.5%) women were found positive for HIV-1 and/or HIV-2, including 335 (8.1%) for HIV-1 only, 69 (1.7%) for HIV-2 only, and 29 (0.7%) for both HIV-1 and HIV-2.

HIV serostatus was associated with a number of demographic factors, including being currently divorced, separated or widowed, lack of contraceptive use, birthplace outside of Senegal, lower educational attainment, and work as a commercial sex worker (CSW) (Table 1.1). Women infected with HIV-2 alone, as compared to HIV-1 alone, tended to be older. Women with HIV-1 alone had lower CD4 levels and higher CD8 levels than women infected with HIV-2 alone, while women co-infected with HIV-1 and HIV-2 had levels intermediate to those of women singly infected with HIV-1 or HIV-2. Nearly half of the women infected with HIV-1 had CD4 counts below 200 cells/μL.

Risk of HPV Detection Associated With HIV-1, HIV-2, and HIV-1 and HIV-2 Co-Infection

HPV DNA was detected in 1,207 (29.7%) of 4,059 sufficient cervical swab samples, including 766 (18.9%) specimens in which oncogenic ('high risk') HPV types were detected. Women infected with HIV-1, HIV-2, or both HIV-1 and HIV-2 were significantly more likely to have HPV DNA detected, with HPV DNA found in 25.3% of HIV-negative women, 69.1% of HIV-1 positive women, 61.8% of HIV-2 positive women, and 67.8% of co-infected women (Table 1.2; $p<0.001$). High risk HPV types were detected in 15.0% of HIV-negative women as compared to 53.6% of women with HIV-1 alone, 41.2% of women with HIV-2 alone, and 60.7% of women co-infected with HIV-1 and HIV-2 ($p<0.001$).

Multivariable logistic regression was used to evaluate the independent associations between type of HIV infection and detection of high risk HPV types. In analyses adjusting for the potentially confounding effects of marital status, contraceptive method, and employment as a CSW, women infected with HIV-1 alone (OR=7.3, 95% CI=5.5-9.7), HIV-2 alone (OR=4.7, 95% CI=2.7-8.3), and both HIV-1 and HIV-2 (OR=10.2, 95% CI=4.3-24.4) were significantly more likely than HIV negative women to have high-risk HPV types (with or without low-risk types) detected.

Risk of Cervical Neoplasia Associated with HIV-1, HIV-2, and HIV-1 and HIV-2 Co-Infection

Overall, 162 (3.9%) of 4,119 cervical Pap smears were unsatisfactory for diagnosis. Of the remaining 3,957 smears, 3,241 (81.9%) were classified as negative, 498 (12.6%) as ASCUS, 131 (3.3%) as LSIL, 75 (1.9%) as HSIL, and 12 (0.3%) as ICC. HIV was

strongly associated with presence of LSIL or worse, as 4.0% of HIV-negative women had cytology diagnosis of LSIL or worse as compared to 17.2% of women with HIV-1 alone, 19.5% of women with HIV-2 alone, and 34.5% of women co-infected with both HIV-1 and HIV-2 (Table 1.2; $p<0.001$). HSIL was detected in 1.4% of women without HIV infection, 4.5% of women with HIV-1 alone, 10.5% of women with HIV-2 alone, and 13.8% of women co-infected with HIV-1 and HIV-2 ($p<0.001$).

Separate multiple logistic regression models were utilized to evaluate the risks of LSIL and HSIL (each compared to negative cytology) associated with HIV infection. After adjusting for age, marital status, history of prior Pap screening, employment as a CSW, and cytology reader, women with HIV-1 alone (OR=6.2, 95% CI=3.8-10.1) and HIV co-infection (OR=10.6, 95% CI=3.4-32.7) were at greatly increased risk for LSIL, while those with HIV-2 alone were somewhat, although, not significantly, more likely to have LSIL (OR=2.5, 95% CI=0.8-7.5). However, women with HIV-1 alone (OR=3.7, 95% CI=1.9-7.4), HIV-2 alone (OR=7.1, 95% CI=2.9-17.4) and HIV co-infection (OR=14.4, 95% CI=4.2-49.6) were all at greatly increased risk for HSIL (Figure 1.1).

As expected, detection of high risk HPV types was highly associated with HSIL both among HIV-negative (adjusted OR=5.0, 95% CI=2.8-9.2) and HIV-positive women (adjusted OR=10.1, 95% CI=2.9-40.0, each odds ratio adjusted for, age, marital status, employment as a CSW, and cytology reader). Furthermore, HIV infection was associated with HSIL only among women with high risk HPV types detected. Among those women infected with high risk HPV types, those infected with HIV-1 alone (OR=2.7, 95%

CI=1.2-6.4), HIV-2 alone (OR=6.9, 95% CI=2.1-22.5), or co-infected with both HIV-1 and HIV-2 (OR=9.8, 95% CI=2.3-41.6) were significantly more likely to have HSIL than were HIV-negative women. However, among women without high risk HPV types detected, no such association with HIV positivity was seen (OR=1.4, 95% CI=0.4-4.9).

Risk of Invasive Cervical Cancer Associated With HIV-1, HIV-2, and HIV-1 and HIV-2 Co-Infection

Cytologic evidence of invasive cervical cancer (ICC) was found in 12 women, including 7 (0.20%) of 3552 women without HIV, 2 (0.65%) of 309 women with HIV-1 alone, 2 (2.99%) of 67 women with HIV-2 alone, and 1 (3.45%) of 29 women co-infected with HIV-1 and HIV-2 ($p<0.001$; Table 1.2). Unfortunately, only one of the 10 women with cytologic evidence of ICC was biopsied; for this case, the cytologic diagnosis was histologically confirmed. However, ICC was diagnosed by histologic evaluation of biopsy tissue obtained from an additional 9 women with cytologic diagnoses of either LSIL or HSIL. Of these 21 cases of ICC diagnosed by cytology or histology, 10 cases (0.28%) occurred among the 3,552 women without HIV, 6 (1.94%; OR=6.7, 95% CI=2.1-21.7) in 309 women with HIV-1 alone, 3 (4.48%; OR=16.0, 95% CI=3.8-67.7) in 67 women with HIV-2 alone, and 2 (6.9%; OR=37.2, 95% CI=6.6-210) in 29 women co-infected with HIV-1 and HIV-2 (Table 1.3).

Effect of HIV-Induced Immunosuppression on Detection of High Risk HPV Types

We next evaluated relationships between HPV DNA detection, CD4 count and HIV plasma viral load in order to assess whether the increased risk for HPV detection among

HIV positive women was associated with HIV-induced immunosuppression. CD4 counts were performed for 216 (65%) of 335 women with HIV-1 alone, 52 (75%) of 69 women with HIV-2 alone, 17 (59%) of 29 co-infected with both HIV-1 and HIV-2, and 352 (9.5%) of the 3,686 HIV-negative women, with mean counts (cells/μL) of 273, 368, 269, and 870 observed among these groups, respectively. In women infected with HIV-1 alone, mean CD4 counts were somewhat lower among those with (254 cells/μL), as compared to without (289 cells/μL) high risk HPV types detected (p=0.2; Table 1.4). However, this relationship was more pronounced among women infected with HIV-2 alone (258 versus 440 cells/μL, p=0.01) or co-infected with both HIV-1 and HIV-2 (184 versus 442 cells/μL, p=0.01). Among HIV-positive women, as compared to women with CD4 counts above 500 cells/μL, those with CD4 counts between 200 and 500 cells/μL were 2.7 times more likely (95% CI=1.2-6.0), and those with CD4 counts below 200 cells/μL were 3.5 times more likely (95% CI=1.6-7.8) to be infected with high risk HPV types, after adjusting for age, marital status, birth control method, prostitution and HIV type.

HIV-1 plasma viral loads were performed for 217 (65%) of 335 women infected with HIV-1 alone, and for 17 (59%) of 29 women co-infected with both HIV-1 and HIV-2. HIV-2 plasma viral loads were performed for 48 (70%) of 69 women infected with HIV-2 alone, and for 15 (52%) of the 29 co-infected women. Women with and without HIV-1 and/or HIV-2 plasma loads were similar with regard to age, marital status, and birthplace (data not shown). Among women infected with HIV-1 alone, mean $\log_{10}$ HIV-1 plasma

RNA levels were significantly higher among those with (5.07), as compared to without (4.63) high risk HPV DNA types detected (p=0.02; Table 1.4). Moreover, high risk HPV types were detected among 56% versus 29% of women with HIV-1 plasma viral loads above as compared to below 10,000 copies/mL, respectively (p=0.002). Among women infected with HIV-2 alone, mean $\log_{10}$ HIV-2 plasma RNA levels were somewhat higher among those with (3.69), as compared to without (2.02) high risk HPV DNA types detected (p=0.10). However, high risk HPV types were detected among 60% versus 30% of women with HIV-2 plasma viral loads above as compared to below 1,000 copies/mL, respectively (p=0.04).

Multivariable logistic regression analyses were performed to simultaneously assess the effect of HIV-1 plasma RNA level, CD4 count, and co-infection with HIV-2 on detection of high risk HPV types among women infected with HIV-1. Detection of high risk HPV DNA types was significantly associated with HIV-1 plasma RNA levels above 10,000 copies/mL (OR=3.7, 95% CI=1.1-12.3 compared to below 10,000 copies/mL), and was marginally associated with CD4 counts between 200-500 cells/μL (OR=1.8, 95% CI=0.7-4.4) and CD4 counts below 200 cells/μL (OR=2.1, 95% CI=0.9-5.2) compared to CD4 counts above 500 cells/μL. Compared to women with HIV-1 alone, women with co-infection with HIV-1 and HIV-2 (OR=2.1, 95% CI=0.5-8.6) was also at marginally increased increased risk for detection of high risk HPV DNA. Similar trends were seen among women infected with HIV-2. Detection of high risk HPV DNA types was marginally associated with HIV-2 plasma RNA levels above 1,000 cells/mL (OR=2.0,

95% CI=0.4-9.3), CD4 counts between 200 and 500 cells/μL (OR=3.0, 95% CI=0.5-17.3), CD4 counts below 200 cells/μL (OR=5.2, 95% CI=0.7-40.0), and co-infection with HIV-1 and HIV-2 (OR=1.9, 95% CI=0.4-8.8).

Effect of HIV-Induced Immunosuppression on Presence of Cervical Neoplasia

The presence of any dysplasia (LSIL or worse) detected by cytology was strongly correlated with decreased CD4 counts, in women with HIV-1 ($p<0.001$), HIV-2 alone ($p<0.001$), and HIV-1 and HIV-2 co-infection ($p=0.02$). Among HIV-1 positive women, LSIL or worse was detected among 23.5% of those with CD4 counts below 200 cells/μL, among 10.8% of those with CD4 counts between 200 and 500 cells/μL, but in none of the women with CD4 counts greater than 500 cells/μL ($p=0.003$, Table 1.5). HSIL was detected among 6.1% of those with CD4 counts below 200 cells/μL and 4.1% of those with CD4 counts between 200 and 500 cells/μL. Similarly, among HIV-2 infected women, LSIL or worse was detected among 33.3% of those with CD4 counts below 200 cells/μL, among 23.5% of those with CD4 counts between 200 and 500 cells/μL, and in none of the women with CD4 counts greater than 500 cells/μL ($p=0.05$). HSIL was detected among 22.2% of those with CD4 counts below 200 cells/μL and 11.8% of those with CD4 counts between 200 and 500 cells/μL. Moreover, ICC detected by cytology or histology was seen among 5.7% of 123 women with CD4 counts below 200 cells/μL and among 1.0% of 99 women with CD4 counts between 200 and 500 cells/μL.

Presence of cervical Pap smear abnormalities was also associated with higher plasma HIV RNA levels among women infected with HIV-1 ($p<0.001$), and among women infected with HIV-2 ($p<0.001$; Table 1.5). LSIL or greater was diagnosed in 22.4% of HIV-1 infected women with plasma HIV-1 RNA levels greater than 10,000 copies/mL, as compared to none of the women with HIV-1 RNA levels less than 10,000 copies/mL ($p<0.001$). HSIL was detected among 5.6% of those with plasma HIV-1 RNA levels greater than 10,000 copies/mL. Moreover, the only HIV-1 positive woman with ICC diagnosed by cytology had greater than 1,000,000 copies/mL of HIV-1 plasma RNA. Among HIV-2 infected women, LSIL or greater was diagnosed in 47% of women with plasma HIV-2 RNA levels greater than 1,000 copies/mL, as compared to 7.4% of women with HIV-2 RNA levels less than 1,000 copies/mL ($p=0.004$). Moreover, HSIL was detected among 26% of those with plasma HIV-2 RNA levels greater than 1,000 copies/mL, and among 7.4% those with RNA levels less than 1,000 copies/mL. As with HIV-1, the only HIV-2 positive woman with ICC diagnosed by cytology had a very high HIV-2 plasma load (4.71 logs).

Multivariable logistic regression analysis was used to quantify the risk of HSIL and ICC associated with decreasing CD4 counts and HIV type. After adjusting for age, HIV type, and cytology reader, each 100 cell/μL decrease in CD4 level below 500 cells/μL was associated with a 1.65-fold increased risk of HSIL (95% CI=1.1-2.4) and more than a doubling of risk for invasive cervical cancer as detected by cytology or histology (OR=2.6, 95% CI=1.2-5.4). After adjustment for CD4 level, age level, and cytology reader, infection with HIV-2, compared to HIV-1, was independently associated with

increased detection of HSIL (OR=3.3, 95% CI=0.9-12.4) and ICC (OR=7.9, 95% CI=1.1-56.8).

DISCUSSION

To our knowledge, this study is the first to demonstrate a significantly increased risk for detection of high risk HPV types, HSIL, and ICC associated with infection with HIV-1, HIV-2 and HIV-1 and HIV-2 co-infection in Africa. In women with high risk HPV types, HIV-1 was associated with nearly three times the risk, while women with HIV-2 infection or dually infected with HIV-1 and HIV-2 were at seven and ten times the risk, respectively, for HSIL compared to women without HIV infection. Furthermore, HIV-positive women were over 10 times more likely to have invasive cervical cancer identified by either histology or cytology. In the absence of high risk HPV, infection with HIV-1, HIV-2, or dual infection with HIV-1 and HIV-2 was not associated with increased risk for HSIL or invasive cancer.

This study, conducted in Senegal, represents a unique opportunity to assess the relative importance of infection with HIV-1 and HIV-2, HIV-associated immunosuppression, as measured by CD4 count and HIV plasma RNA load, and HPV in relation to presence of high grade cervical intraepithelial lesions in a large group of women who generally have not previously undergone cytology screening. HIV-2 is infrequently detected in the United States, but is common in many West African countries along with HIV-1. We were able to assess HSIL, which is more likely to develop into invasive cervical cancer in comparison to any SIL [Melnikow, 1998; Holowaty, 1999] as an outcome of interest in

this study due to the large numbers of women screened. Hence, detection of HSIL likely represents a more relevant outcome with respect to risk of developing invasive disease than does LSIL.

Previous studies in Africa regarding associations between HIV-1 and cervical neoplasia have shown inconsistent results, and many have not been able to specifically evaluate between risk associated with HSIL as opposed to any SIL. Kreiss et al. [1992] found no relationship between HIV-1 seropositivity and prevalence of CIN among commercial sex workers in Kenya, although this study was limited by small sample size and a large number of uninterpretable Pap smears. Laga et al. [1992] found a strong association between HIV positivity and CIN among 82 Zairean prostitutes, although only 3 of the 12 CIN cases were high grade lesions. Maggwa et al. [1993] reported a nearly 3-fold increased risk for any CIN associated with HIV infection in 4058 women attending family planning clinics in Kenya, but results were not stratified by degree of neoplasia. In postpartum women in Malawi, HIV-1 was associated with twice the risk for any SIL, but there was no apparent association with high grade lesions [Miotti, 1996]. Temmerman [1999] reported a nearly five-fold risk (OR=4.8, 95% CI=1.8-12.4) for HSIL associated with HIV-1 seropositivity in 513 women at a family planning clinic in Kenya, while in Zimbabwe [Womack, 2000], HSIL prevalence was nearly three times higher in HIV-1 positive women compare with HIV-negative women.

Few studies have evaluated associations between SIL and both HIV-1 and HIV-2. Coll-Seck et al. [1994] conducted a small case-control study in an infectious disease clinic in

Senegal and reported a strong association between SIL and both HIV-1 (OR=23.3, 95% CI=2.9-209) and HIV-2 (OR=9.3, 95% CI=1.1-79). Langley et al. [1996] found, in a cross-sectional study among Senegalese commercial sex workers, no relationship between HSIL and either HIV-1 (OR=1.4, 95% CI=0.3-5.3) or HIV-2 (OR=1.1, 95% CI=0.3-4.7). In the Ivory Coast [La Ruche, 1998], compared to HIV-negative women, HIV-1, but not HIV-2 positive outpatients at a gynecology clinic were at higher risk for HSIL (OR=5.8, 95% CI=3.6-9.6). Vernon, et al. [1999] reported a significant association between SIL and HIV-1 (OR=11.0, 95% CI=1.1-112) but not HIV-2 (OR=3.9, 95% CI=0.4-40) or HIV dual infection (OR=7.1, 95% CI=0.6-9.4) in 223 post-partum (with presumedly low lifetime number of sex partners) women in the Ivory Coast, although the number of HIV-2 and dually infected women was small. Further, among 208 female sex workers included in that study, no association was observed between SIL and HIV-1 (OR=0.6) or dual HIV infection (OR=0.4). Interpretation of results from that study are difficult, however, as the authors presented risk estimates which were adjusted for the presence of low and high risk HPV types, factors in the causal pathway to SIL, in an attempt to evaluate HIV as an "independent risk factor" for cervical neoplasia. In unadjusted analyses of data presented in that paper, HIV-1 and coinfection with HIV-1 and HIV-2 were significantly associated (OR=27.9 and 22.7, respectively), and infection with HIV-2 marginally associated (OR=9.0, p=0.07) with SIL in women with low numbers of sex partners but not commercial sex workers (OR=2.6, 0.0, and 1.8 in those with HIV-1 alone, HIV-2 alone, and HIV-1 and HIV-2 dual infection, respectively). Occupation as a commercial sex worker may modify the association between HIV, HPV, and SIL, as two studies not showing a strong relationship between HIV and SIL [Kriess,

1992; Langley, 1996] were also conducted among commercial sex workers in Africa. In this study, few women reported exchange of sex for money or goods, thus, we could not evaluate this hypothesis.

We found that in HIV-infected women, increased HIV plasma RNA loads and decreased CD4 levels were significantly associated with detection of high risk HPV types as well as the degree of cervical abnormality, both in women with HIV-1 infection and in women with HIV-2 infection. Among HIV-positive women, SIL was seen only in subjects with CD4 counts below 500 cells/μL, and invasive disease was seen only in women with CD4 counts below 200 cells/μL. CD4 counts and plasma HIV RNA levels were strongly correlated in these subjects, and in multivariable analyses, CD4 counts, but not plasma RNA levels, were the best predictors of cervical lesions.

Few other studies have evaluated CD4 counts and plasma RNA levels in relation to SIL. Among HIV-1 infected women in the Ivory Coast [LaRuche, 1998], the prevalence of LSIL increased with decreasing CD4 cell counts ($p<0.001$), although this was not seen in women in regard to HSIL ($p=0.3$). Vernon [1999] reported no significant association between low CD4 counts (<500 cells/μl) and SIL in either postpartum women or commercial sex workers, although trends in each group were apparent. In Tanzania [Kapiga, 1999], risk of SIL was significantly increased among 661 pregnant women with CD4 counts <200 cells/μl (OR=6.2, 95% CI=1.2-41) compared to CD4counts >500

cells/μl. To our knowledge, this is the first report evaluating CD4 levels and HIV-2 plasma RNA level in subjects infected with HIV-2.

Cross-sectional studies in non-African populations have generally found very strong associations between HIV-1 infection and presence of high grade cervical lesions [Wright, 1994; Sun, 1997; Maiman, 1998; Massad, 1999; Hocke, 1998; Six, 1998]. Those investigators assessing the role of HIV-induced immunosuppression have also found that risk of high grade lesions increases with decreasing CD4 count [Wright, 1994; Six, 1998] and increasing viral load [Massad, 1999]. Other studies assessing risk of any SIL (as opposed to high grade SIL) associated with HIV infection have generally found that prevalence of SIL increases with increasing immunosuppression [Kapiga, 1999; Heard, 2000; Delmas, 2000], although Moscicki et al. [2000] did not find such an association in HIV-infected adolescent girls. Luque et al. [1999] reported a significant association between any abnormal Pap findings (ASCUS or worse) and increased HIV-1 plasma RNA > 10,000 copies/mL (RR=2.1, p=0.01) but not decreased CD4 cell counts. In a recent retrospective study, Davis [2001] reported significant associations between both HIV-1 plasma viral load and CD4 counts associated with SIL. Similar to our findings, Duerr et al. [2001] found both HIV-1 plasma viral loads and CD4 counts to be associated with SIL in separate analyses among women enrolled in the Human Immunodeficiency Virus Research Study (HER) cohort, but only HIV-associated immunosuppression remained independently associated in multivariate analyses.

In this study, HIV-positive women were ten times more likely to have invasive cervical cancer identified by cytology or biopsy, with even greater risk associated with HIV-2 or dual HIV-1 and HIV-2 infection. In Africa, cross-sectional data from the early 1990s failed to document large increases in the incidence of invasive cervical cancer due to the greater numbers of women infected with HIV. However, the lack of AIDS and cancer registries in the developing world make it difficult to conduct population-based studies or track changes in cervical cancer rates over time, and many cases of cervical cancer are never diagnosed. As a result, cross-sectional sero-prevalence studies among cervical cancer cases and controls have generally been performed to estimate the risk for cervical cancer associated with HIV. In a study conducted by Rogo et al. [1990], only 3 (1.5%) of 200 Kenyan patients with cervical carcinoma were positive for HIV, compared to 2% in the general population. In Tanzania, ter Meulen et al. [1992] found evidence of HIV infection in 3% of cervical cancer patients compared to 12.8% among non-cancer patients. In a hospital-based case-control study conducted among black patients in South Africa, Sitas et al. [1997] found an HIV prevalence rate of 8.9% among controls who had cancers not believed to be infective in origin as compared to 3.9% among cases with invasive cervical cancer.

It is hypothesized that a lack of increase in HIV-related cervical cancer rates may be due to the competing risk of mortality from other HIV-associated conditions in settings where anti-retroviral therapies are unavailable [ter Meulen, 1992; Kuhn, 1999]. This seems plausible, as the estimated 10 to15-year interval between the appearance of a high grade lesion and invasive cervical cancer, [van Oortmarssen, 1995], is longer than the average

survival of HIV-infected African subjects. Evidence in support of this hypothesis includes the findings of La Ruche et al. [1998] who found an association between ICC and HIV-2 but not HIV-1, possibly because women with HIV-2 live significantly longer after infection with HIV than women with HIV-1. Further support of HIV as a factor associated with the development of ICC is a study by Lomalisa et al. [2000], who reported that HIV-positive patients presented with invasive cervical cancer almost 10 years earlier than HIV-negative patients, and HIV-seropositive patients with CD4 cell counts below 200 cells/μl had more advanced cancer than HIV-seronegative patients.

Three recently published studies have used National registries and databases to assess the risk of developing invasive cervical cancer associated with HIV infection in the United States. Data from the Sentinel Hospital Surveillance System for HIV Infection [Chin, 1998] estimated the prevalence of invasive cervical cancer to be 10.4 versus 6.2 cases per 1000 HIV-infected and 1000 HIV-uninfected women, respectively, suggesting a slightly increased risk (relative risk=1.7, 95% CI=1.1-2.5) associated with HIV infection. Selik et al. [1998] examined death certificates between 1990-1995 for women, ages 25 to 44, and used ICD-9 codes to estimate cervical cancer morality due to HIV infection. Using "plausible ranges" of the number of HIV-infected persons to determine denominators, Selik reported that HIV infection was associated with a 5.5-fold increased risk of cervical cancer mortality (95% CI 4.3 to 7.1) after age and race-standardizing. A report from the National Cancer Institute's AIDS-Cancer Match Study Group [Goedert, 1998] indicated that the risk of invasive cervical cancer was increased five-fold among women with early-AIDS as compared to pre-AIDS, but a weaker, non-significant association was

noted when comparing risks in the post-AIDS period. Similar findings were observed in an update of this study [Frisch, 2001]. When looking specifically at HPV-associated cancers, this same study group [Frisch, 2000] reported a significantly increased risk both for in situ and invasive cervical cancers when comparing post to pre-AIDS time periods. However, in this study, risk for these malignancies did not increase with decreasing CD4 counts. These changes over time may reflect recent, more aggressive screening efforts and treatment of precursor lesions in HIV-infected women in the United States, resulting in increases in detection of pre-invasive lesions but no subsequent significant increase in cervical cancer due to AIDS. However, in Africa, there is considerably more HIV infection, less access to general medical care, and very little screening or treatment. The situation in Africa may be analogous to what we have seen in unscreened (by anal cytology) gay men in the developed world, where the incidence of invasive anal cancer has been steadily increasing among HIV-positive gay men, and is now approximately twice that of HIV-negative gay men [Palefsky, 2000].

In the present study, we were able to assess the relationship between HIV and cervical intraepithelial lesions and invasive cancer in an untreated HIV-infected population, across a wide range of levels of immunosuppression. Currently in Senegal (and most of the developing world), the vast majority of HIV-infected individuals do not have access to combination therapies, including protease inhibitors. Treatment with highly active antiretroviral therapies (HAART), in a manner parallel to treatment of low grade SIL, may disrupt the relationships between HIV, HPV DNA detection, and the presence and progression of cervical lesions. In an early study, Heard [1998] examined 49 women

who started triple-combination antiretroviral therapy prior to and after treatment, and early regression of both high and low-grade lesions was observed. Those who regressed had a significantly higher treatment-associated increase in absolute CD4 cells compared to women who did not regress. In another small, more recent study [Robinson, 2001], among HIV-infected women with CIN, exposure to HAART was associated with lower rates of lesion recurrence, persistence and progression.

An important limitation of this cross-sectional study is the potential misclassification (mostly, through false negative findings) of our primary study endpoint (HSIL) due to measurement error in the utilization of conventional cytology in classifying our outcomes. While the Pap smear is an excellent indicator of invasive cervical cancer, the reproducibility of less severe abnormalities is low, with inter-observer agreement ranging between 30-50% for LSIL, and 40-60% for HSIL [Sedlacek, 1999; Joste, 1996; La Ruche, 1999; Stoler, 2001]. This potential misclassification of our primary outcome, if non-differential, results in an underestimate of the true relative risk. However, there has been concern that the rates of cytologic abnormalities reported may be underestimating the true prevalence of cervical disease in HIV-positive women, as the performance of Pap smears may be unusually poor in HIV-positive women [Maiman, 1991; Wright, 1996; La Ruche, 1999], although this has not been confirmed by other studies [Wright, 1994; Tweddel, 1994; Korn 1994; Fink, 1994; Adachi, 1993; Boardman, 1994; Spinillo, 1998; Branca, 2001].

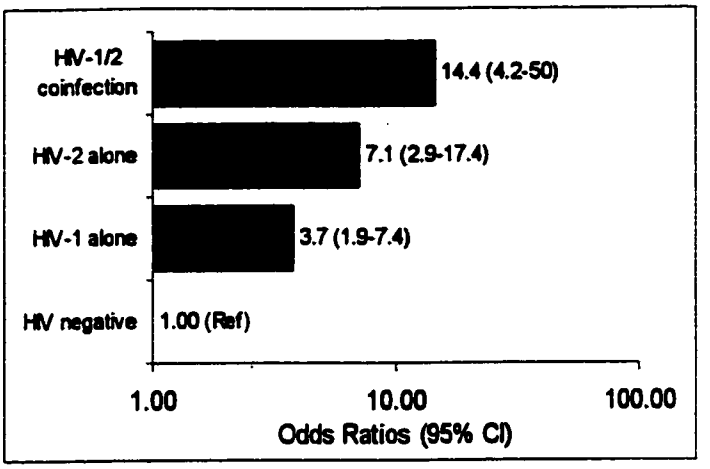
Potential misclassification might be reduced through the use of histologic confirmation of cytologically-identified cases of SIL. By study design, women with HSIL or worse were to undergo biopsy confirmation of our cytologic findings. Unfortunately, due to the need to re-stain and re-read a majority of the Pap smears due to inconsistencies in diagnoses between study cytopathologists, the diagnosis of high grade neoplasia was often noted long after initial screening, and only 32 women had cervical biopsies performed. However, similar findings were present when analyses were restricted to subjects who underwent biopsies, and a recent study has suggested that histopathology of cervical biopsies is not more reproducible than monolayer cytology [Stoler, 2001].

Another limitation of this study is the relatively small number of women with HSIL and especially invasive cervical cancer, even though this study includes one of the largest groups of women studies for HIV, HPV and SIL. In addition, measurements of HIV plasma RNA levels were not conducted on all study participants, although those with and without laboratory measurements were similar with regards to most factors.

In summary, this study includes one of the largest groups of women screened for HIV, HIV-related immunosuppression, HPV DNA, and cervical abnormalities in a previously unscreened population, and one of the first to describe an increased risk for invasive cervical cancer in women infected with HIV-1 and/or HIV-2. HIV was associated with increased risk of HSIL in women with detectable high risk HPV types, but was not associated with increased risk in those without HPV infection. Furthermore, in both

HIV-1 and/or HIV-2 infected women, those with high plasma RNA loads and low CD4 levels were at highest risk for HSIL and invasive disease.

Figure 1.1 Risk of HSIL Associated with HIV Type*



*Adjusted for age, marital status, prostitution, and cytology reader

Table 1.1. Demographic Characteristics of the Study Population, by HIV Serostatus. (Number (%))

	HIV-negative (n=3686)	HIV-positive			p-value
		HIV-1 only (n=335)	HIV-2 only (n=69)	HIV-1 and HIV-2 co-infected (n=29)	
Age (mean years±s.d)	31.9±7.9	31.0±8.1	36.1±8.0	33.8±7.4	<0.001
Marital status					<0.001
Never married	411 (11%)	20 (6%)	5 (7%)	0 (0%)	
Married – monogamous	1849 (51%)	108 (33%)	30 (44%)	7 (26%)	
Married – polygamous	1013 (28%)	53 (16%)	14 (20%)	6 (22%)	
Divorced/Separated	304 (8%)	48 (15%)	7 (10%)	2 (7%)	
Widowed	57 (2%)	80 (24%)	8 (12%)	10 (37%)	
Place of birth – Senegal	3442/3654 (94%)	302/333 (91%)	59 (86%)	25/27 (93%)	0.002
Any formal education	1473/2313 (64%)	101/233 (43%)	19/45 (42%)	4/16 (25%)	<0.001
Commercial sex worker	6 (0.2%)	21 (6.3%)	5 (7.2%)	2 (6.9%)	<0.001
Contraception					<0.001
None	2055 (56%)	290 (88%)	53 (78%)	22 (79%)	
Condoms	100 (3%)	9 (3%)	4 (6%)	1 (4%)	
OC	538 (15%)	13 (4%)	7 (10%)	2 (7%)	
Injections	159 (4%)	5 (2%)	1 (2%)	0 (0%)	
Traditional/Other	809 (22%)	14 (4%)	3 (4%)	3 (11%)	
Parity (mean±s.d.)	4.0±3.1	3.4±2.7	5.4±3.3	3.8±2.4	<0.001
Smoking	156/3676 (4%)	21/333 (6%)	7 (10%)	0 (0%)	0.02
Alcohol use	69/3677 (2%)	10/333 (3%)	2 (3%)	0 (0%)	0.4
CD4 level (cells/μL)	n=352	n=216	n=52	n=17	<0.001
>500	316 (90%)	34 (15%)	16 (31%)	2 (12%)	
200-500	32 (9%)	78 (36%)	17 (33%)	8 (47%)	
<200	4 (1%)	104 (48%)	19 (37%)	7 (41%)	
CD8 level (cells/μL)	493±259	957±568	666±588	843±483	<0.001

Table 1.2. HPV DNA Detection, Cytology and Histology Results, by HIV Serostatus. (Number (%))

		HIV-positive		
	HIV-negative	HIV-1 only	HIV-2 only	HIV-1 and HIV-2 co-infected
HPV DNA	**(n=3633)**	**(n=330)**	**(n=68)**	**(n=28)**
No HPV detected	2715 (74.7%)	102 (30.9%)	26 (38.2%)	9 (32.1%)
HPV 6/11 only	13 (0.4%)	3 (0.9%)	1 (1.5%)	0 (0.0%)
Untyped HPV only	360 (9.9%)	48 (14.6%)	13 (19.1%)	2 (7.1%)
High risk HPV types	545 (15.0%)	177 (53.6%)	28 (41.2%)	17 (60.7%)
Screening Cytology Diagnosis†	**(n=3552)**	**(n=309)**	**(n=67)**	**(n=29)**
Negative	2991 (84.2%)	195 (63.1%)	41 (61.2%)	14 (48.3%)
ASCUS	419 (11.8%)	61 (19.7%)	13 (19.4%)	5 (17.2%)
LSIL	85 (2.4%)	37 (12.0%)	4 (6.0%)	5 (17.2%)
HSIL	50 (1.4%)	14 (4.5%)	7 (10.5%)	4 (13.8%)
Invasive Cancer	7 (0.2%)	2 (0.7%)	2 (3.0%)	1 (3.4%)
Invasive Cancer Diagnosed By:				
Histology only	3 (0.08%)	4 (1.3%)	1 (1.5%)	1 (3.4%)
Cytology and/or Histology	10 (0.3%)	6 (1.9%)	3 (4.5%)	2 (6.9%)

† ASCUS, atypical cells of uncertain significance; LSIL, low grade squamous intraepithelial lesions; HSIL, high grade squamous intraepithelial lesions [NCI Workshop, 1989]

Table 1.3. Relative Odds of Invasive Cervical Cancer Associated with HIV Type (Odds Ratio*, (95% Confidence Interval))

	Invasive Cervical Cancer Diagnosed By:		
	Cytology	Cytology or Histology	Histology
HIV status			
HIV-negative	1.0 (reference)	1.0 (reference)	1.0 (reference)
HIV-positive	5.6 (1.4-22.1)	10.2 (3.8-27.7)	10.7 (3.0-38.3)
HIV type			
HIV-negative	1.0 (reference)	1.0 (reference)	1.0 (reference)
HIV-1 alone	2.9 (0.5-17.4)	6.7 (2.1-21.7)	13.0 (2.8-60.7)
HIV-2 alone	10.9 (1.7-69.9)	16.0 (3.8-67.7)	18.6 (1.8-192)
HIV-1 and 2 co-infection	18.4 (1.7-198)	37.2 (6.6-210)	65.1 (6.1-692)

* Multivariate logistic regression, adjusting for age, marital status, employment as a commercial sex worker, and cytology reader, comparing those with invasive cancer to those with negative screening cytologies

Table 1.4. CD4 Counts and Log_{10} HIV Plasma RNA Levels Among Women With and Without High Risk HPV Types Detected, Stratified by Type of HIV Infection (Number (row %))

	High Risk HPV Types		
	Absent	**Present**	**p-value***
HIV-1 only	n=106	n=108	
CD4 count (mean cells/μl)	289±232	254±211	0.2
<100	27	28 (51)	0.13
100-200	20	27 (57)	
200-500	38	40 (51)	
>500	21	12 (36)	
Log HIV-1 Plasma RNA (mean)	4.63±1.55	5.07±1.09	0.02
$<10^4$	30	12 (29)	0.005
$10^4 - 10^5$	20	31 (61)	
$10^5 - 10^6$	42	48 (53)	
$>10^6$	14	17 (55)	
HIV-2 only	n=32	n=19	
CD4 count (mean cells/μl)	440±253	258±230	0.01
<100 cells/ml	2	7 (78)	0.02
100-200	6	3 (33)	
200-500	11	6 (35)	
>500	13	3 (19)	
Log HIV-2 Plasma RNA (mean)	2.02±1.84	3.69±0.82	0.10
$<10^3$	19	8 (30)	0.054
$10^3 - 10^4$	3	6 (67)	
$>10^4$	5	6 (55)	
HIV-1 and HIV-2	n=7	n=9	
CD4 count (mean cells/μl)	442±213	184±149	0.01
Log HIV-1 Plasma RNA (mean)	4.14±2.05	3.37±1.88	0.4
Log HIV-2 Plasma RNA (mean)	0.90±1.47	2.37±1.23	0.06

* T-test for comparison of means; Mantel-Haenszel chi-square test for trend for comparison of distributions.

Table 1.5. CD4 Counts and Log_{10} HIV Plasma RNA Levels Among Women With and Without Cervical Pap Smear Abnormalities, Stratified by Type of HIV infection (Number (row %))

	Cytology[†] Result					
	Negative	ASCUS	LSIL	HSIL	ICC	p-value[‡]
HIV-1 only	n=130	n=42	n=22	n=7	n=1	
CD4 count (mean cells/μl)	313±237	228±191	151±133	142±102	164	<0.001
<100	26 (50)	14 (27)	10 (19)	2 (4)	0 (0)	<0.001
100-200	28 (61)	7 (15)	7 (15)	3 (7)	1 (2)	
200-500	51 (69)	15 (20)	5 (7)	3 (4)	0 (0)	
>500	25 (83)	5 (17)	0 (0)	0 (0)	0 (0)	
Log HIV-1 Plasma RNA (mean)	4.57±1.4	5.04±1.4	5.53±0.5	5.26±0.7	6.09	<0.001
$<10^4$	35 (85)	6 (15)	0 (0)	0 (0)	0 (0)	<0.001
10^4 - 10^5	32 (67)	10 (21)	2 (4)	4 (8)	0 (0)	
10^5 - 10^6	48 (55)	15 (17)	21 (24)	3 (4)	0 (0)	
$>10^6$	10 (39)	10 (39)	4 (15)	1 (4)	1 (4)	
HIV-2 only	n=32	n=10	n=4	n=2	n=2	
CD4 count (mean cells/μl)	400±239	436±317	191±178	207±30	37±6	<0.001
<100	3 (38)	0 (0)	2 (25)	1 (13)	2 (25)	0.02
100-200	6 (60)	3 (30)	0 (0)	1 (10)	0 (0)	
200-500	12 (71)	1 (6)	2 (12)	2 (12)	0 (0)	
>500	11 (73)	4 (27)	0 (0)	0 (0)	0 (0)	
Log HIV-2 Plasma RNA (mean)	2.05±1.9	2.10±1.7	3.87±0.4	3.71±0.8	4.71	<0.001
$<10^3$	20 (74)	5 (19)	0 (0)	2 (7)	0 (0)	0.02
10^3 – 10^4	2 (25)	1 (13)	3 (38)	2 (25)	0 (0)	
$>10^4$	6 (55)	1 (9)	1 (9)	2 (18)	1 (9)	

Table 1.5. continued

HIV-1 and HIV-2	n=10	n=2	n=4	n=2	n=1	
CD4 count (mean cells/μl)	353±220	368±105	115±155	56	145	0.02
Log HIV-1 Plasma RNA (mean)	4.09±2.1	3.41±1.2	4.11±1.2	0.30	4.38	0.7
Log HIV-2 Plasma RNA (mean)	2.22±1.6	1.36±1.5	0.30	2.67±0.4	NA	0.5

† ASCUS, atypical cells of uncertain significance; LSIL, low grade squamous intraepithelial lesions; HSIL, high grade squamous intraepithelial lesions; ICC, invasive cervical [NCI Workshop, 1989]

‡ T-test for difference between those without dysplasia (Negative or ASCUS) compared to those with dysplasia or worse (LSIL/HSIL/ICC). Mantel-Haenszel chi-square test for trend for comparison of distributions.

CHAPTER 2 – The association of human papillomavirus, HIV-1, and HIV-2 with development of high grade squamous intraepithelial lesions.

INTRODUCTION

Cancer of the uterine cervix remains a major cause of morbidity and mortality among women [Parkin, 1999; Pisani, 1999], especially in developing countries, where access to Pap smear screening is largely unavailable. Furthermore, we now know that human papillomavirus (HPV) plays a central role in the development of cervical cancers worldwide [Bosch, 1995; Cuzick, 1998]. In addition, we and others have shown that infection with HIV-1 is associated with increased risk of both HPV infection and HPV-associated lesions, termed squamous intraepithelial lesions (SIL), which are precursors to invasive cervical cancer (ICC) [Feingold, 1990; Vermund, 1991; Laga, 1992; Klein, 1994; Coll-Seck, 1994; Wright, 1994; Miotti, 1996; Langley, 1996; La Ruche, 1998; Six, 1998; Vernon, 1999; Massad, 1999; Ellerbrock, 2000]. In 1993, case reports and small series of rapidly progressive invasive cervical cancers in HIV-infected women led the Centers for Disease Control to add invasive cervical cancer to the AIDS surveillance case definition as an AIDS-defining condition [CDC Update, 1993].

The underlying causes for the association between HIV and cervical cancer remain mostly unknown, although HIV-associated systemic immunosuppression, impaired local immunity, and the resulting establishment of chronic or persistent HPV infections have been hypothesized as the mechanism by which HIV infected women are at increased risk for pre-invasive lesions [Vermund, 1991; Wright, 1994; Sun, 1995; Barberis, 1998;

Ellerbrock, 2000; Ahdieh, 2000; Cardillo, 2001]. However, much of the previous work regarding HIV and risk for squamous intraepithelial lesions have not distinguished between cases of LSIL (low grade squamous intraepithelial lesions), which most often regresses spontaneously, and HSIL (high grade squamous intraepithelial lesions), which are more likely to progress to invasive cervical cancer. Furthermore, most prior studies have been cross-sectional in study design, making evaluation of the role of HPV persistence in the development of HSIL impossible. While a few prospective studies have evaluated HIV-related immunosuppression as it relates to incident SIL [Six, 1998; Delmas, 2000; Ahdieh, 2000; Ellerbrock, 2000], none were conducted in Africa or assessed the risks for SIL associated with infection with HIV-2.

Preliminary reports from West Africa describe HIV-2 as a risk factor for prevalent LSIL [Coll-Seck, 1994; Langley, 1996]. Little is known about the risk of HSIL associated with HIV-2 as compared to HIV-1, although an increased rate of invasive cancer, but not HSIL has been reported in a study conducted among HIV-2 infected women in the Ivory Coast [La Ruche, 1998]. It is unclear whether, or to what extent, HIV-associated immunosuppression and plasma viral loads among those infected with HIV-1 or HIV-2 are associated with increased risk for HSIL. Among those co-infected with both HIV-1 and HIV-2, it is unknown whether the presence of one HIV virus modifies the effect of the other virus on the presence of HPV and/or SIL.

We conducted a prospective cohort study in Dakar, Senegal, West Africa to assess the association of HIV-1, HIV-2, and HIV-1 and HIV-2 co-infection, HPV detection and

persistence, HIV plasma load, and immunosuppression, as they relate to development of high grade cervical intraepithelial neoplasia.

MATERIALS AND METHODS

Study Population

Between October 1994 and November 1997, all women older than 15 years presenting to the University of Dakar Infectious Disease Clinic (n=4,349), as well as commercial sex workers (CSWs) attending one of two sexually transmitted disease clinics in Dakar (n=773) and M'Bour (n=270), were offered serologic testing for HIV-1 and HIV-2, and cervical HPV DNA and cytologic screening. Four weeks after this screening visit, women returned for their HIV, HPV and Pap smear results. Persons found to be positive for HIV infection were counseled according to locally established guidelines, and all women with HIV or HPV infection were invited to participate in a longitudinal study. Of the 5392 women screened at the three study sites, 1348 (25.0%) were positive for HIV-1 and/or HIV-2 or had high risk HPV types detected in cervical swabs, and thus were eligible for inclusion into the longitudinal study. Of these eligible, 939 (69.7%) enrolled. In addition, a subset of HIV-negative women without HPV infection (n=177) was also asked to enroll in the study, in order to represent a reference group at low risk for development of HSIL during follow-up. Enrollment of eligible women varied by site of recruitment, as 63% of eligible women from the outpatient infectious disease clinic enrolled, compared to 85% and 88% of eligible women from the STD clinics serving CSWs. Enrollment rates also varied by HIV status, as 76% of HIV-positive women enrolled compared to 64% of HIV-negative women with high risk HPV detected.

However, after adjusting for differing study site and HIV status, enrollment of eligible women did not vary by age, marital status, birth control method, number of children, or alcohol or tobacco use.

Collection of Specimens and Study Procedures During Follow-Up

At the screening visit, blood was collected for HIV-1 and HIV-2 serologic assays, and cervical cellular samples were obtained for cytologic screening as previously described [Langley, 1996]. Subjects completed a short standardized interview, including questions on medical and sexual history, and underwent a general physical examination. At the return visit four weeks later, enrolled subjects filled out a more detailed study questionnaire regarding their sexual history, including marital history, contraception, age at first intercourse, number of lifetime sexual partners, as well as a medical history containing information about previous hospitalizations and medication usage. In addition, blood was obtained for HIV-1 and/or HIV-2 qualitative and quantitative RNA and DNA assays, and for lymphocyte subset analysis.

Follow-up visits were scheduled every four months. At each follow-up visit, subjects received a brief physical exam, including cervical sampling for Pap smear screening for cervical abnormalities and HPV detection. Blood was collected from HIV-infected subjects for HIV-1 and/or HIV-2 qualitative and quantitative RNA and DNA assays and for determination of CD4 and CD8 counts. Serologic tests for HIV antibodies were conducted at every other visit for HIV-negative subjects. In addition, all subjects completed a short questionnaire regarding recent sexual history including changes in

marital status, contraception and new sexual partners, travel outside of Senegal, as well as illnesses, hospitalizations, and medication usage.

Cytology Screening

Pap smears judged sufficient for diagnosis were interpreted and classified according to the Bethesda System [NCI Workshop, 1989] as negative, atypical squamous cells of uncertain significance (ASCUS), LSIL, HSIL, or ICC. Between 1994 and March 1998, conventional Pap smears were utilized. Slides were initially read by a pathologist in Senegal, and later sent to Seattle for a second reading by a cytotechnologist, if the slide was available. All conventional slides classified as LSIL or worse by either pathologist were subsequently re-stained, re-covered and re-read by the pathologist in Seattle. For analysis purposes, the final cytology diagnosis was that made by the Seattle pathologist; however if the slide was not re-read or re-classified in Seattle, the diagnosis made by the Senegalese pathologist was used. Beginning in March 1998, all Pap smears were obtained using the Thin-Prep system.

HPV DNA Detection and Typing

Polymerase chain reaction (PCR) assays for the detection of HPV DNA were performed using HPV L1 consensus primers and HPV type-specific oligonucleotide probes to HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52 and 56 and a generic probe as previously described [Kuypers, 1993]. At baseline, screening for high risk ('oncogenic') HPV types was done with a 10 probe mix, testing for DNA from HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, or 56 by PCR using consensus primers MY09 and MY11 to a highly conserved

region in the L1 open reading frame. Subsequently, samples were re-amplified in order to assess presence of specific HPV types. First, samples were tested with a generic probe to detect the presence of any HPV DNA. Positive samples were then re-dotted for 12 HPV types in primer groups for HPV 6/11 ('low risk' HPV types), 16, 18, 31/33/35/39, 45/56, and 51/52 ('high risk' HPV types). Samples positive by the generic probe but negative by the type-specific probes were called positive, but untyped. Samples positive by the initial 10 probe screening mix, but not positive by the type-specific probes were classified as 'high risk' HPV types.

During follow-up, the initial step of screening for high risk types using the 10 probe mix was skipped, and all samples were tested for specific HPV types. Between October 1994 and March 1998, samples were tested for 12 HPV types in primer groups for HPV 6/11, 16, 18, 31/33/35/39, 45/56, and 51/52. Beginning in April 1998, HPV types were detected through probe hybridization using 27 HPV type specific oligonucleotide probes immobilized onto a filter strip, using the reverse line blot strip-detection method as described by Gravitt et al. [Gravitt, 1998]. The HPV types identified by this method include HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, 82, 83, and 84. The PCR testing for HPV DNA was conducted without knowledge of clinical or other laboratory data.

HIV Serology and Lymphocyte Subset Analysis

Initial screening of serum samples for HIV-1 or HIV-2 antibodies was performed using a microwell plate enzyme immunoassay that detects antibodies to both HIV types in one

well (HIV 1/2 EIA, Sanofi Diagnostics Pasteur, Redmond, WA). Positive samples were confirmed using a rapid, HIV peptide-based membrane immunoassay that distinguishes between antibodies to HIV-1 and HIV-2 (Multispot, Genetic Systems, Redmond, WA). Whole blood collected in EDTA tubes was analyzed using the FACSCount analyzer (Becton Dickinson Biosciences, San Jose, CA) to determine the number of CD4, CD8, and CD3 cells per microliter of blood. Cell counts were performed for all visit samples from HIV-infected women, and on the baseline samples from HIV-negative subjects.

Quantitation of HIV-1 and HIV-2 Plasma RNA

Quantitative and qualitative assays for HIV-1 RNA were performed as follows. RNA was extracted and quantified from EDTA-anticoagulated blood as described for the AMPLICOR™ Monitor HIV-1 Test (Roche Molecular Systems, Pleasanton, CA), with the exception that two additional primers were added to the mastermix. These two primers, SK145 and SK 151, are identical in length to the first generation primers (SK462 and SK431) and hybridize to the identical regions. The added primers were designed to have fewer mismatches with variant non-subtype B isolates. Whereas SK462 and SK431 inefficiently amplify some divergent isolates, these additional primers provide accurate quantification of HIV-1 RNA in plasma samples from individuals infected with different genetic subtypes [Aleus, 1999; Triques, 1999]. For qualitative detection of HIV RNA, plasma samples were prepared as described for the quantitative assay, except that plasma samples were suspended in 200 µl of specimen diluent. RNA amplifications were performed as described for the quantitative assays but with an increased number of cycles

(N=35). The amplified products were analyzed undiluted in microwell plates coated with the respective HIV and quantitation standard (QS) probes. A positive reaction was determined using a cut-off absorbance value at 450 nm of $\geq$0.350. This quantitative RNA assay can detect as few as 80 copies of HIVRNA/ml, and has a reproducible sensitivity of 400 HIV RNA copies/mL. The qualitative assay was performed on all available samples that were negative with the quantitative assay. The RNA assay can detect as few as 40 copies of HIV RNA/mL, and has a reproducible sensitivity of 200 HIV RNA copies/mL.

The HIV-2 assays use primers RAR 1000 (5'GCTGGCAGATTGAGCCCTGGGAGGTTCTCT 3') and RARO4 (5'GAATGACCAGGCGGCGACTAGGAGAGAT 3') to amplify a 201 base pair fragment of the LTR region. These primers hybridize to a region of the HIV-2 LTR that is highly conserved among the known HIV-2 and SIV isolates; the upstream primer has no more than a two base mismatch while the downstream primer has no mismatches with the sequenced isolates. As with the HIV-1 assays, an internal quantitation standard is used to monitor reaction variability. The QS harbors the HIV-2 primer binding sites but has different probe sequences to allow differentiation from the true target. The RT-PCR reactions were amplified using the following thermal cycling parameters: (a) 1 cycle at 60°C for 30 min for reverse transcription, (b) 5 cycles at 94°C for 20 sec, 60°C for 20 sec, and 72°C for 10 sec, (c) 35 cycles at 90°C for 10 sec, 65°C for 20 sec and 72°C for 20 sec, (d) hold at 72°C. The HIV-2 quantitative assay is comparable to the HIV-1 RNA test in sensitivity, accuracy, linearity, and reproducibility and can detect as few as 40 copies of HIV-2 RNA/mL and has a reproducible sensitivity of 200 HIV-2 RNA copies/mL. For

qualitative detection of HIV-2 RNA, plasma samples were prepared as described above for the HIV-1 qualitative RNA assays. This qualitative HIV-2 RNA assay can detect as few as 20 copies of HIVRNA/mL, and has a reproducible sensitivity of 100 HIV RNA copies/mL.

Statistical Methods

HIV serostatus was considered a categorical variable with four levels (HIV-negative, HIV-1 alone, HIV-2 alone, HIV-1/2 co-infected). HPV DNA types detected by PCR from cytology swab specimens initially were categorized according to their known associations with invasive cervical cancer. "High risk" HPV types refers to the presence of any of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, and 56, while "low risk" HPV types refers to detection of any of HPV types 6, 11, 40, 42, 53, 54, 57, 66, or 84. "Other HPV" refers to known high risk HPV types included in the Roche 27-type strips but not included in the earlier 12-type mix (HPV types 26, 55, 56, 58, 59, 68, 73, 82, and 83) or the presence of untyped HPV using a generic probe. "No HPV" refers to the absence of any HPV by either method. For some analyses, "high risk" and "other" HPV types were combined into one group of oncogenic HPV types. Persistence of an HPV risk type ("high", "low", or "other") was defined as the presence of that risk type, regardless of the specific type detected, over two consecutive visits, while an incident HPV infection for a given risk type was the defined as the occurrence of an HPV risk type at a visit when that risk type was not detected at a previous visit.

Incidence rates for development of HSIL, by baseline HIV types and detection of high risk HPV types, were estimated using Kaplan-Meier methodology, with HSIL defined as the presence of a single high grade SIL detected by Pap smear. Women with LSIL or worse at initial cytologic diagnosis were excluded from this analysis. Multivariable survival analysis techniques, including Cox regression analysis, were performed to evaluate the independent effects of HIV status and HPV on development of HSIL, adjusting for factors found to confound this relationship as well as the number of follow-up visits. Factors such as prostitution, age, marital status, birth control method, smoking, and alcohol use were examined as possible confounders of the relationship between HIV infection and detection of HPV as they related to development of high grade SIL and were included in models if they changed the relevant coefficient by more than 10%. Factors which were measured at baseline and during follow-up, such as HPV DNA detection and types, new sex partners, CD4 counts, and HIV plasma RNA levels were included as time-dependent variables. Data analyses were performed using the SAS statistical software package, version 8.0 (SAS Institute, Cary, NC).

RESULTS

Characteristics of the Study Population

At baseline, 132 (11.8%) of the 1,116 enrolled women had a diagnosis of LSIL or worse as detected on cervical Pap smear and thus were excluded from further analyses. In addition, 66 women had either no Pap smear or an unsatisfactory Pap smear at the initial study visit. Of these, 32 women were excluded from analysis because they either never had a satisfactory Pap smear (n=17), had LSIL or worse at the time of their first

satisfactory Pap smear (n=5), or they did not have further follow-up after their first satisfactory Pap smear (n=10). For the remaining 34 women who were included in the analysis, the subject's first satisfactory negative or atypical Pap smear following the initial unsatisfactory Pap smear(s) was considered to be the baseline visit. Of the remaining 952 women, 630 women (249 HIV-positive, 381 HIV-negative) had at least one follow-up visit and were included in the final analysis.

The 249 HIV-positive and 381 HIV-negative women were similar in regard to site of recruitment, age, number of children, current smoking, religion, age of first sexual intercourse, employment as a commercial sex worker, and length of study follow-up (Table 2.1). However, HIV-positive women were more likely to be divorced or separated, to have been born outside of Senegal, to use no contraception, to drink alcohol, had less formal education, and had more follow-up visits compared to HIV-negative women. Among non-CSWs, the lifetime number of sex partners did not vary significantly by HIV status (data not shown); 56% of non-CSWs reported one lifetime sex partner and only 4% reported more than 5 lifetime partners. Among HIV-positive women, those with HIV-2 were older, more likely to be a commercial sex worker, and were more likely to use condoms for birth control than were women with HIV-1 infection. Length of follow-up among women with HIV-2 alone was longer than for women with HIV-1 alone ($p=0.03$), although the number of follow-up visits did not vary significantly by HIV type ($p=0.2$).

By study design, in order to enroll a group of women at high risk for development of HSIL during followup, we over-sampled HIV-negative women with respect to the presence of high risk HPV infection. Thus, HIV-negative women were more likely to have high risk HPV types detected at baseline (Table 2.2) compared to women with HIV-infection.

Among HIV-infected women, those with HIV-1, HIV-2 and dual HIV-1 and HIV-2 infection were similar with regards to the presence of any or high risk HPV DNA, CD3 cell counts, and yeast or clue cells detected on vaginal wet mount. However, women with HIV-1 had lower CD4 but higher CD8 cell counts, and were less likely to have T. vaginalis detected on wet mount. Twenty-five percent of women with HIV-1 alone, compared to 9% of women with HIV-2 alone and 9% of women dually infected with HIV-1 and HIV-2, had CD4 counts below 200 cells/μL. No HIV-infected women in this cohort received any antiviral treatments. Among subjects with HIV-1 infection, plasma HIV-1 RNA levels were lower in those dually infected with HIV-1 and HIV-2 compared to HIV-1 alone ($p<0.001$). However, in subjects with HIV-2 infection, HIV-2 plasma RNA levels were similar in those with HIV-2 alone as compared to levels among women co-infected with HIV-1 and HIV-2 ($p=0.4$).

Cumulative Incidence of HSIL

The mean follow-up for this study cohort consisted of five visits conducted over 2.3 years, with a maximum of 5.7 years and 17 follow-up visits. Seventy-nine percent of women had at least one year of follow-up, and 84% of women had more than one follow-

up visit. During the study follow-up period, 71 (11.3%) of 630 women developed HSIL detected by cytology, for an incidence rate of 5.0 cases per 100 person-years of follow-up. Biopsies were performed on 38 (54%) of 71 women developing cytologic evidence of HSIL. High grade neoplasia was diagnosed in 18 (47%) of these biopsy tissues, low grade neoplasia was diagnosed in 6 (16%) cases, and atypical squamous cells in 2 (5%) cases. In 9 (24%) of 33 biopsy tissue samples, histologic findings were in the normal range, while 3 (8%) samples were insufficient for diagnosis.

Development of HSIL, as detected by cytology, varied greatly by detection of high risk HPV at baseline and HIV infection status. By three years of follow-up, HSIL had developed in 6% of women without HIV or HPV detected at baseline (95% CI=0%-15%), 6% (95% CI=2%-11%) of women with HIV without high risk HPV, 10% (95% CI=6%-14%) of HIV-negative women with high risk HPV, and 34% (95% CI=20%-48%) of women infected with both HIV and high risk HPV (Figure 2.1, $p<0.001$). Among women with high risk HPVs detected at baseline, development of incident HSIL was more likely in women with HIV-1 alone or dually infected with HIV-1 and HIV-2, compared to women without HIV infection or infected with HIV-2 infection alone (Figure 2.2). At 3 years, 37% of women with HIV-1 alone (95% CI=19%-55%) had developed HSIL as detected by cytology.

The rate of development of HSIL was associated with HIV type in women who were HIV-1 and/or HIV-2 positive (Figure 2.3). Women with HIV-2 were less likely to develop HSIL detected by cytology than were women with HIV-1 alone or women dually

infected with HIV-1 and HIV-2 (p=0.04). By 3 years, 18% of women with HIV-1 alone and 16% of women with HIV-1 and HIV-2, but only 3% of women with HIV-2 alone, regardless of HPV status, had developed HSIL as detected by cytology.

Development of HSIL varied by baseline CD4 level among women with HIV infection. At 3 years of follow-up, HSIL had developed in 6% (95% CI=0%-11%) of women with baseline CD4 counts above 500 cells/mL, compared to 20% (95% CI=10%-30%) in women with baseline CD4 counts between 200 and 500 cells/mL and 39% (95% CI=14%-64%) in women with baseline CD4 counts below 200 cells/mL ($p<0.001$; Figure 2.4).

Risk Factors for Development of HSIL

Because we over-sampled HIV-negative women with respect to the presence of high risk HPV infection, we can not directly compare the risks for development of HSIL in women with and without HIV infection without adjusting for HPV infection. However, we are able to evaluate the effect of HPV type and persistence, and the effect of HIV after adjusting for HPV, on development of HSIL. In order to do this, we performed multivariable Cox regression analysis, using HPV DNA detection and persistence, CD4 counts, and HIV plasma RNA levels as time dependent covariates. We compared the risks for development of HSIL in women with incident and persistent high risk HPV, as well as among women with untyped or low risk HPV types detected. Analyses were adjusted for age, employment as a commercial sex worker, parity, and birthplace in Senegal.

Women with incident or persistent infection with high risk HPV types were much more likely to develop HSIL than were women without any HPV DNA detected (crude RR for incident and persistent infection = 14.9 and 27.9, respectively; data not shown). After adjusting for age, work as a CSW, parity, birthplace in Senegal, cytology reader and type of Pap smear, HIV status, and the presence of other HPV types present, women with incident high risk (RR=3.0, 95% CI=1.1-8.1) and incident infection with other HPV types (RR=2.8, 95% CI=1.4-5.7) were both at significantly increased risk of developing HSIL (Table 2.3). Women with persistent infection with other HPV types (RR=3.5, 95% CI=1.8-6.9), and especially persistent high risk HPV types (RR=10.6, 95% CI=6.1-18.4) were at greatly increased risk for development of high grade lesions detected by cytology. However, after adjustment for the presence and persistence of HPV DNA, infection with HIV-1, HIV-2 or dual HIV-1 and HIV-2 were not significantly associated with development of HSIL.

In HIV positive women, we evaluated risk of developing HSIL associated with factors related to HIV-associated immunosuppression, including HIV type, CD4 count, and HIV plasma RNA level, in addition to incident and persistent HPV infection. We performed multiple Cox regression analysis, with CD4 counts, HIV plasma RNA levels, and HPV DNA detection as time-dependent covariates, and in all analyses we adjusted for age, employment as a CSW, parity, birthplace in Senegal, and cytology reader and type of Pap smear. In crude analyses (not adjusting for HPV or other HIV-related factors), women with HIV-2 (RR=0.3, 95% CI=0.1-0.8), but not dual infection with HIV-1 and HIV-2

(RR=1.4, 95% CI=0.5-4.1), were at a reduced risk for development of HSIL compared to women with HIV-1 infection (Table 2.4). Risk for development of HSIL increased with decreasing CD4 counts, as those with CD4 counts between 200 and 500 cells/μl (RR=2.4, 95% CI=0.8-6.8) and CD4 counts below 200 cells/μl (RR=5.6, 95% CI=2.1-15.5) were at increased risk for HSIL compared to women with CD4 counts above 500 cells/μl. Persistent, but not incident, detection of low risk (RR=3.2, 95% CI=1.1-9.5), other (RR=4.2, 95% CI=1.8-9.8), and high risk (RR=11.3, 95% CI=5.0-25.2) HPV DNA was associated with increased risk for development of HSIL. In adjusted analyses, persistent detection of HPV DNA, but not low CD4 cell counts or infection with HIV-2, remained independently associated with development of HSIL.

Similar findings were seen when stratifying by type of HIV infection. Among HIV-1 positive women, in crude analyses (not adjusting for HPV or other HIV-related factors), CD4 counts were strongly associated with development of HSIL (Table 2.5). Compared to women with CD4 counts above 500 cells/μl, women with CD4 counts between 200 and 500 cells/μl (RR=1.9, 95% CI=0.8-8.5) and CD4 counts below 200 cells/μl (RR=4.6, 95% CI=1.5-14.6) were at increased risk for HSIL. Increased HIV-1 plasma RNA level was associated with risk for HSIL, as each $\log_{10}$ increase or plasma load was associated with a 42% increase in risk for development of HSIL (RR=1.42, 95% CI=1.05-1.93). Incident high risk HPV infection was not associated with risk for HSIL (RR=1.6, 95% CI=0.6-13.3), while persistent infection was strongly associated (RR=11.2, 95% CI=4.5-27.8). Women co-infected with HIV-1 and HIV-2 were not at increased risk for HSIL

compared to women with HIV-1 alone. In multivariable analyses adjusting for all the HIV and HPV-related factors of interest, only persistent infection with high risk HPV (RR=9.2, 95% CI=3.4-25.2) remained independently associated with development of HSIL in HIV-1 infected women.

Similar analyses were performed in women infected with HIV-2, and similar associations were noted, although for most factors, the smaller sample size resulted in wide confidence intervals (Table 2.6). Compared to women with CD4 counts above 500 cells/μl, women with CD4 counts between 200 and 500 cells/μl (RR=2.6, 95% CI=0.3-26.2) and CD4 counts below 200 cells/μl (RR=12.5, 95% CI=1.2-126) were at increased risk for HSIL. Increased HIV-2 plasma RNA level was associated with risk for HSIL at a similar magnitude as it was in HIV infection, as each $\log_{10}$ increase or plasma load was associated with a 31% increase in risk for development of HSIL (RR=1.31, 95% CI=0.77-2.2), although this was not statistically significant. Incident high risk HPV infection was not associated with risk for HSIL while persistent infection was strongly associated (RR=18.6, 95% CI=2.7-127). Women co-infected with HIV-1 and HIV-2 were at increased risk for HSIL compared to women with HIV-2 alone (RR=8.4, 95% CI=1.5-46.8). In multivariable analyses adjusting for all the HIV and HPV-related factors of interest, CD4 counts below 200 cells/ml, co-infection with HIV-1 and HIV-2, and persistent infection with high risk HPV showed trends towards increased risk for development of HSIL in HIV-2 infected women, although none of these factors were statistically significant.

DISCUSSION

This study is the first to evaluate risk for development of HSIL associated with infection with HIV-1, HIV-2 and HIV-1 and HIV-2 co-infection, in addition to that associated with presence and persistence of high and low risk HPV types in Africa. We found, among women without high risk HPV types detected at study entry, similar risk of HSIL in women with and without HIV infection. However, among women with high risk HPV types detected, HIV infection was associated with a 3-fold increased risk of incident HSIL, although this was confined to women with HIV-1 rather than HIV-2 infection. Furthermore, among HIV-positive women, regardless of HIV type, immune suppression as measured by decreased CD4 count, was associated with increased risk for HSIL. Because of our large sample size and long follow-up time, we were able to assess factors associated with HSIL, which is more likely to develop into invasive cervical cancer as compared to LSIL [Melnikow, 1998; Holowaty, 1999].

HPV detection is significantly more common, more likely to be persistent, and is more likely to include multiple types of HPV in HIV-positive as compared to HIV negative men and women [Kiviat, 1993; Vernon, 1994; Williams, 1994; Miotti, 1996; Minkoff, 1998; Palefsky, 1998; Critchlow, 1998; Sun, 1998; Eckert, 1999; Ahdieh, 2000]. We found that after adjusting for HPV persistence of specific high risk HPV types, neither HIV-1, HIV-2, nor dual infection with HIV-1 and HIV-2 remained significantly associated with risk for development of HSIL. These findings are similar to those found in a study of the ALIVE cohort [Ahdieh, 2000], where no association between HIV infection and cervical neoplasia was observed after adjustment for repeated HPV

positivity. However, this is in contrast to findings of Ellerbrock et al. [2000], who reported a higher SIL incidence rate amongHIV infected women, even after adjustment for HPV presence and persistence. Six et al. [1998] reported that after adjustment for HPV infection, HIV-positive women with CD4 counts above 500 cells/μl did not have a significantly increased risk of SIL, but immunodeficient HIV-positive women maintained a three to four-fold increase in risk. Similar to our study, Duerr et al. [2001] found no increased risk for SIL among HIV-infected women in the Human Immunodeficiency Virus Research Study (HER) cohort in the absence of detectable HPV. However, these studies were limited in their ability to characterize the persistence of high risk HPV detection, which have been shown to be an important predictor of development of HSIL and cervical cancer [Remmink, 1995; Ho, 1995; Chua, 1996; Londesborough, 1996; Ho, 1998; Wallin, 1999; Ahdieh 2000].

Among women with HIV infection, HIV-2 appeared to be associated with a 70% reduced risk for development of HSIL compared to HIV-1. However, this association was explained by the higher CD4 counts and lower degree of HPV incidence and repeated detection experienced by women with HIV-2 compared to HIV-1. We found that increased immunosuppression, as measured by CD4 counts over time, was associated with increased risk for development of incident HSIL. However, in HIV infected individuals, HPV detection and persistence and HPV viral load are strongly associated with increased immune suppression [Minkoff, 1998; Palefsky, 1999; Heard, 2000; Ahdieh, 2000]. In our study, the relationship between CD4 level and development of HSIL was greatly attenuated by adjustment for HPV type and persistence.

This study, conducted in Senegal, represents a unique opportunity to assess the relative importance of infection with HIV-1 and HIV-2, HIV-associated immunosuppression, as measured by CD4 count and HIV plasma RNA load, and HPV detection in relation to incident high grade cervical intraepithelial lesions in a large group of women who generally have not previously undergone cytology screening. HIV-2 is infrequently detected in the United States, but is common in many West African countries along with HIV-1 and is associated with an extended period of subclinical infection, lower plasma RNA loads, and a slower rate of CD4 decline. At the time of this study, no women in Senegal were being treated for HIV infection with highly active antiretroviral therapy, so this did not confound the interpretation of factors associated with HIV-related immunosuppression.

Few studies have previously evaluated associations between SIL and both HIV-1 and HIV-2 [Coll-Seck, 1994; Langley, 1996; La Ruche, 1998; Vernon, 1999], and none have been longitudinal in study design. Furthermore, few studies have evaluated CD4 counts and plasma RNA levels in relation to SIL and most of these have been cross-sectional in study design as well. Among HIV-1 infected women in the Ivory Coast [LaRuche, 1998], the prevalence of LSIL increased with decreasing CD4 cell counts ($p<0.001$), although this was not seen in women with regards to HSIL ($p=0.3$). Vernon [1999] reported no significant association between low CD4 counts (<500 cells/μl) and SIL in either postpartum women or commercial sex workers, although trends in each group were apparent. In Tanzania [Kapiga, 1999], risk of SIL was significantly increased among 661

pregnant women with CD4 counts <200 cells/μl (OR=6.2, 95% CI=1.2-41) compared to CD4counts >500 cells/μl. To our knowledge, this is the first report evaluating CD4 levels and HIV-2 plasma RNA level in subjects infected with HIV-2.

Few studies have evaluated factors associated with incident SIL in HIV-infected women. In France [Six, 1998], 160 HIV-positive and 128 HIV-negative women were followed for development of SIL. After one year, the cumulative incidence of SIL in HIV-positive women was 20.5%, compared to only 4.9% in HIV-negative women. In women not receiving antiretroviral therapy, incidence was higher in those with CD4 counts below 500 cells/μl. In multivariate analysis, HIV and HPV remained independently associated with SIL incidence. However, HPV was evaluated only at baseline, and only 6 of 33 incident lesions identified in this study were high grade lesions.

The European Study Group on Natural History of HIV Infection in Women followed 229 HIV-infected women from 23 centers every six months for a median time of two years [Delmas, 2000]. While seventy women developed SIL during follow-up, only six high grade lesions were identified. In multivariate analyses, incident SIL was associated with HPV positivity at baseline, and marginally associated with decreasing baseline CD4 cell counts. However, this study evaluated HPV and HIV-associated immunosuppression at only one visit, included women receiving antiretroviral therapy, and was limited by the small number of women developing HSIL.

In the ALIVE cohort [Ahdieh, 2000], 144 HIV-positive and 43 HIV-negative women were followed every 6 months for HPV persistence for up to four years. A vast majority (74%) of HIV-negative women cleared HPV infection during study follow-up, in contrast to 41% of HIV-positive women with CD4 counts above 200 cells/μl and only 16.1% of HIV-positive women who had CD4 counts below 200 cells/μl. HPV persistence was strongly associated with detection of SIL, and in stratified analyses, repeated HPV positivity explained the observed association between HIV infection and SIL. However, only 11 lesions were observed in this cohort, allowing only limited power to evaluate specific risk factors for SIL.

Finally, 328 HIV-positive and 325 HIV-negative women were followed for development of SIL in the New York Cervical Disease Study [Ellerbrock, 2000]. Biopsy-confirmed SIL developed in 83 women, including 20% of HIV-infected women compared to 5% of HIV-uninfected women. Approximately half of the HIV-positive cohort were on antiretroviral therapy, and only 10 cases of HSIL were seen. By 54 months, incident SIL was observed in 7% of HIV-negative women without HPV compared to 15% of HIV-positive women without HPV detected at baseline. In comparison, 12% of HIV-negative, compared to 54% of HIV-positive women with HPV 16 or 18 developed SIL. Persistence of HPV during follow-up was strongly associated with HIV infection. Surprisingly, CD4 cell level was not significantly associated with incident SIL, even in univariate analyses. Similar to our findings, in women with HIV infection, both transient and persistent infection with HPV were significantly associated with SIL development. However, in contrast to our study, HIV seropositivity was associated with a significantly

higher incidence of SIL, even after controlling for the presence and persistence of HPV infection. This discrepancy may be due to differing collection techniques for HPV; we utilized cervical swab specimens while the Ellerbrock study detected HPV in cervicovaginal lavage specimens. HPV detection from cervicolavage specimens may be less sensitive and more likely to yield uninterpretable results compared to cervical swab specimens [Wheeler, 1996].

Our study is limited in several important ways. Although we initially planned to utilize histologic evidence of HSIL as our outcome of interest in this study, biopsies were obtained from only half of the women developing HSIL by cytology. In subjects from whom biopsies were obtained, a majority of cytologically-diagnosed high grade lesions were confirmed as HSIL by histologic diagnosis. However, in many cases, HSIL was not identified in histologic samples. Ultimately, because of the incompleteness of biopsies taken in women with HSIL identified by cytology, and because of the delay in taking these samples in some women who did undergo biopsy, we used HSIL identified by cytology as our outcome of interest. This may have led to misclassification of our outcome of interest in some cases. Recent evidence, however, suggests that that histopathology of cervical biopsies may not be more reproducible than monolayer cytology [Stoler, 2001] and whether this potential misclassification could have been differential in women with and without HIV infection is unclear [Maiman, 1991; Wright, 1996; LaRuche, 1999; Branca, 2001].

As is the case with all prospective studies, some eligible participants choose not to enroll, and others drop out during the follow-up period. While our participation rate was high (over 70%), as was the retention rate for women enrolled (79% of enrolled women were followed for more than one year), loss to follow-up may have biased our study findings, if these losses were differential between either women with and without our HIV-associated factors of interest or among women who did and did not develop HSIL. However, the length of follow-up was similar between women with and without HIV infection, and analyses were adjusted for the number of follow-up visits in an attempt to adjust for opportunity to assess relevant exposures and HSIL outcome.

Finally, our evaluation of development of HSIL in women with HIV-2 infection and women co-infected with HIV-1 and HIV-2, compared to women with HIV-1 infection, was limited by the small number of women with these infections enrolled and followed for development of incident HSIL. While similar trends were seen in women with HIV-2 or dual HIV-1/HIV-2 infection compared to women with HIV-1, few of these associations achieved statistical significance.

In summary, this study represents one of the largest groups of HIV-positive and HIV-negative women followed for HSIL in a generally unscreened population. HPV and HIV-associated immunosuppression, as measured by CD4 counts and HIV viral loads, were quantified both at baseline and during follow-up. HIV was associated with significantly increased risk for HSIL, the immediate precursor lesion to cervical cancer, in women with detectable high risk HPV types, but not among women without HPV

infection. However, after adjustment for the presence and persistence of HPV infection, HIV infection was not associated with increased risk for HSIL. HIV-1 positive women appeared at higher risk for incident HSIL compared to HIV-2 positive women, most likely because of their decreased levels of immune competence. In both HIV-1 and HIV-2 positive women, those with high plasma RNA loads and low CD4 levels were at highest risk for HSIL, and this increased risk was explained through repeated detection of high risk HPV DNA.

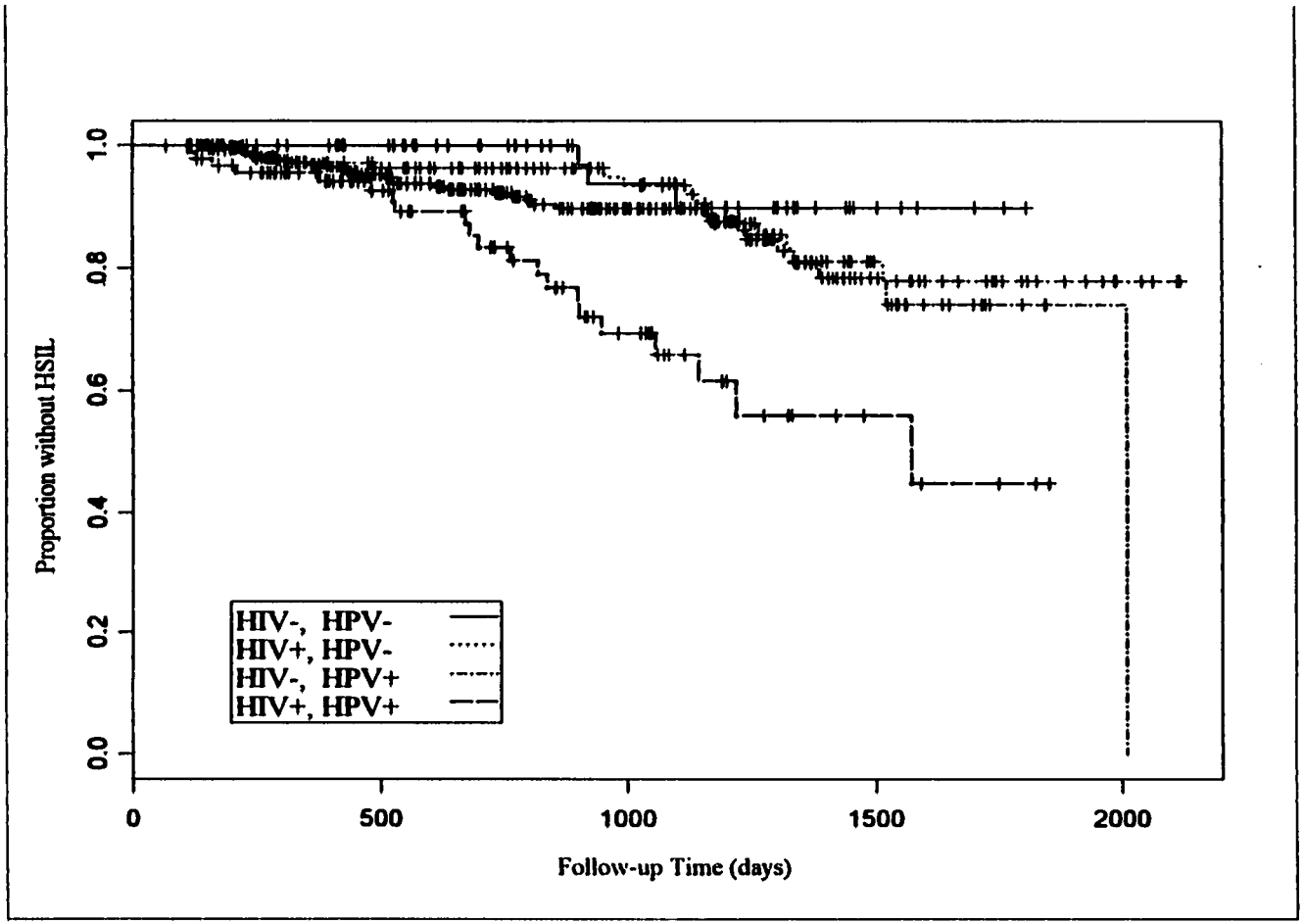


Figure 2.1. Cumulative Incidence of HSIL by HIV and HPV Status at Baseline. Group sample sizes are 71, 157, 310, and 92 for those without HIV or HPV, with HIV only, with HPV only, and with both HIV and HPV, respectively.

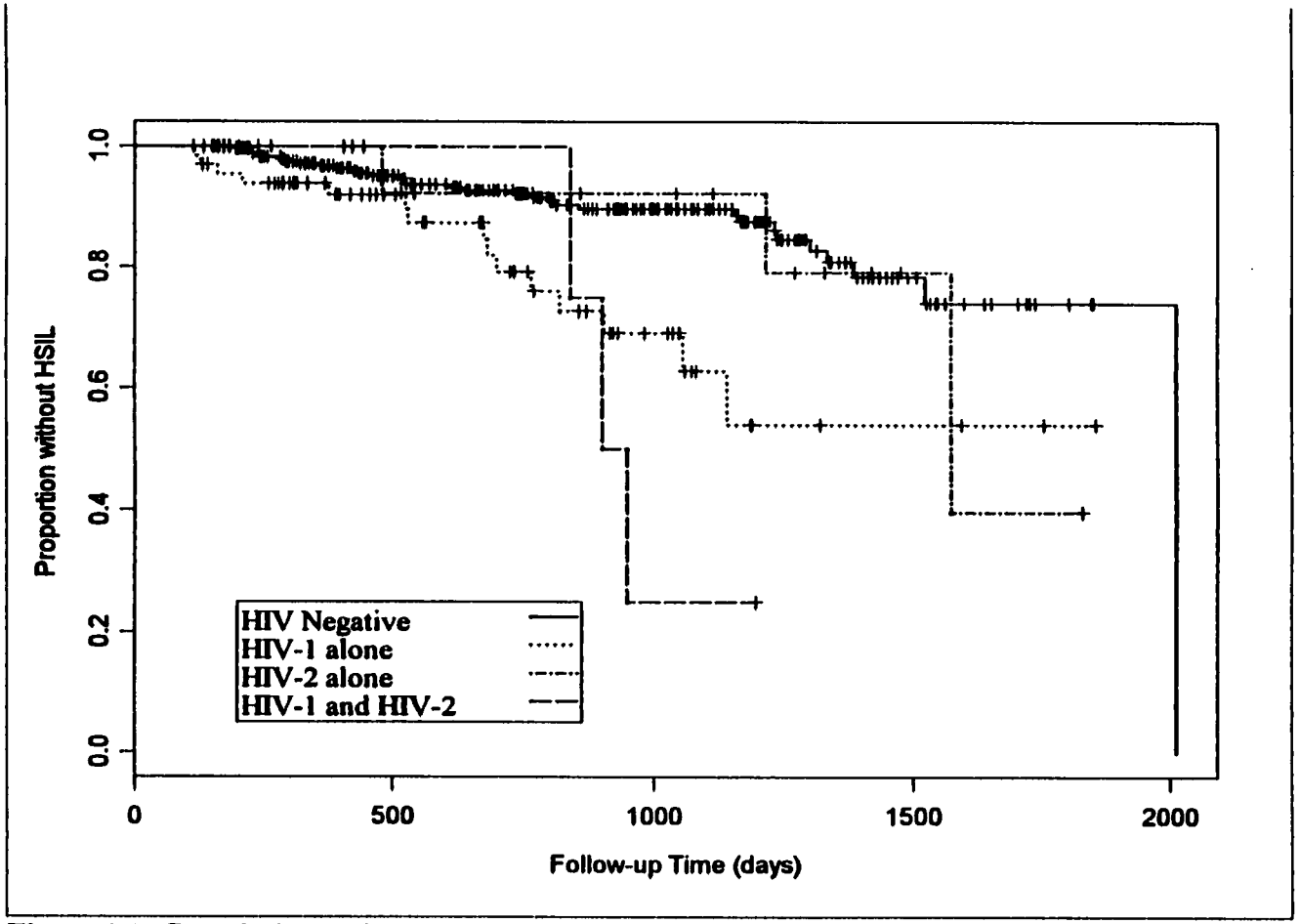


Figure 2.2. Cumulative Incidence of HSIL by HIV type among women infected with high risk HPV. Group sample sizes are 310, 67, 17, and 8 for those HIV-negative, or infected with HIV-1 alone, HIV-2 alone, and both HIV-1 and HIV-2, respectively.

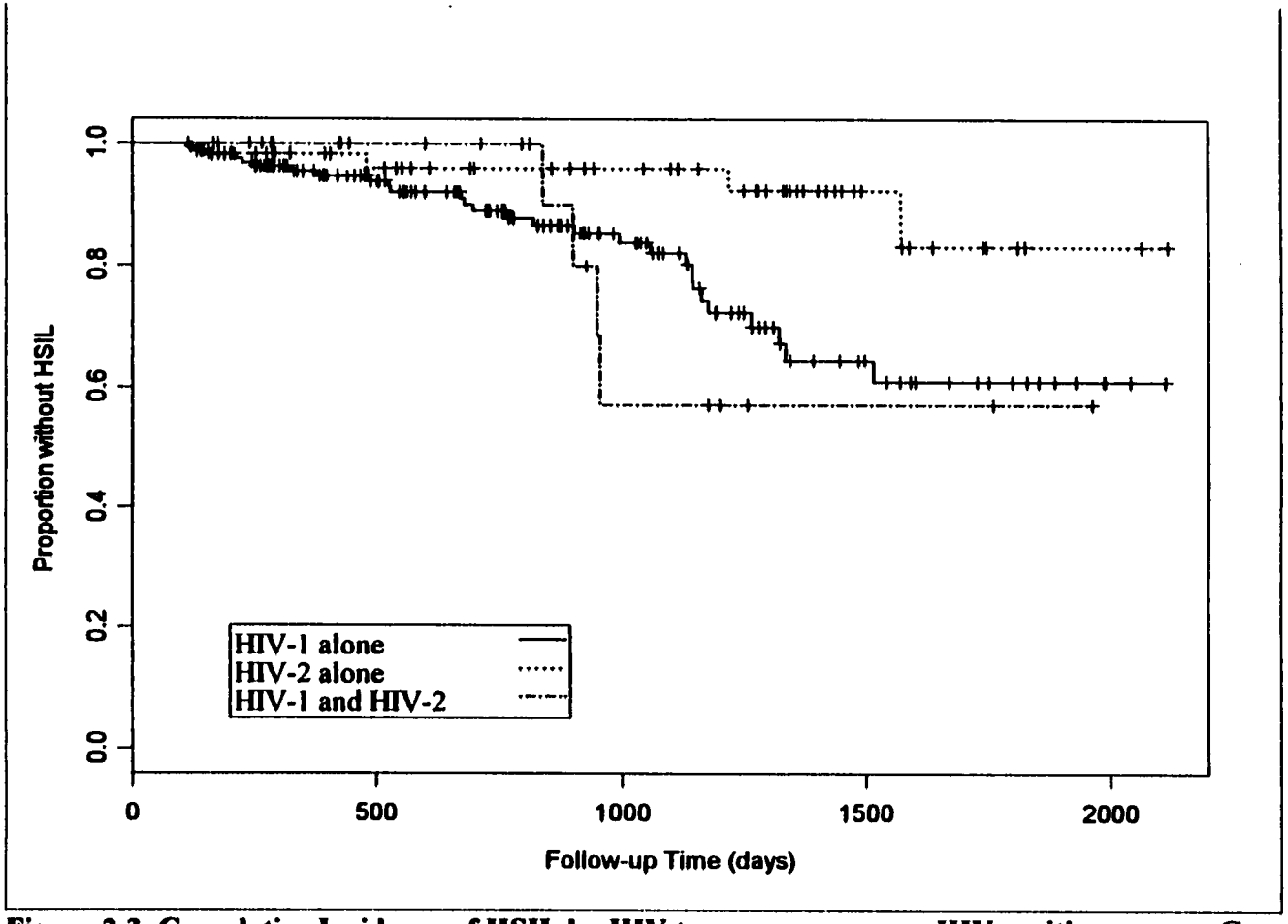


Figure 2.3. Cumulative Incidence of HSIL by HIV type among women HIV positive women. Group sample sizes are 165, 57, and 24 for those infected with HIV-1 alone, HIV-2 alone, and both HIV-1 and HIV-2, respectively.

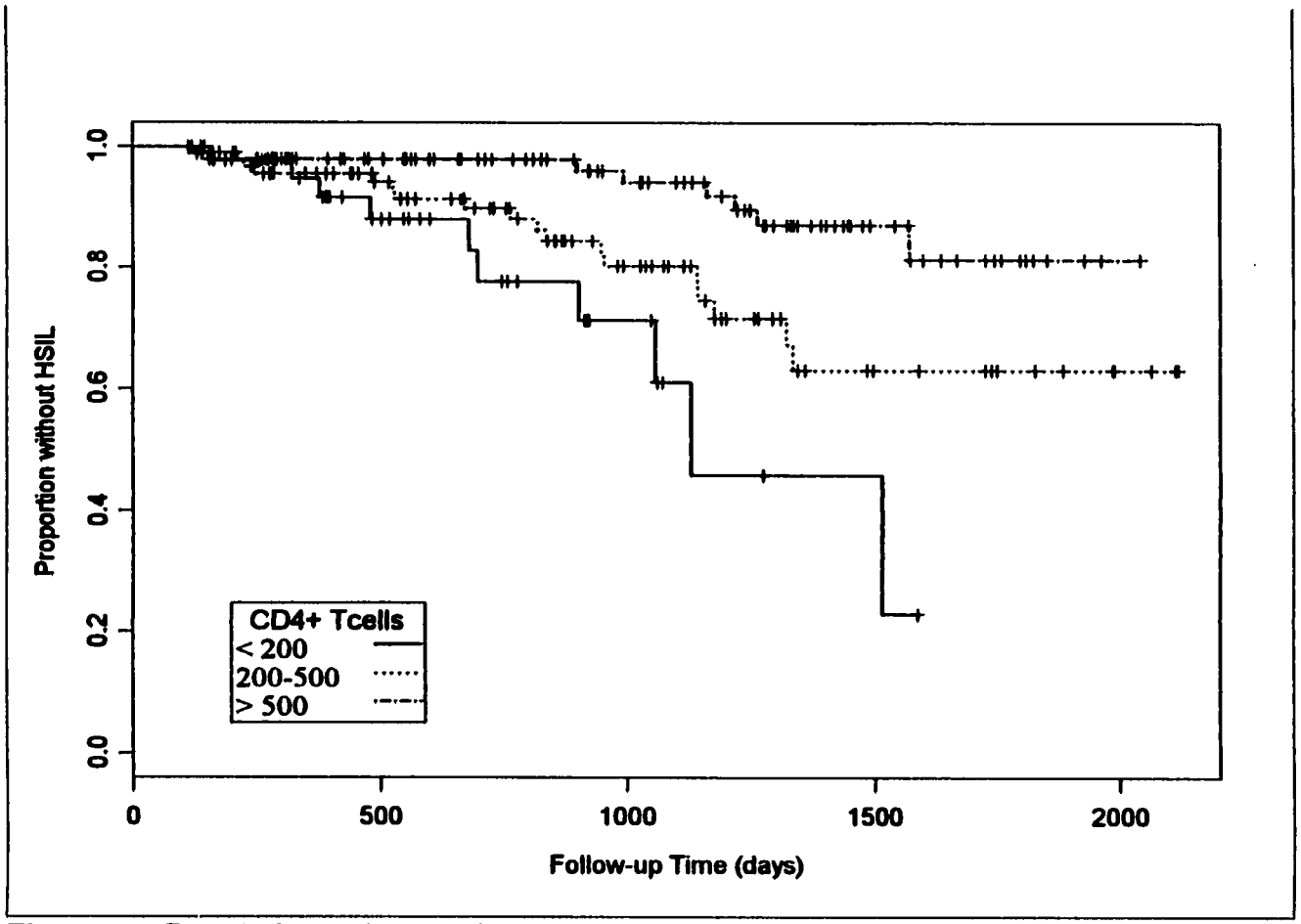


Figure 2.4. Cumulative Incidence of HSIL by Baseline CD4 level among women with HIV infection. Group sample sizes are 49, 96, and 102 for those with CD4 counts below 200, between 200 and 500, and above 500 cells/μl, respectively.

Table 2.1. Demographic Characteristics of Women Enrolled and Followed in Longitudinal Study of Development of HSIL in Senegal, n (%).

	HIV-negative (n=381)	HIV-1 positive (n=168)	HIV-2 positive (n=57)	HIV-1 and HIV-2 coinfected (n=24)	Total (n=630)	P[1]	p[2]
Site of Recruitment						0.3	0.002
Dakar STD Clinic	108 (28)	37 (22)	15 (26)	12 (50)	172 (27)		
M'Bour STD Clinic	39 (10)	18 (11)	14 (25)	3 (13)	74 (12)		
Dakar outpatient ID Clinic	234 (62)	113 (67)	28 (49)	9 (37)	384 (61)		
Age						0.03	0.02
<20	9 (2)	6 (4)	0 (0)	0 (0)	15 (2)		
20-29	181 (48)	74 (44)	13 (23)	8 (33)	276 (44)		
30-39	141 (37)	62 (37)	32 (56)	11 (46)	246 (39)		
40-49	45 (12)	22 (13)	10 (18)	5 (21)	82 (13)		
50+	5 (1)	4 (2)	2 (4)	0 (0)	11 (2)		
Mean Age	30.5	31.0	34.2	32.6	31.0	0.02	0.02
Marital Status						<0.001	0.3
Never married	81 (21)	23 (14)	7 (12)	4 (17)	115 (18)		
Married – monogamous	128 (34)	48 (29)	17 (30)	3 (13)	196 (31)		
Married – polygamous	52 (14)	19 (11)	4 (7)	5 (21)	80 (13)		
Divorced/Separated	113 (29)	48 (29)	24 (42)	10 (42)	195 (31)		
Widowed	6 (2)	30 (18)	5 (9)	2 (8)	43 (7)		
Place of Birth – Senegal	339 (89)	132 (79)	51 (89)	20 (83)	542 (86)	0.006	0.2
Commercial Sex Worker	151 (40)	63 (38)	30 (53)	15 (63)	259 (41)	0.4	0.02
Contraception						<0.001	0.02
None	139 (37)	109 (66)	24 (43)	9 (39)	281 (45)		
Condoms	91 (24)	31 (19)	22 (39)	12 (52)	156 (25)		
OC	63 (16)	13 (8)	6 (11)	1 (4)	83 (13)		

Table 2.1 continued

Injections	15 (4)	4 (2)	1 (2)	0 (0)	20 (3)		
Traditional/Other	72 (19)	9 (5)	3 (5)	1 (4)	85 (14)		
Any Children	333 (88)	142 (85)	52 (91)	21 (88)	548 (87)	0.7	0.5
Number of children (mean)	3.3	3.1	3.7	3.0	3.3	0.6	0.3
Smoking	91 (24)	34 (20)	20 (35)	7 (30)	152 (24)	0.8	0.06
Alcohol use	49 (13)	25 (15)	13 (23)	7 (29)	94 (15)	0.07	0.13
Age first sex (mean)	17.5	17.1	16.4	17.6	17.3	0.09	0.3
Years of followup (mean)	2.2	2.2	2.7	2.1	2.3	0.5	0.03
Followup visits	4.6	5.7	6.4	4.7	5.1	<0.001	0.2

p^1 P-value for difference between HIV- and HIV+ women
p^2 P-value for difference between 3 types of HIV infection among HIV+ women

Table 2.2. Laboratory Characteristics of Women Enrolled and Followed in Longitudinal Study of Development of HSIL in Senegal, n (%).

	HIV-negative (n=381)	HIV-1 positive (n=168)	HIV-2 positive (n=57)	HIV-1 and HIV-2 coinfected (n=24)	Total (n=630)	p^1	p^2
HPV DNA						<0.001	0.3
No HPV	45 (12)	76 (46)	25 (45)	12 (52)	158 (25)		
HPV 6/11 only	1 (0)	0 (0)	1 (2)	0 (0)	2 (0)		
Untyped HPV	22 (6)	23 (14)	13 (23)	3 (13)	61 (10)		
High Risk HPV	310 (81)	67 (41)	17 (30)	8 (33)	402 (65)		
Cell counts						<0.001	<0.001
>500 cells/ml	321 (91)	53 (32)	37 (65)	12 (52)			
200-500 cells/ml	29 (8)	72 (43)	15 (26)	9 (39)			
<200 cells/ml	4 (1)	42 (25)	5 (9)	2 (9)			
CD4 (mean)	910	399	639	583		<0.001	<0.001
CD8 (mean)	488	953	694	909		<0.001	0.002
CD3 (mean)	1486	1453	1395	1587		0.5	0.5
Log_{10} plasma HIV-1 RNA	NA	4.38	NA	2.97	NA	NA	<0.001
Log_{10} plasma HIV-2 RNA	NA	NA	1.66	1.34	NA	NA	0.4
RPR positive	28 (8)	17 (10)	8 (14)	3 (13)	56 (9)	0.13	0.7
Vaginal wet mount							
T vaginalis	42 (12)	23 (14)	8 (15)	9 (41)	82 (14)	0.11	0.005
Yeast	57 (16)	33 (20)	6 (11)	2 (9)	98 (16)	0.7	0.2
Clue cells	82 (23)	42 (25)	15 (27)	6 (27)	145 (24)	0.8	0.9

p^1 P-value for difference between HIV- and HIV+ women

p^2 P-value for difference between 3 types of HIV infection among HIV+ women

Table 2.3. Cox Regression Analysis of risks of HIV and HPV associated with Development of High Grade Cervical Dysplasia

	Adjusted RR* (95% CI)
Age	
<30	1.0 (ref)
30-39	1.26 (0.7-2.2)
40-49	1.56 (0.7-3.3)
50+	4.72 (1.1-20.5)
Prostitute	0.53 (0.3-1.01)
Any Children	2.80 (0.98-8.0)
Born in Senegal	1.79 (0.5-5.8)
HIV Status	
HIV negative	1.0 (ref)
HIV-1 only	0.85 (0.5-1.5)
HIV-2 only	0.60 (0.2-1.7)
HIV-1 and HIV-2	1.07 (0.3-3.4)
HPV DNA	
No Low Risk HPV DNA	1.0 (ref)
Incident Low Risk HPV DNA	1.39 (0.5-4.0)
Persistent Low Risk HPV DNA	2.22 (0.87-5.6)
No other HPV DNA**	1.0 (ref)
Incident other HPV DNA**	2.79 (1.4-5.7)
Persistent other HPV DNA**	3.49 (1.8-6.9)
No High Risk HPV DNA	1.0 (ref)
Incident High Risk HPV DNA	2.99 (1.10-8.1)
Persistent High Risk HPV DNA	10.56 (6.1-18.4)

*Multivariate Cox Regression analysis, with time-dependent covariates, adjusting for all other factors in the table as well as cytology reader.
**other HPV refers to HPV types 26, 39, 51, 52, 55, 56, 58, 59, 68, 73, 82, 83 or untyped HPV

Table 2.4. Cox Regression Analysis of risks of HIV type and HPV associated with Development of HSIL in HIV positive women

	Crude RR* (95% CI)	Adjusted RR** (95% CI)
Age		
<30	NA	1.0 (ref)
30-39	NA	1.56 (0.7-3.6)
40-49	NA	1.53 (0.4-5.5)
50+	NA	8.26 (0.9-78)
Prostitute	NA	0.35 (0.1-0.99)
Any Children	NA	2.07 (0.5-7.8)
Born in Senegal	NA	1.30 (0.3-5.8)
HIV Status		
HIV-1 only	1.0 (ref)	1.0 (ref)
HIV-2 only	0.27 (0.1-0.8)	0.93 (0.3-3.3)
HIV-1 and HIV-2	1.39 (0.5-4.1)	1.53 (0.5-5.0)
CD4 count		
>500	1.0 (ref)	1.0 (ref)
200-500	2.37 (0.8-6.8)	1.92 (0.6-6.5)
<200	5.64 (2.1-15.5)	1.58 (0.4-5.7)
HPV DNA		
No Low Risk HPV DNA	1.0 (ref)	1.0 (ref)
Incident Low Risk HPV DNA	1.82 (0.5-6.5)	1.74 (0.5-6.3)
Persistent Low Risk HPV DNA	3.15 (1.05-9.5)	2.93 (0.97-8.8)
No other HPV DNA***	1.0 (ref)	1.0 (ref)
Incident other HPV DNA***	2.46 (0.8-7.2)	2.45 (0.8-7.4)
Persistent other HPV DNA***	4.22 (1.8-9.8)	3.95 (1.6-9.8)
No High Risk HPV DNA	1.0 (ref)	1.0 (ref)
Incident High Risk HPV DNA	1.05 (0.1-8.5)	1.06 (0.1-8.8)
Persistent High Risk HPV DNA	11.23 (5.0-25.2)	10.36 (4.1-26.3)

*Multivariate Cox Regression, adjusting for age, work as a prostitute, any children, cytology reader, and birth in Senegal.

**Multivariate Cox Regression, adjusting for all other factors in the table as well as cytology reader.

***other HPV refers to HPV types 26, 39, 51, 52, 55, 56, 58, 59, 68, 73, 82, 83 or untyped HPV

Table 2.5. Multivariate Cox Regression Analysis of Development of High Grade Cervical Dysplasia in HIV-1 positive Women

	Crude RR* (95% CI)	Adjusted RR** (95% CI)
CD4 count		
>500	1.0 (ref)	1.0 (ref)
200-500	1.9 (0.8-8.5)	1.1 (0.3-4.7)
<200	4.6 (1.5-14.6)	0.8 (0.3-4.7)
HIV-2 coinfection	1.3 (0.4-4.0)	1.4 (0.4-4.8)
Log_{10} Plasma HIV-1 RNA level	1.42 (1.05-1.9)	1.32 (0.92-1.9)
HPV DNA		
No Low Risk HPV DNA	1.0 (ref)	1.0 (ref)
Incident Low Risk HPV DNA	1.79 (0.5-6.5)	1.77 (0.5-6.6)
Persistent Low Risk HPV DNA	2.77 (0.90-8.5)	2.89 (0.92-9.0)
No other HPV DNA***	1.0 (ref)	1.0 (ref)
Incident other HPV DNA***	1.55 (0.5-5.2)	1.60 (0.5-5.5)
Persistent other HPV DNA***	2.96 (1.2-7.5)	2.88 (1.10-7.6)
No High Risk HPV DNA	1.0 (ref)	1.0 (ref)
Incident High Risk HPV DNA	1.06 (0.1-8.8)	1.06 (0.1-9.0)
Persistent High Risk HPV DNA	9.89 (4.1-24.0)	9.52 (3.4-26.3)

*Multivariate Cox Regression, adjusting for age, work as a prostitute, any children, cytology reader, and birth in Senegal.

**Multivariate Cox Regression, adjusting for all other factors in the table as well as age, work as a prostitute, any children, cytology reader, and birth in Senegal.

***other HPV refers to HPV types 26, 39, 51, 52, 55, 56, 58, 59, 68, 73, 82, 83 or untyped HPV

Table 2.6. Multivariate Cox Regression Analysis of Development of High Grade Cervical Dysplasia in HIV-2 positive Women

	Crude RR* (95% CI)	Adjusted RR** (95% CI)
CD4 count		
>500	1.0 (ref)	1.0 (ref)
200-500	2.6 (0.3-26.2)	1.1 (0.1-18.5)
<200	12.5 (1.2-126)	5.0 (0.2-163)
HIV-1 coinfection	8.4 (1.5-46.8)	1.8 (0.1-54)
Log_{10} Plasma HIV-2 RNA level	1.31 (0.77-2.2)	1.10 (0.53-2.3)
HPV DNA		
No Low Risk HPV DNA	1.0 (ref)	1.0 (ref)
Incident Low Risk HPV DNA	0.0	0.0
Persistent Low Risk HPV DNA	30.8 (0.6-1505)	14.7 (0.2-934)
No other HPV DNA***	1.0 (ref)	1.0 (ref)
Incident other HPV DNA***	16.4 (1.3-202)	11.9 (0.9-160)
Persistent other HPV DNA***	11.9 (0.6-227)	22.4 (0.8-625)
No High Risk HPV DNA	1.0 (ref)	1.0 (ref)
Incident High Risk HPV DNA	0.0	0.0
Persistent High Risk HPV DNA	34.7 (2.2-554)	16.9 (0.5-524)

*Multivariate Cox Regression, adjusting for age, work as a prostitute, any children, cytology reader, and birth in Senegal.

**Multivariate Cox Regression, adjusting for all other factors in the table as well as age, work as a prostitute, any children, cytology reader, and birth in Senegal.

***other HPV refers to HPV types 26, 39, 51, 52, 55, 56, 58, 59, 68, 73, 82, 83 or untyped HPV

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APPENDIX A

SCREENING INTERVIEW FORM

Site: _ _ _ Numéro d'étude: _ _ _ _ _ _

MIN = Fann **MBN** = M'Bour **DKN** = Dakar **AS** = ASBEF **MIH** = MI Hommes)
MB = M'Bour (ancien) **DK** = Dakar (ancien) **MTN** = Matam

Date: _ _/_ _/_ _ Sexe: _ (1 = Homme; 2 = Femme)

Si MIN ou MIH, Groupe: _:
(1 = Hôpitalisé(e); 2 = Consultation externes; 3 = Epouse/Co-épouse séro+; 4 = Sero+ déjà connue)

Si épouse/co-épouse HIV séropositif, numéro d'étude du cas initial: _ _ _ _ _ _ _ _ _

Prénom(s): _

Nom de famille: _

Adresse: Ville: _____ Quartier:

Rue: _____ Chef du Quartier:

Village: _____ Chef du Village:

Chef de maison:

Contact: _____ Tél:

1. Date de naissance: _ _/_ _/_ _

2. Statut matrimonial: _ 1 = célibataire; 2 = marié(e) monogame; 3 = marié(e) polygame; 4 = divorcé(e); 5 = séparé(e); 6 = veuf(ve); 7 = concubinage

3. Lieu de naissance: _ 1 = Sénégal; 2 = Guinée Bissau; 3 = Ghana; 4 = Rep de Guinée; 5 = Gambie; 6 = Mauritanie; 7 = Mali; 8 = Autres pays de l'Afrique Ouest; 9 = Afrique Centrale; 10 = Afrique de l'Est; 11 = Europe; 12 = Autre: _____

4. Ethnie: _ (1 = Wolof; 2 = Pulaar; 3 = Serere; 4 = Sarakhole; 5 = Mandjack; 6 = Diola; 7 = Autre)

5. Religion: _ (1 = Musulman; 2 = Chrétien; 3 = Animist; 4 = Autre; 5 = Aucune)

6. Profession: _ 1 = Secteur Primaire; 2 = Secteur Secondaire; 3 = Secteur Tertiaire; 4 = Prostituée; 5 = Sans profession; 6 = Etudiant(e); 7 = Militaire; 8 = Retraité

7. Instruction: _ (0 = non; 1 = oui)

   Si oui, a) Type d'instruction: _ (1 = Francais; 2 = Arabe; 3 = Autres: _____)

   b) Niveau d'instruction: _ (1 = Primaire; 2 = Secondaire; 3 = Supérieur; 4 = Autodidacte)

8. (*Pour les femmes*)
   a) Date des dernières règles: __ __/__ __/__ __

   b) Nombre de grossesses: __ __

   c) Contraception: __
      0 = aucune; 1 = préservatifs; 2 = contraceptifs oraux; 3 = contraceptifs injectables;
      4 = toilette intime; 5 = méthodes traditionnelles; 6 = autres: _____

   d) Frottis dans le passé: __ (0 = non; 1 = oui) Si oui, depuis combien de mois: __ __

9. Fumeur: __ (0 = non; 1 = oui) Alcool: __ (0 = non; 1 = oui)

10. Visite chez le dentiste dans le passé: __ (0 = non; 1 = oui)

    Si oui, a) depuis combien de mois: __ __

    b) raison de la visite: __
       (1 = routine; 2 = ulcéres; 3 = saignement; 4 = douleur; 5=autre: _____)

11. Est-ce que vous voyagez souvent hors du Senegal? __ (0 = non, 1 = oui)

    Si oui, dans quels pays? __ __, __ __, __ __
    2 = Guinée Bissau; 3 = Ghana; 4 = Rep de Guinée; 5 = Gambie; 6 = Mauritanie; 7 = Mali; 8 = Autres pays de l'Afrique Ouest; 9 = Afrique Centrale; 10 = Afrique de l'Est; 11 = Europe; 12 = Autre: _____

12. Est-ce que votre epoux (se) voyage souvent hors du Senegal? __ (0 = non, 1 = oui)

    Si oui, dans quels pays? __ __, __ __, __ __
    2 = Guinée Bissau; 3 = Ghana; 4 = Rep de Guinée; 5 = Gambie; 6 = Mauritanie; 7 = Mali; 8 = Autres pays de l'Afrique Ouest; 9 = Afrique Centrale; 10 = Afrique de l'Est; 11 = Europe; 12 = Autre: _____

13. Motif de la consulation: __

14. Si MI ou Matam,
    hypothèse diagnostique: __

EXAMEN GYNECOLOGIQUE—SCREENING VISIT (LES FEMMES)

Site: __ __ __ Numéro d'étude: __ __ __ __ __
MIN = Fann **MBN** = M'Bour **DKN** = Dakar **AS** = ASBEF
MTN = Matam **MB** = M'Bour (ancien) **DK** = Dakar (ancien)

Date: __ __/__ __/__ __ Sexe: 2 (**1** = Homme; **2** = Femme)

Prénom(s): __

Nom de famille: __

Date de naissance: __ __/__ __/__ __

Excisée? __ (**0** = non; **1** = oui) Gros Rein __ (**0** = non; **1** = oui)

Pertes vaginales? __ (**0** = non; **1** = oui) Si oui, décrivez: __ __ __ __ __ __ __ __ __ __ __ __

pH (vaginale): __ __.__ Fissures? __ (**0** = non; **1** = oui)

Verrues génitales? __ (**0** = non; **1** = oui) Si oui, précisez: (**0** = non; **1** = oui)

sur la vulve? __ dans le vagin? __ autrepart? __ Si autrepart, précisez: _____

Ulcère génital? __ (**0** = non; **1** = oui) Si oui, douloureux? __ (**0** = non; **1** = oui)

Herpes génitaux (vesicules douleureuses)? __ (**0** = non; **1** = oui)

Col? __ (**0** = normale; **1** = anormale) Si anormale, précisez: (**0** = non; **1** = oui)

fragilisé? (saigne au contact)? __ ectropion oedémateux? __ autre? __

Si autre, précisez: __ __ __ __ __ __ __ __ __ __ __ __

Pertes purulentes du col? __ (**0** = non; **1** = oui) Leukoplasie? __ (**0** = non; **1** = oui)

Ectropion: proportion1: __ __% proportion2: __ __%

Visualisation du col (présence de CIN 2-3) (**0** = pas CIN 2-3; **1** = CIN 2-3; **2** = ne sait pas; **9** = ne fait pas):

sans le gynéscope? __ avec le gynéscope? __ avec le colposcope? __

Inflammation intrapelvienne? __ (**0** = non; **1** = oui) Masse pelvienne? __ (**0** = non; **1** = oui)

TRAITEMENT

1 = antifungal; **2** = amoxicillin; **3** = bactrim; **4** = cefixime; **5** = ceftriaxone; **6** = doxycycline; **7** = erythromycin; **8** = metronidazole; **9** = penicillin; **10** = autre

Medicament	Dose (mg/jour)	Traité pour combien de jours?
__ __	__ __ __ __	__ __

LES RESULTATS DE LABORATOIRE DU SCREENING

Site: __ __ __ Numéro d'étude: __ __ __ __ __

MIN = Fann **MBN** = M'Bour **DKN** = Dakar **AS** = ASBEF
MIH = MI (Hommes) **MB** = M'Bour (ancien) **DK** = Dakar (ancien) **MTN** = Matam

Date de rendevous: __ __/__ __/__ __ Date de la visite du screening: __ __/__ __/__ __

Sexe: __ (1 = Homme; 2 = Femme)

Prénom(s): __

Nom de famille: __

Age: __ __

Adresse: Ville: _____ Quartier:

Rue: _____ Chef du Quartier:

Village: _____ Chef du Village:

HIV: __ (0 = négatif; 1 = VIH=1; 2 = VIH-2; 3 = VIH1+VIH-2; 4 = indéterminé)

Examen orale: __ (0 = normale, 1 = anormale)

LES FEMMES:

Cytologie: __ 1 = négatif; 2 = négatif/réactif ou inflammatoire; 3 = atypie/non-réactif; 4 = CIN1;
5 = CIN2-3; 6 = peu satisfaisant/insuffisant/inadequat; 9 = renseignements incomplets

HPV: __ (0 = négative/risque bas; 1 = risque haut)

LES HOMMES ET LES FEMMES:

Le (la) malade est revenue pour son resultat? __ (0 = non; 1 = oui)

Etude: Dysplasie sur le col (A. Coll):

Eligible: __ (0 = non; 1 = oui) Malade participera: __ (0 = non; 1 = oui; 2 = pas demandé)

Etude: Dentaire (C. Faty):

Eligible: __ (0 = non; 1 = oui) Malade participera: __ (0 = non; 1 = oui; 2 = pas demandé)

Etude: Couples (A. Coll):

Eligible: __ (0 = non; 1 = oui, époux, épouse ou co-épouse est HIV+)

Malade participera: __ (0 = non; 1 = oui; 2 = pas demandé)

Numéro d'étude de l'époux/épouse/co-épouse: __ __ __ (Site) __ __ __ __ __ (Numéro)

VISITE DE PREMIER ENREGISTREMENT (LES FEMMES)

Site: __ __ __ Numéro d'étude: __ __ __ __ __

MIN = Fann **MBN** = M'Bour **DKN** = Dakar **AS** = ASBEF
MTN = Matam **MB** = M'Bour (ancien) **DK** = Dakar (ancien)

Date: __ __/__ __/__ __ Sexe: 2 (1 = Homme; 2 = Femme)

Si MIN, Groupe: __
(1 = Hôpitalisée; 2 = Consultation externes; 3 = Epouse/Co-épouse séro+; 4 = Sero+ déjà connue)

QUESTIONNAIRE - INFORMATIONS GENERALES

1. Quelle est votre date de naissance? __ __/__ __/__ __

2. a) Dans quel pays êtes-vous née? __ __ 1 = Sénégal; 2 = Guinée Bissau; 3 = Ghana; 4 = Rep de Guinée; 5 = Gambie; 6 = Mauritanie; 7 = Mali; 8 = Autres pays de l'Afrique Ouest; 9 = Afrique Centrale; 10 = Afrique de l'Est; 11 = Europe; 12 = Autre: _____

   b) Etes vous née dans une ville ou une zone rurale? __ (1 = ville; 2 = zone rurale)

3. a) Présentement, vivez-vous dans une ville ou une zone rurale? __ (1 = ville; 2 = zone rurale)

   b) Depuis combien d'années vivez-vous dans cette ville/village? __ __

4. Nationalité: __ __ 1 = Sénégal; 2 = Guinée Bissau; 3 = Ghana; 4 = Rep de Guinée; 5 = Gambie; 6 = Mauritanie; 7 = Mali; 8 = Autres pays de l'Afrique Ouest; 9 = Afrique Centrale; 10 = Afrique de l'Est; 11 = Europe; 12 = Autre: _____

5. Ethnie: __ (1 = Wolof; 2 = Pulaar; 3 = Serere; 4 = Sarakhole; 5 = Mandjack; 6 = Diola; 7 = Autre)

6. Quel est votre religion? __ (1 = Musulman; 2 = Chrétien; 3 = Animist; 4 = Autre; 5 = Aucune)

7. Quelle est votre profession? __ 1 = Secteur Primaire; 2 = Secteur Secondaire; 3 = Secteur Tertiaire; 4 = Prostituée; 5 = Sans profession; 6 = Etudiante; 7 = Militaire; 8 = Retraité

8. Instruction? __ (0 = non; 1 = oui)

   Si oui, a) Type d'instruction: __ (1 = Francais; 2 = Arabe; 3 = Autres: _____)

   b) Niveau d'instruction: __ (1 = Primaire; 2 = Secondaire; 3 = Supérieur; 4 = Autodidacte)

9. a) Buvez-vous de l'alcool? __ (0 = non; 1 = oui)

   b) Si oui, combien de verres par jour? __ (1 = 1 verre/jour; 2 = >1 verre/jour; 3 = occasionnellement)

   c) Précisez le type d'alcool que vous buvez la plupart du temps: __ (1 = bière, 2 = vin, 3 = autres)

10. Avez-vous déjà fumé? __ (0 = non; 1 = oui)

11. Fumez-vous actuellement? __ (0 = non; 1 = oui)

    Si oui, a) combien? __ (1 = 1-10 cigs/jour; 2 = 11-20 cigs/jour; 3 = >20 cigs/jour)

    b) à quel âge avez-vous commencé à fumer régulièrement? __ __

12. Quel est votre statut matrimonial? __ 1 = Célibataire; 2 = Mariée monogame; 3 = Mariée polygame; 4 = Séparée; 5 = Divorcée; 6 = Veuve; 7 = Concubinage

13. Curriculum matrimonial (si vous êtes mariée ou si vous avez été mariée)

			1 = Sénégal; 2 = Guinée Bissau; 3 = Ghana; 4 = Rep de Guinée; 5 = Gambie; 6 = Mauritanie; 7 = Mali; 8 = Autres pays de l'Afrique Ouest; 9 = Afrique Centrale; 10 = Afrique de l'Est; 11 = Europe; 12 = Autre	1 = mariage monogame; 2 = mariage polygame; 3 = separée; 4 = divorcée; 5 = décédé
Mari	Année du mariage	L'âge du mari au mariage	Lieu de naissance du mari	Statut matrimonial
1	19__ __	__ __	__ __	__
2	19__ __	__ __	__ __	__
3	19__ __	__ __	__ __	__
4	19__ __	__ __	__ __	__

14. Si vous êtes dans un mariage polygame, combien d'autres co-épouses avez-vous? __ __

15. Combien de fois est-ce que votre mari s'est-il marie (en dehors de vous-même et des autres co-épouses (actuelles))? __ __

16. Antécédents: (0 = non; 1 = oui)

Hôpitalisation: __ Si oui, la cause: __
1 = grossesse (avortement) pathologique; 2 = maladie infectieuse; 3 = diarrhée chronique; 4 = chirurgie; 5 = trauma; 6=autre

Transfusion: __ Si oui, année de la transfusion: 19 __ __

Utilisation des droques: __ Si oui, (i) à quelle époque? __ (1=actuellement; 2 = passé)

(ii) quel type de droque? __ (1 = IV; 2 = autre; 3 = les deux)

Tuberculose: __

Dermocorticoides: __ Si oui, depuis combien de temps? __ __ __ mois

Médicaments actuellement utilisés: __ Si oui, précisez: __ __ __ __ __ __ __ __ __ __ __ __

17. a) Est-ce que vous avez voyagé hors du Senegal depuis la visite du screening? __ (0 = non, 1 = oui)

Si oui, dans quels pays? __ __, __ __, __ __
2 = Guinée Bissau; 3 = Ghana; 4 = Rep de Guinée; 5 = Gambie; 6 = Mauritanie; 7 = Mali; 8 = Autres pays de l'Afrique Ouest; 9 = Afrique Centrale; 10 = Afrique de l'Est; 11 = Europe; 12 = Autre: _____

b) Est-ce que votre epoux a voyagé hors du Senegal depuis la visite du screening? __ (0 = non, 1 = oui)

Si oui, dans quels pays? __ __, __ __, __ __
2 = Guinée Bissau; 3 = Ghana; 4 = Rep de Guinée; 5 = Gambie; 6 = Mauritanie; 7 = Mali; 8 =

Autres pays de l'Afrique Ouest; 9 = Afrique Centrale; 10 = Afrique de l'Est; 11 = Europe; 12 = Autre: _____

18. Etes-vous informé qu'un membre de votre famille est diagnostiqué du SIDA ou VIH? __ (0 = non; 1 = oui)

    Si oui, qui? __, __, __ (1 = mari; 2 = enfant; 3 = co-épouse; 4 = autre: _____)

MATERNITE ET ANTECEDANTS SEXUELS

19. Date des dernières règles: __ __/__ __/__ __

20. Etes-vous actuellement enceinte? __ (0 = non; 1 = oui; 2 = ne sait pas)

21. Combien de grossesses? __ __ d'enfants vivants? __ __ d'avortements? __ __

22. a) L'âge de la malade à sa première grossesse? __ __ b) A sa dernière grossesse? __ __

23. Avez-vous vu un médecin pendant votre dernière grossesse? __ (0 = non; 1 = oui)

24. Avez-vous déjà été opérée de vos organes génitaux? __ (0 = non; 1 = oui)

25. Avez-vous déjà fait un frottis cervico-vaginal? __ (0 = non; 1 = oui)

    Si oui, combien de fois pendant ces cinq dernières années? __ __

26. Quel âge aviez-vous lors de votre premier rapport sexuel? __ __

27. Combien de partenaires avez-vous eu? __ (1 = 1; 2 = 2-5; 3 = 6-10; 4 = >10)

28. Combien de fois par semaine avez-vous des rapports sexuels? __ __

29. A combien de jours remonte votre dernier rapport sexuel? __ __

30. A combien de jours remonte votre dernière toilette intime? __ __

31. Utilisez-vous des produits intravaginaux pour: (0 = non; 1 = oui)

    la contraception? __ Si oui, precisez: __ __ __ __ __ __ __ __ __ __ __ __

    les leucorrhées? __ Si oui, precisez: __ __ __ __ __ __ __ __ __ __ __ __

    autres? __ Si oui, precisez: __ __ __ __ __ __ __ __ __ __ __ __

32. Avez-vous déjà eu des rapports sexuels avec un homme non-Sénégalais? __ (0 = non; 1 = oui)

    Si oui, est-ce qu'il était non-circoncis? __ (0 = non; 1 = oui)

33. Pendant les 4 derniers mois, avez-vous eu un nouveau partenaire? __ (0 = non; 1 = oui)

    Si oui, à quelle fréquence utilisiez-vous les préservatifs? __
    (0 = jamais; 1 = rarement; 2 = quelques fois; 3 = souvent; 4 = toujours)

34. Avez-vous déjà eu des rapports sexuels avec un homme qui est atteint de VIH ou de SIDA? __
    (0 = non; 1 = oui; 2 = ne sait pas)

Si oui, à quelle fréquence utilisiez-vous les préservatifs? __
(0 = jamais; 1 = rarement; 2 = quelques fois; 3 = souvent; 4 = toujours)

35. Quelle méthode de contraception utilisez-vous actuellement? __
0 = aucune; 1 = préservatifs; 2 = contraceptifs oraux; 3 = contraceptifs injectables; 4 = toilette intime;
5 = méthodes traditionnelles; 6 = autre: _____

36. Quelle méthodes de contraception avez-vous utilisé dans le passé __, __, __, __, __
0 = aucune; 1 = préservatifs; 2 = contraceptifs oraux; 3 = contraceptifs injectables; 4 = toilette intime;
5 = méthodes traditionnelles; 6 = autre: _____

37. Avez-vous jamais eu un saignement pendant les relations sexuelles? __ (0 = non; 1 = oui)

38. Recevez-vous de l'argent en échange de relations sexuelles? __ (0 = non; 1 = oui)
Si oui:
a) Etes-vous enregistrée? __ (0 = non; 1 = oui)

b) Depuis combien de mois vous prostituez-vous? __ __ __

c) Combien (CFA) gagnez-vous par relation? __
(1 = ≤500; 2 = 501-2500; 3 = 2501-5000; 4 = >5000)

39. Pendant les 4 derniers mois,
avez-vous jamais eu des rapports sexuels pendant les règles? __ (0 = non; 1 = oui)

40. Pendant les 4 derniers mois, avez-vous jamais eu des rapports sexuels:

a) par voie orale? __ (0 = non; 1 = oui) b) par voie rectale? __ (0 = non; 1 = oui)

41. Pour les maladies suivantes:

	0 = non; 1 = oui	0 = non; 1 = oui	1 = médecin; 2 = sage-femme; 3 = pharmacien(ne); 4 = biologist(e); 5 = guerrisseur
Nom de la maladie	Avez-vous jamais eu cette maladie?	Traité pour cette maladie?	Traité par qui?
Ecoulement	__	__	__
Ulcérations	__	__	__

LES ATTITUDES: VIH

42. Vous a-t-on jamais proposé le test du VIH? __ (0 = non; 1 = oui; 2 = ne sait pas).

Si oui: a) quel était le résultat? __
0 = négatif; 1 = VIH-1; 2 = VIH-2; 3 = VIH-1+2; 4 = indéterminé;
5 = positif, type inconnu; 9 = inconnu

b) vous a-t-on proposé le test:
(i) pendant une grossesse? __ (0 = non; 1 = oui)

(ii) pendant une visite à la clinique (pas pendant une grossesse)? __ (0 = non; 1 = oui)

(iii) parce que un partenaire sexuel est atteint de VIH/SIDA __ (0 = non; 1 = oui)

43. Vous a-t-on dit que le VIH pourrait être transmis au bébé pendant la grossesse? __ (0 = non; 1 = oui)

44. Si vous étiez diagnostiquée VIH+, est-ce que: __
1 = vous essayeriez d'éviter une grossesse; 2 = de concevoir un bébé;
3 = ni essayer d'éviter une grossesse ni concevoir un bébé

POUR LES FEMMES VIH+

45. Depuis combien de mois vous savez-vous VIH+? __ __ __

46. Depuis que vous vous savez séropositif:
a) combien de partenaires sexuels avez-vous eu? __ __ __

b) quelle méthode de contraception utilisez-vous? __
0 = aucune; 1 = préservatifs; 2 = contraceptifs oraux; 3 = contraceptifs injectables; 4 = toilette intime;
5 = méthodes traditionnelles; 6 = autre

c) avez-vous parlé à votre mari (vos partenaires sexuels) du diagnostic? __ (0 = non; 1 = oui)

47. Si vous utilisez des préservatifs pendant vos rapports sexuels, avec quelle fréquence les utilisez-vous? __
(0 = jamais; 1 = rarement; 2 = quelques fois; 3 = souvent; 4 = toujours)

48. Depuis que vous vous savez VIH+, êtes-vous tombée enceinte? __ (0 = non; 1 = oui)

EXAMENS CLINIQUES—PREMIERE VISITE—(LES HOMMES ET LES FEMMES)

Site: __ __ __ Numéro d'étude: __ __ __ __ __

MIN = Fann **MBN** = M'Bour **DKN** = Dakar **AS** = ASBEF
MIH = MI (Hommes) **MB** = M'Bour (ancien) **DK** = Dakar (ancien) **MTN** = Matam

Date: __ __/__ __/__ __ Sexe: __ (**1** = Homme; **2** = Femme)

Prénom(s): __

Nom de famille: __

Date de naissance: __ __/__ __/__ __

SIGNES FONCTIONNELS

Toux plus que 1 mois __ (**0** = non; **1** = oui) Diarrhée plus que 1 mois __ (**0** = non; **1** = oui)

SIGNES GENERAUX

Poids: __ __ __ (kg) Temp: __ __.__ (°C) Tension Systol: __ __ __ Diastol: __ __ __

Taille: __ __ __ (cm) Pouls: __ __ __ Amaigrissement __ (**0** = non; **1** = oui)

SIGNES PHYSIQUES

Poumon: Pneumonie: __ (**0** = non; **1** = oui) Suspicion de TB: __ (**0** = non; **1** = oui)

Digestif: Candidose (bouche): __ (**0** = non; **1** = oui) Cheilite angulaire: __ (**0** = non; **1** = oui)

Leucoplasie orale: __ (**0** = non; **1** = oui) Ulcération orale: __ (**0** = non; **1** = oui)

Hépatomegalie: __ (**0** = non; **1** = oui) Splenomegalie: __ (**0** = non; **1** = oui)

Dermatologie examen: __ (**0** = normale; **1** = anormale)
Si pas normale, précisez: Dermatose prurigineuse: __ (**0** = non; **1** = oui)

Ichtyose générale: __ (**0** = non; **1** = oui) Zona: __ (**0** = non; **1** = oui)

Herpes: __ (**0** = non; **1** = oui) Abcès: __ (**0** = non; **1** = oui)

Psoriasis: __ (**0** = non; **1** = oui) Suspicion de Kaposi sarcome: __ (**0** = non; **1** = oui)

Autre: __ (**0** = non; **1** = oui) Si oui, précisez: __ __ __ __ __ __ __ __ __ __

Cheveux: __ (**0** = normale; **1** = anormale) Si anormale, précisez: __ __ __ __ __ __ __ __

Ganglions: Adénopathie: __ (**0** = non; **1** = oui) Si oui, précisez: (**0** = non; **1** = oui)

cervical? __ axillaire? __ au-dessus de la clavic? __ inguinal? __ autrepart? __

Si autrepart, précisez: __ __ __ __ __ __ __ __ __ __ __ __

DIAGNOSTIC SIDA

Stade OMS: __ (**1**, **2**, **3**, **4**) Classification CDC: __ (**1** = I; **2** = II; **3** = III; **4** = IV)

QUESTIONS—SUIVI (LES FEMMES)

Site: __ __ __ Numéro d'étude: __ __ __ __ __

MIN = Fann **MBN** = M'Bour **DKN** = Dakar **AS** = ASBEF
MTN = Matam **MB** = M'Bour (ancien) **DK** = Dakar (ancien)

Date: __ __/__ __/__ __ Sexe: 2 (**1** = Homme; **2** = Femme)

INFORMATIONS GENERALES

1. Pendant les 4 derniers mois: (**0** = non; **1** = oui)

   a) Hôpitalisation? __ Si oui, la cause? __ **1** = grossesse ou avortement pathologique; **2** = maladie infectieuse; **3** = diarrhée chronique; **4** = chirurgie; **5** = trauma; **6** = autre

   b) Transfusion? __

   c) Utilisation des droques: __ Si oui,
      (i) à quelle époque? __ (**1**=actuellement; **2** = passé)
      (ii) quel type de droque? __ (**1** = IV; **2** = autre; **3** = les deux)

   d) Tuberculose: __

   e) Dermocorticoides: __ Si oui, depuis combien de temps? __ __ __ mois

   f) Médicaments actuellement utilisés: __

   Si oui, précisez: __ __ __ __ __ __ __ __ __ __ __ __ __

2. a) Est-ce que vous avez voyagé hors du Senegal depuis la dernière visite? __ (**0** = non, **1** = oui)

   Si oui, dans quels pays? __ __, __ __, __ __
   2 = Guinée Bissau; **3** = Ghana; **4** = Rep de Guinée; **5** = Gambie; **6** = Mauritanie; **7** = Mali; **8** = Autres pays de l'Afrique Ouest; **9** = Afrique Centrale; **10** = Afrique de l'Est; **11** = Europe; **12** = Autre: _____

   b) Est-ce que votre epoux a voyagé hors du Senegal depuis la dernière visite? __ (**0** = non, **1** = oui)

   Si oui, dans quels pays? __ __, __ __, __ __
   2 = Guinée Bissau; **3** = Ghana; **4** = Rep de Guinée; **5** = Gambie; **6** = Mauritanie; **7** = Mali; **8** = Autres pays de l'Afrique Ouest; **9** = Afrique Centrale; **10** = Afrique de l'Est; **11** = Europe; **12** = Autre: _____

3. Pendant les 4 derniers mois, êtes-vous informé qu'un membre de votre famille est diagnostiqué du SIDA ou VIH? __ (**0** = non; **1** = oui)

   Si oui, qui? __, __, __ (**1** = mari; **2** = enfant; **3** = co-épouse; **4** = autre: _____)

4. Comment vous sentez-vous depuis la dernière visite? __ (**1**=mieux; **2** = plus fatigué; **3** = autre)

   Si autre, précisez: __ __ __ __ __ __ __ __ __ __ __ __ __

MATERNITE ET ANTECEDANTS SEXUELS

5. Date des dernières règles: __ __/__ __/__ __

6. Etes-vous actuellement enceinte? __ (0 = non; 1 = oui; 2 = ne sait pas)

7. Quelle méthode de contraception utilisez-vous actuellement? __
   0 = aucune; 1 = préservatifs; 2 = contraceptifs oraux;
   3 = contraceptifs injectables; 4 = toilette intime; 5 = autre: _____

8. Combien de partenaires avez-vous eu pendant les 4 derniers mois? __
   (1 = 1; 2 = 2-5; 3 = 6-10; 4 = >10)

9. Pendant les 4 derniers mois, avez-vous eu un nouveau partenaire? __ (0 = non; 1 = oui)

   Si oui, à quelle fréquence utilisiez-vous les préservatifs? __
   (0 = jamais; 1 = rarement; 2 = quelques fois; 3 = souvent; 4 = toujours)

10. Pendant les 4 derniers mois, avez-vous déjà eu des
    rapports sexuels avec un homme atteint de VIH ou de SIDA? __ (0 = non; 1 = oui; 2 = ne sait pas)

    Si oui, à quelle fréquence utilisiez-vous les préservatifs? __
    (0 = jamais; 1 = rarement; 2 = quelques fois; 3 = souvent; 4 = toujours)

11. Combien de fois par semaine avez-vous des rapports sexuels? __ __

12. A combien de jours remonte votre dernier rapport sexuel? __ __

13. A combien de jours remonte votre dernière toilette intime? __ __

14. Depuis la dernière visite, avez-vous utilisé des produits intravaginaux pour: (0 = non; 1 = oui)

    la contraception? __ Si oui, precisez: _ _ _ _ _ _ _ _ _ _ _ _ _

    les leucorrhées? __ Si oui, precisez: _ _ _ _ _ _ _ _ _ _ _ _ _

    autres? __ Si oui, precisez: _ _ _ _ _ _ _ _ _ _ _ _ _

15. Pendant les 4 derniers mois,
    avez-vous jamais eu des rapports sexuels pendant les règles? __ (0 = non; 1 = oui)

16. Pendant les 4 derniers mois, avez-vous eu un saignement pendant les relations sexuelles? __
    (0 = non; 1 = oui)

17. Pendant les 4 derniers mois, avez-vous jamais eu des rapports sexuels: (0 = non; 1 = oui)

    a) par voie orale? __ b) par voie rectale? __

18. Avez-vous remarqué au cours des 4 derniers mois: (0 = non; 1 = oui)

    a) des écoulements du vagin? __ b) des ulcérations dans la région génitale? __

VITA

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Education:

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1993	**M.S., Biostatistics**, University of Washington, Seattle, WA. Thesis Title: Hydrogen Peroxide-Producing Lactobacilli in the Prevention of Bacterial Vaginosis, *Trichmonas Vaginalis*, and Vaginal Yeast Infection.
2001	**Ph.D., Epidemiology**, University of Washington, Seattle, WA. Dissertation Title: HIV-1, HIV-2, and dual infection with HIV-1 and HIV-2 are associated with increased risk for human papillomavirus (HPV) and high grade cervical intraepithelial lesions (HSIL) in Senegal, West Africa

Professional Positions:

1988-1991	**Research Assistant**, Biostatistics Center, The George Washington University, Rockville, MD.
1991-1993	**Research Assistant**, Department of Biostatistics, Center for AIDS and STDs, University of Washington, Seattle, WA.
1993-1996	**Research Scientist**, Department of Medicine, Center for AIDS and STDs; HPV Research Group, University of Washington, Seattle, WA.
1996-present	**Research Scientist**, Department of Pathology, HPV Research Group, University of Washington, Seattle, WA.

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