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Cervical cancer screening for HIV-infected women in Zambia.

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CERVICAL CANCER SCREENING FOR HIV-INFECTED WOMEN IN ZAMBIA

by

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A DISSERTATION

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ABSTRACT

Women infected with the human immunodeficiency virus (HIV) are at elevated risk for the development of cervical intraepithelial neoplasia (CIN) induced by oncogenic human papillomavirus (HPV) and need screening for the early detection and treatment of CIN. The first chapter of this dissertation includes a systematic review of literature to assess the accuracy of cervical cytology for screening HIV-infected women. The results of a meta-analysis yielded a pooled sensitivity of 68% and specificity of 81% at the $\geq$ CIN1 diagnostic threshold and a pooled sensitivity of 40% and specificity of 96% at the $\geq$ CIN2 threshold. These estimates suggest that cytology is sub-optimally sensitive for screening for CIN in HIV-infected women even in the most controlled settings, underscoring the necessity for the development of alternative screening tests. The second manuscript assesses the accuracy of visual inspection with acetic acid (VIA), a low-cost, low-technology alternative to cytology, for screening HIV-infected women. In a cross-sectional study among 150 HIV-infected women in Lusaka, Zambia, VIA had a lower sensitivity (73% versus 91%) than cytology, but higher specificity (62% versus 59%), and a lower overall efficiency (65% versus 66%), although none of these were statistically significant ($p>0.5$). When considered in series combination, the efficiency of the combination increased to 76%. The data strongly justify the use of VIA as an independent or adjunct screening test especially since it allows for screening and treatment to be linked in the same clinic visit, thereby preventing loss to follow-up. In the third manuscript, polymerase chain reaction-based typing of HPV in the 150 participating

HIV-infected women in our cross-sectional study is discussed. The mean number of HPV types per participant increased with increasing severity of cervical cytological lesions and decreasing CD4+ cell counts (worsening HIV-clinical/immunological status). The relative rarity of HPV-16 and 18 vis-à-vis other genital HPV-types strongly suggest the need for additional research on the utility of prophylactic vaccines in this high-risk population. In conclusion, it is important to develop evidence-based guidelines for preventing cervical cancer in HIV-infected women living in resource-limited settings like Zambia in the era of antiretroviral therapy.

DEDICATION

This dissertation is a humble attempt to make a change in the lives of millions of women worldwide who are at elevated risk for cervical cancer. This work is in memory of every woman who dies of cervical cancer, each one of whom represents one of the greatest public health failures of our times.

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HIGH-PREVALENCE OF GENITAL HPV INFECTION AND MULTIPLE HPV GENOTYPES IN HIV-INFECTED WOMEN IN LUSAKA, ZAMBIA

LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ASCUS	Atypical squamous cells of undetermined significance
CIN	Cervical intraepithelial neoplasia
DOR	Diagnostic odds ratio
ECC	Endocervical curettage
FN	False Negatives
FP	False Positives
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
ICC	Invasive cervical cancer
LEEP	Loop electrosurgical excision procedure
PCR	Polymerase chain reaction
ROC	Receiver operating characteristic
SCC	Squamous cell carcinoma
SIL	Squamous intraepithelial lesions
TN	True Negatives
TP	True Positives
VIA	Visual inspection with acetic acid

INTRODUCTION

Cancer of the uterine cervix, or cervical cancer, is caused by oncogenic types of human papillomavirus (HPV). Invasive cervical cancer represents the leading cause of cancer-related morbidity and mortality among women living in resource-constrained settings of the developing world. In these same settings, the HIV/AIDS pandemic has overwhelmed health care systems and is increasingly affecting women in the reproductive age group. HIV-infected women are at increased risk for the development, progression and recurrence of HPV-induced cervical neoplasia. As access to life-prolonging antiretroviral therapy for HIV/AIDS increases worldwide, HIV-infected women may live long enough for malignancies like cervical cancer to manifest and progress. Thus, it is critical to monitor HIV-infected women for the development and recurrence of cervical pre-neoplastic disease through the use of cost-effective and accurate tests and protocols.

Zambia, one of the sub-Saharan African nations hardest hit by the AIDS pandemic also has some of the highest background prevalence rates of invasive cervical cancer in the world. It has been difficult to establish and sustain cervical cytology based screening programs in resource-limited settings like Zambia due to lack of resources and manpower, coupled with the lack of awareness and education. Given these challenges, there is interest in assessing the utility of non-cytological methods, including Visual Inspection with Acetic Acid (VIA) that has emerged as a low-cost, low-technology, and a

single-visit alternative to cervical cytology. Recent research has focused on comparison of VIA and other alternative screening methods, however, estimates of the accuracy of VIA in HIV-infected women are as yet unknown.

Another intervention for cervical cancer control that is already on the horizon is primary prevention through HPV-prophylactic vaccines. The current vaccine constructs are based only on two HPV types (16 and 18), although more than 40 types infect the genital tract and over a dozen types are implicated as being oncogenic. The relative prevalence of various HPV types infecting genital types in HIV-infected women in settings like Zambia is as yet unknown, precluding an understanding of the usefulness of currently available vaccines for preventing cervical cancer in these high-risk women.

This dissertation tackles the theme of cervical cancer screening for HIV-infected women through three inter-connected manuscripts. The first assesses the accuracy of cervical cytology among HIV-infected women through a systematic literature review and meta-analytic techniques, the second assesses the relative accuracy of VIA for screening HIV-infected women in a cross-sectional study in Zambia and the third manuscript reports the relative-prevalence of HPV types among HIV-infected women in Zambia, and their association with cytology and immunosuppressant state. The findings of this study provide the rationale for undertaking research on this important high-risk population. The development of appropriate guidelines for the prevention of cervical cancer among HIV-infected women is critical considering the significant morbidity and mortality due to both HIV/AIDS and cervical cancer in resource constrained settings.

ACCURACY OF CYTOLOGY TO DETECT CERVICAL INTRAEPITHELIAL NEOPLASIA AMONG HIV-INFECTED WOMEN: A SYSTEMATIC REVIEW

by

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ABSTRACT

Background: There is mixed evidence regarding the accuracy of cervical cytology to detect cervical intraepithelial neoplasia (CIN) among HIV-infected women. Test accuracy is especially important for immunosuppressed women whose disease course may be especially rapid. We performed a systematic review to assess the accuracy of cytology to detect low and high grade CIN in HIV-infected women.

Methods: Data were extracted from articles published from 1981-2005 that reported results of screening cytology followed by performing a reference diagnostic investigation (colposcopy with/without histopathological confirmation) in HIV-infected women. Nineteen studies met the inclusion criteria in which twelve studies allowed the comparison of the accuracy measures at the high-grade (≥CIN 2) threshold and 13 studies permitted these measures for low-grade (≥CIN 1) disease. We used meta-analytic techniques to compute pooled estimates of sensitivity, specificity, and diagnostic odds ratios, and studied the extent of interstudy heterogeneity. We assessed the influence of key study and subject characteristics on the variation in the pooled sensitivity and specificity in subgroup meta-analyses.

Results: Meta-analysis yielded a pooled sensitivity of 68% (95% CI: 64, 71) and specificity of 81% (95% CI: 77, 83) at the ≥CIN1 diagnostic threshold and a pooled sensitivity of 40% (95% CI: 33, 47) with specificity of 96% (95% CI: 94, 97) at the ≥CIN 2 threshold. The pooled estimates of the diagnostic odds ratio were 12.7 (5.2-30.4) for

cytology to predict disease at the ≥CIN1 threshold and 10.6 (5.0-22.5) at the ≥CIN2 threshold.

Conclusion: Compared to similar meta-analyses among the HIV-uninfected women, pooled sensitivity of cytology (for ≥CIN2) is lower for HIV-infected women, thereby. The discriminatory capacity was superior at the ≥CIN1 threshold, as expected. Given the complexity of cytology as a screening tool and the high risk for HIV-infected women in resource-limited settings, the evaluation, use and implementation of alternative screening strategies is needed.

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INTRODUCTION

Cervical cancer screening has relied on the microscopic detection of cellular changes by directly examining a sample of desquamative cells taken from the cervix. This sampling procedure was first described by George Papanicolaou in 1943. (1) The 'Pap smear' has been the mainstay of cervical cancer screening programs in modern health systems, resulting in a plummeting of cervical cancer rates in industrialized nations over the past six decades. (2-3) The Pap smear is one of the few interventions to receive an "A" rating as a prevention tool from the U.S. Preventive Services Task Force, even though there are no randomized trials demonstrating its effectiveness. (2) HIV-infected women represent one of the highest risk populations for the development, progression, persistence, and recurrence of HPV-induced cervical neoplasia. (4-7) Evaluations of reliable, affordable,

and practical means of screening for cervical cancer precursors in immunosuppressed women are necessary to develop and implement better preventive guidelines. There has been mixed evidence as to the accuracy of cervical cytology among HIV-infected populations. Although some authors have suggested unusually high rates of false negative results (and a correspondingly poor sensitivity), (9,10,11) many other studies have failed to substantiate this finding. (12,13). However, many studies have involved small numbers of cases and controls and lack adequate statistical power.

To evaluate the accuracy of cytology as a stand-alone test we conducted a systematic review of published estimates of the accuracy of cytology for screening HIV-infected women. We used meta-analytic techniques to derive pooled estimates of sensitivity and specificity for cytology among HIV-infected women.

METHODS

We used meta-analytic tools to extract from the literature all available data concerning diagnostic accuracy parameters (i.e., clinical sensitivity and clinical specificity), studied variation in a systematic way, and obtained summary measures.

Research Questions: We addressed the following questions using meta-analytic techniques: 1) What is the accuracy of cervical cytology testing to detect colposcopic-histologically confirmed cervical intraepithelial neoplasia (CIN) among HIV-infected women (at thresholds of $\geq$CIN1, and $\geq$CIN2)? 2) What is the influence of key study and subject characteristics on the pooled estimates of accuracy in these high-risk women?

Target population: The primary target population was HIV-infected women regardless of their place of residence. Women were screened by cytology as a primary screening

procedure. All women had also undergone a reference investigation by colposcopy, with or without histological confirmation.

Data Sources: The PubMed/Medline database of the US National Library of Medicine was the primary source of data. PubMed is linked to the Cancer Lit and AIDSLINE databases. The database was searched through December 2005 (as of January 31, 2006) by using an electronic search strategy described below. A manual search of newly published relevant journals, bibliographies of relevant studies, and recent systematic reviews was also performed. To locate unpublished studies, we contacted three researchers and clinicians working in the field.

Retrieval Strategy: A targeted systematic search strategy using population and intervention terms was developed. Primary elements included: HIV/AIDS and Cervical Cancer, while secondary elements included Screening tests, Type of study designs, Human studies and studies in females. MeSH (Medical Subject Headings) and title key words were combined in an algorithm that maximized the yield of the search strategy (Appendix 1).

Inclusion criteria: The inclusion criteria for studies were:

i. HIV-infected women or adolescents
ii. Primary screening for cervical cancer using cytology or repeat screening/referral evaluation to confirm a prior screening test of concern
iii. Reference investigation done on all women by colposcopy and/or some form of histological confirmation (through biopsy, endocervical curettage, loop electrosurgical excision procedure (LEEP), cervical conization or hysterectomy)

iv. Availability of the numbers of true positives, false positives, true negatives, and false negatives detected by the screening test relative to the colposcopy/histological standard from the study population.

Thresholds for cytology (screening test positivity): We considered two thresholds for cytology: low and high grade squamous intraepithelial lesions (SIL) (≥LSIL and ≥HSIL respectively), that theoretically correspond to the histological thresholds of ≥CIN 1 and ≥CIN 2 respectively. The 1991 version of the Bethesda Reporting System was used for cytological classification. (14) If not reported as a separate category, Atypical Squamous Cells of Undetermined Significance (ASCUS) results were considered together with LSIL for dichotomization purposes. Although this may be judged controversial among HIV-uninfected women in light of the ASCUS Low-grade SIL Triage Study (ALTS), (15), recent well-controlled studies have confirmed the high-rates of progression of ASCUS to LSIL among HIV-infected women (16,17) recommending colposcopic/histological referral and aggressive follow-up for HIV-infected women with ASCUS results on cytology

Disease outcome: The colposcopic-histological result was used as the gold standard. We assumed that histological examination provided complete ascertainment of the considered disease status; whenever histological confirmation was unavailable, the colposcopic diagnosis was deemed definitive. Throughout our systematic review, we have used the CIN nomenclature for colposcopic-histological outcomes.

Covariate Information: Key study properties were summarized in comprehensive tables (Table 1 and 2). These included: characteristics of the study population (place of recruitment, study population being screened, exclusion criteria, study size, and age

distribution), collection devices for cytology sample (Ayre's spatula, cotton swab, or endocervical cytobrush), reports of quality control/verification of histological outcome, whether histologic interpreters were blinded to screening and/or colposcopy results, and concurrent evaluation of HIV-negative or other controls.

Definition of Accuracy Measures: The numbers of true-positives, false-negatives, false-positives, and true-negatives defined at the considered thresholds were extracted from each study, and sensitivity and specificity were calculated. Sensitivity or true positivity rate (TPR) referred to the number of diseased cases that had a positive test result (TPR=true-positives/ [true-positives plus false-negatives]) while Specificity or true negativity rate (TNR) referred to the number of non-diseased cases that had a negative test result (TNR= true-negatives/ [true-negatives plus false-positives]). We also calculated the Diagnostic Odds Ratio (DOR) since it effectively combines sensitivity and specificity estimates into a single measure, thereby allowing comparison of paired indicators of cytology with other tests. This measure is especially useful in cases where either of the sensitivity or specificity is outperformed by those of the other test being compared. Thus, using DOR has been recommended in systematic reviews and meta-analyses. (18). The DOR can be interpreted as the ratio of the odds of disease among test positives relative to the odds of disease among test negatives, or alternatively, as the ratio of the odds of positivity in the diseased relative to the odds of positivity in the non-diseased. Thus, DOR = [(sensitivity/1-sensitivity)] / [(1-specificity)/specificity].

Statistical Analysis: We accounted for the variability in the diagnostic threshold by pooling sensitivity and specificity estimates at each CIN threshold separately. The sensitivity and specificity were pooled using notations and formulae preferred for meta-

analyses of diagnostic tests (Appendix 2). (19) The variation in accuracy measures in the individual studies and in the pooled measures were displayed graphically using forest plots. We calculated the Mantel Haenszel Chi-Square estimate of the Cochran's Q-statistic of homogeneity to assess the heterogeneity between studies. Random-effects models (DerSimonian Laird method) were used for pooling the diagnostic odds ratios at both reference thresholds (20). We plotted the summary receiver operating characteristics (SROC) curve (sensitivity against 1-specificity) for both thresholds and calculated the areas under the curve (AUC) as a summary measure of accuracy of cytology at both thresholds. We also used the Inconsistency index (I^2) to quantify the heterogeneity in the studies. The I^2 index is the percentage of total variation across studies explained due to heterogeneity rather than chance. (21) Meta-analyses were performed using Meta-DiSc for Windows™ v 1.2 , a Cochrane-collaboration recommended software package for the Meta-analysis of Diagnostic Tests. (22) Subgroup meta-analysis was carried out to assess the influence of study and subject characteristics on the outcome. (23)

RESULTS

Study Characteristics: Our sensitive PubMed search strategy retrieved 1697 potential articles for inclusion. We excluded ≈1100 articles that were not relevant. Through manual review of the remaining articles, we retrieved the articles that met our inclusion criteria i.e., only those where individual numbers of true positives, false positives, false negatives and true negatives were reported as raw data (either in the text or the figures/tables) were included in the meta-analysis. Nineteen studies met our inclusion criteria. (9-13, 24-37) After disregarding studies for some analyses that did not permit null values for any of the

parameters of interest at any threshold the accuracy of cytology at the ≥CIN2 (Colposcopic-histology) threshold was calculated for 12 studies (10-13, 24,28,29,31,33,35-37), and at the ≥CIN1 threshold for 13 studies (10-13, 25, 29-31, 33-37) (Tables 1-2).

Study Size: In the 19 studies, 1823 women underwent cytological screening and simultaneous reference investigations. Nine studies (9, 24-30, 32, 35) contributed <50 women each, six studies contributed between 50-200 women (10, 30, 31, 33, 34, 36), and the four largest studies each contributed >200 women. (11-13, 37)

Clinical setting, patient and study characteristics: The studies varied in their recruitment criteria from HIV-infected women screened either as a primary screening or those referred to colposcopy clinics from gynecologists/providers due to a suspicious Pap smear result. Only four of the 19 studies reported excluding women with a history of CIN, cervical surgery, biopsy, or other treatment for cervical pathology. (10,28,36,37) Other exclusion criteria included refusal to undergo colposcopy (26). Two studies reported including pregnant women, (29,32) one study reporting excluding pregnant women, (30), and the rest did not report on the women's pregnancy status. Women were concurrently recruited from other clinical studies/trials related to HIV/HPV in three studies. (26,35,37) All 19 studies were done in western industrialized countries viz., USA, Italy, Germany, and the United Kingdom, although women accessing these services ranged considerably from women seeking gynecologic care on their own (24,31,13), women being treated for drug dependence (12, 26), STD patients (10,25) and women being treated for HIV (9,-12, 36-37). Twelve studies reported evaluating HIV-negative women concurrently, (10-13, 26-32, 34, 35) while seven studies were designed

for evaluating HIV-infected women exclusively. (9,24,25, 32,33,36,37). In six studies, it was explicitly stated that the histological interpretation was blinded to the triage test results. (12,11,13,35-37) In seven studies, the histopathologic diagnosis was subjected to quality verification/review by another histopathologist/s. (9,12,32,11,35-37). The types of collection devices for collecting cytology samples included Ayre's spatula (10 studies), (10, 13, 25-28, 30, 31, 34, 35), cervical cotton swab (3 studies), (26, 10, 28), Dacron swab (2 studies), (33,36) and endocervical cytobrush in 8 studies.(27,30,31,13,33-36) Seven studies did not report the type of collection device used. (9, 11, 12, 24,29, 32, 37)

Meta-Analysis: On plotting the sensitivities and specificities on the forest plots (Figs.1 and 2), and computing the Cochran's Q-statistic, it was noted that the interstudy variation in sensitivity and specificity was large at both thresholds ($p < 0.05$). Meta-analysis yielded a pooled sensitivity of 68% (95% CI: 64, 71) and a specificity of 81% (95% CI: 77, 83) at the ≥CIN1 diagnostic threshold from the 13 included studies. (Table 3) At the ≥CIN2 threshold, the 12 included studies results in a pooled sensitivity of 40% (95% CI: 33, 47) and a pooled specificity of 96% (95% CI = 94, 97) at the >CIN 2 threshold. (Table 4) The pooled estimates of the DOR were 12.7 (5.2-30.4) at the ≥CIN1 threshold and 10.57 (4.97-22.5) at the ≥CIN2 threshold. (Tables 3,4). The Areas under the SROC curve (AUC) were 0.85 (SE: ± 0.03) at the ≥CIN1 threshold and 0.83 (SE: ±0.04) at the ≥CIN2 threshold. (Figures 3, 4) The I^2 measures of inconsistency were 62.3% for the sensitivity and 83.2% for the specificity at the ≥CIN 1 threshold and 93.7% for the sensitivity and 93.9% for the specificity at the ≥CIN2 threshold. (Figures 1,2)

Sub-group Meta Analysis: We determined the change in pooled sensitivity and specificity over technical and design variables for cytology at both thresholds. (Table 5, 6) The following variables were assessed:

i. *Prevalence of disease:* The prevalence of disease ranged from 12% to 85% at the ≥CIN1 threshold, and 8% to 50% at the ≥CIN 2 threshold. We stratified the prevalence of ≥CIN 1 as ≥ 50% and < 50%. We found that both the pooled sensitivity and specificity were much higher (64% versus 77%, and 66% versus 86% respectively) for studies where the prevalence of ≥CIN1 disease less than 50%. At the ≥CIN2 threshold, we stratified the prevalence at the 25% level (into ≥25% and <25%), and found that although the pooled specificity was higher (97% versus 72%) for studies with prevalence of ≥CIN2 disease less than 25%, the pooled sensitivity was conversely lower (36% versus 59%).

ii. *Sampling device* (Ayre's Spatula, Cotton/Dacron swab, Endocervical brush, not reported): Among the methods reported for collection of cytological samples, the ones using cervical cytobrush reported the highest pooled sensitivity (72% and 49% at the ≥CIN1 and ≥CIN 2 thresholds). The studies reporting use of a cytobrush also reported the highest specificity (97%) compared to other collection methods at ≥CIN 2 threshold, whereas the studies reporting the use of Ayre's spatula had the highest pooled specificity (89%) at the ≥CIN 1 threshold. Studies that reported use of cotton or a Dacron swab had lower estimate of pooled sensitivity (56%) and specificity (66%) at the ≥CIN 1 threshold than estimates of studies using other collection devices.

iii. *Quality review of histological outcome:* Studies that reported having some form of quality control/verification for histological diagnosis had lower estimates of sensitivity but higher specificity (at both thresholds) than studies not reporting such quality review.

iv. *Blinding of outcome verification:* Studies that reported the blinding of histologists to cytological test results and other clinical characteristics report having a lower sensitivity but higher specificity, and a larger DOR at both reference thresholds.

v. *Concurrent evaluation of HIV-negative controls:* Studies that concurrently evaluated and reported HIV-negative controls reported contrasting results at the two thresholds. At the ≥CIN1 threshold, these studies had a lower sensitivity (63% vs. 79%), higher specificity (91% vs. 56%) and high DOR (16.8 vs. 5.0), whereas at the >CIN2 threshold, these studies reported a higher sensitivity (42% vs. 36%), lower specificity (95% vs. 96%) and lower DOR (9.1 vs. 15.0) compared to studies that did not have (or did not report) having HIV-uninfected controls for recruitment or pathological analysis.

DISCUSSION

In this systematic review, we evaluated the accuracy of cytology used for screening HIV-infected women, focusing on the internal test validity parameters of sensitivity and specificity because of their more universal properties. Similar meta-analyses among HIV-uninfected women have revealed the pooled estimates (at ≥CIN2 threshold) of the sensitivity of cytology between 47-62% (higher than our 40% estimate) and the specificity between 60-95% (lower than our 96% estimate). (38-40). Cytology is

therefore a relative insensitive test for detection of ≥CIN2 disease in HIV-infected women compared to HIV-uninfected women. The higher specificity is expected in the face of the comparatively low sensitivity.

There was a wide range of sensitivities and specificities for cytology at both CIN thresholds. This was expected because the reproducibility of the cytological interpretation of Pap smears is often reported as moderate to poor. (41,42) This low reproducibility of cytology is especially pronounced at a higher threshold (≥CIN 2) as is observed in the high variability in accuracy observed in the forest plots, and the higher I^2 (inconsistency) statistic for the ≥CIN 2 threshold. (Fig. 1a, 1b) In fact, the overall discriminatory power of cytology was lower at the ≥CIN2 than at the ≥CIN1 thresholds, as is reflected by the lower DOR (10.6 vs. 12.7 respectively) as well as slightly lower AUC value (0.83 vs. 0.85 respectively).

The effect of prevalence of disease on the pooled estimates showed that cytology has a better discriminatory capacity at disease prevalence < 50% at the ≥CIN1 threshold and when the prevalence was < 25% at the ≥CIN2 threshold. The reported prevalence of lesions had significant variability, due to differing study designs (clinical series vs. more formal epidemiological studies) or due to biases in study recruitment (e.g., recruiting women with known cytological abnormalities). The sensitivity (detection capacity) was higher when the test results were not blinded/partially blinded to the diagnostician, presumably because a histopathologist would look harder for a lesion in women with known abnormal cytology. Studies using cervical cytobrush had higher estimates of pooled sensitivity and specificity, as well as a better discriminatory value (DOR) than other studies using other devices for sample collection, confirming results in other studies

in HIV-uninfected women (43-45). A limitation of this analysis has been the inability to assess the impact of age or immunosuppressive state (CD4+ cell counts/HIV viral load) of the women being screened on the extent of study heterogeneity, due to limited number of studies that report these variables.

Almost all reported studies of cervical cancer screening tests suffer from verification bias that arises from incomplete or partial assessment of the screened subjects by a "gold standard" reference diagnosis. (46) Although colposcopy is often an imperfect reference standard, it has been the preferred method for diagnosing and basing treatment decisions for HIV-infected women. Women who are positive on colposcopy are usually referred to invasive procedures (biopsy, loop procedure, cervical conization, or endocervical curettage) for histopathological diagnosis and/or treatment. We based our outcome on a composite colposcopic-histological reference standard that is the standard of care for making diagnostic and treatment decisions in gynecologic oncology. 13 out of the 19 studies in this analysis reported complete verification by histopathology (9-11, 24, 27, 28, 30, 31, 34-37) and six other studies (12,13,24, 26, 32, 33) had greater than 50% of subjects verified by histopathology after positive colposcopy lesions, thereby minimizing this bias in our reported estimates.

Our meta-analysis was restricted to cross-sectional outcomes in which sensitivity and specificity were measured against a simultaneously applied gold standard. Only longitudinal studies estimate the longitudinal sensitivity and specificity of the tests, especially for detecting missed lesions and excluding negative lesions by repeat cytology before invasive cancer occurs. This may be even more pertinent for HIV-infected women since they need to be screened at a greater frequency. Nevertheless, since diagnostic or

treatment decisions in management of cervical disease often rely on single reports of cytology (due to the need to intervene whenever CIN is detected), it is important to develop methods of cytology that enhance its accuracy.

None of our included studies used liquid-based cytology and other newer and more accurate cytological methods (including automated cytology and use of computer algorithms to detect abnormal cells), (47-48) However; the use of these methods in HIV-infected women has mixed estimates regarding their efficiency. (49, 50) The evidence for the value of the use of HPV testing in HIV-infected women is also inconsistent. (33, 51-53) Cervicography has proven disappointing to date. (54) Use of visual inspection with acetic acid is being assessed in Zambia and India, (55, chapter 2 of this thesis) There is a need for well designed and controlled studies, including randomized prevention clinical trials, to evaluate screening protocols for screening methods for HIV-infected women.

All studies included in our analysis are reports from industrialized settings in the US and Europe, but they have significant bearing on the development of research and policy for screening HIV-infected women in resource-limited settings. Given the need to reduce false negative findings from a single visit, cytology is best when offered repetitively or combined with other alternative methods of screening. Cytology, as a stand-alone test, may not represent an affordable, sustainable solution for screening HIV-infected women in resource-limited settings not only due to the operational difficulties but also due to its suboptimal test characteristics.

REFERENCES

1. G. N. Papanicolaou and Herbert Traut: Diagnosis of Uterine Cancer by the Vaginal Smear. New York, 1943. (Monograph).
2. Sherman ME. Future Directions in Cervical Pathology J Natl Cancer Inst Monographs 2003 (31): 72-79
3. U.S. Preventive Services Task Force. *Screening for Cervical Cancer: Recommendations and Rationale.* AHRQ Publication No. 03-515A. January 2003. Agency for Healthcare Research and Quality, Rockville, MD. http://www.ahrq.gov/clinic/3rduspstf/cervcan/cervcanrr.htm
4. The Bethesda system for reporting cervical/vaginal cytologic diagnoses: revised after the second National Cancer Institute Workshop, April 29-30, 1991. Acta Cytol. 1993;37:115-24.
5. Sun XW, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright TC Jr. Human papillomavirus infection in women infected with the human immunodeficiency virus. N Engl J Med 1997; 337: 1343-9.
6. Vermund SH, Kelley KF, Klein RS, Feingold AR, Schreiber K, Munk G, Burk RD. High risk of human papillomavirus infection and cervical squamous intraepithelial lesions among women with symptomatic human immunodeficiency virus infection. Am J Obstet Gynecol. 1991;165(2):392-400
7. Tate DR, Anderson RJ. Recrudescence of cervical dysplasia among women who are infected with the human immunodeficiency virus: a case-control analysis. Am J Obstet Gynecol 2002; 186: 880-2.

8. Mandelblatt JS, Kanetsky P, Eggert L, Gold K. Is HIV infection a cofactor for cervical squamous cell neoplasia? Cancer Epidemiol Biomarkers Prev. 1999;8(1):97-106.

9. Maiman M, Tarricone N, Vieira J, Suarez J, Serur E, Boyce JG. Colposcopic evaluation of human immunodeficiency virus-seropositive women. Obstet Gynecol. 1991;78(1):84-8.

10. Fink MJ, Fruchter RG, Maiman M, Kelly P, Sedlis A, Webber CA, Chen P. The adequacy of cytology and colposcopy in diagnosing cervical neoplasia in HIV-seropositive women. Gynecol Oncol. 1994;55(1):133-7.

11. Maiman M, Fruchter RG, Sedlis A, Feldman J, Chen P, Burk RD, Minkoff H. Prevalence, risk factors, and accuracy of cytologic screening for cervical intraepithelial neoplasia in women with the human immunodeficiency virus. Gynecol Oncol. 1998;68(3):233-9.

12. Wright TC Jr, Ellerbrock TV, Chiasson MA, Van Devanter N, Sun XW. Cervical intraepithelial neoplasia in women infected with human immunodeficiency virus: prevalence, risk factors, and validity of Papanicolaou smears. New York Cervical Disease Study. Obstet Gynecol. 1994;84(4):591-7.

13. Spinillo A, Capuzzo E, Tenti P, De Santolo A, Piazzi G, Iasci A. Adequacy of screening cervical cytology among human immunodeficiency virus-seropositive women. Gynecol Oncol. 1998;69(2):109-13.

14. Luff RD. The Bethesda System for reporting cervical/vaginal cytologic diagnoses: report of the 1991 Bethesda workshop. The Bethesda System Editorial Committee. Hum Pathol 1992;23:719–21

15. Schiffman M, Solomon D. Findings to date from the ASCUS-LSIL Triage Study (ALTS). Arch Pathol Lab Med. 2003;127(8):946-9.

16. Duerr A, Paramsothy P, Jamieson DJ, Heilig CM, Klein RS, Cu-Uvin S, Schuman P, Anderson JR; HIV Epidemiology Research Study. Effect of HIV infection on atypical squamous cells of undetermined significance. Clin Infect Dis. 2006;42(6):855-61.

17. Holcomb K, Abulafia O, Matthews RP, et al. The significance of ASCUS in HIV-positive women. Gynecol Oncol 1999; 75:118–21

18. Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiol. 2003;56(11):1129-35.

19. Devillé WL, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, Bezemer PD. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodo*l 2002; **2**:9.

20. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. Stat Med 2002; 21:1525-1537.

21. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327:557-560.

22. Zamora J, Muriel A, Abraira V. Meta-DiSc for Windows: A Software package for the Meta-analysis of Diagnostic Tests. XI Cochrane Colloquium. Barcelona, 2003. (Available at http://www.hrc.es/investigacion/metadisc_en.htm)

23. Deeks JJ. Systematic reviews of evaluations of diagnostic and screening tests. In Egger M, Smith GD, Altman DG (eds). Systematic Reviews in Health Care. Meta-analysis in context. London: BMJ Books; 2001:248-282.

24. Bradbeer C. Is infection with HIV a risk factor for cervical intraepithelial neoplasia? Lancet. 1987;2(8570):1277-8. 3

25. Byrne MA, Taylor-Robinson D, Munday PE, Harris JR. The common occurrence of human papillomavirus infection and intraepithelial neoplasia in women infected by HIV. AIDS. 1989;3(6):379-82.

26. Adachi A, Fleming I, Burk RD, Ho GY, Klein RS. Women with human immunodeficiency virus infection and abnormal Papanicolaou smears: a prospective study of colposcopy and clinical outcome. Obstet Gynecol. 1993;81(3):372-7.

27. Boardman LA, Peipert JF, Cooper AS, Cu-Uvin S, Flanigan T, Raphael SI. Cytologic-histologic discrepancy in human immunodeficiency virus-positive women referred to a colposcopy clinic. Obstet Gynecol. 1994;84(6):1016-20.

28. Fruchter RG, Maiman M, Sillman FH, Camilien L, Webber CA, Kim DS. Characteristics of cervical intraepithelial neoplasia in women infected with the human immunodeficiency virus. Am J Obstet Gynecol. 1994;171(2):531-7.

29. Johnstone FD, McGoogan E, Smart GE, Brettle RP, Prescott RJ. A population-based, controlled study of the relation between HIV infection and cervical neoplasia. Br J Obstet Gynaecol. 1994 Nov;101(11):986-91. Erratum in: Br J Obstet Gynaecol 1994; 101(12):1035.

30. Korn AP, Autry M, DeRemer PA, Tan W. Sensitivity of the Papanicolaou smear in human immunodeficiency virus-infected women. Obstet Gynecol. 1994;83(3):401-4.

31. Del Priore G, Maag T, Bhattacharya M, Garcia PM, Till M, Lurain JR. The value of cervical cytology in HIV-infected women. Gynecol Oncol. 1995;56(3):395-8.

32. Robinson WR, Barnes SE, Adams S, Perrin MS. Histology/cytology discrepancies in HIV-infected obstetric patients with normal pap smears. Gynecol Oncol. 1997;65(3):430-3. 8

33. Petry KU, Bohmer G, Iftner T, Flemming P, Stoll M, Schmidt RE. Human papillomavirus testing in primary screening for cervical cancer of human immunodeficiency virus-infected women, 1990-1998. Gynecol Oncol. 1999;75(3):427-31.

34. Torrisi A, Del Mistro A, Onnis GL, Merlin F, Bertorelle R, Minucci D. Colposcopy, cytology and HPV-DNA testing in HIV-positive and HIV-negative women. Eur J Gynaecol Oncol. 2000;21(2):168-72.

35. Branca M, Rossi E, Alderisio M, Migliore G, Morosini PL, Vecchione A, Sopracordevole F, Mudu P, Leoncini L, Syrjanen K. Performance of cytology and colposcopy in diagnosis of cervical intraepithelial neoplasia (CIN) in HIV-positive and HIV-negative women. Cytopathology. 2001;12(2):84-93.

36. Cohn JA, Gagnon S, Spence MR, Harrison DD, Kluzak TR, Langenberg P, Brinson C, Stein A, Hellinger J; Cervical Disease Study Group of the American Foundation for AIDS Research Community Based Clinical Trials Network. The role of human papillomavirus deoxyribonucleic acid assay and repeated cervical cytologic examination in the detection of cervical intraepithelial neoplasia among human immunodeficiency virus-infected women. Cervical Disease Study Group of the American Foundation for AIDS Research Community Based Clinical Trials Network. Am J Obstet Gynecol. 2001;184(3):322-30.

37. Robinson WR, Luck MB, Kendall MA, Darragh TM. The predictive value of cytologic testing in women with the human immunodeficiency virus who have low-grade squamous cervical lesions: a substudy of a randomized, phase III chemoprevention trial. Am J Obstet Gynecol. 2003;188(4):896-900.

38. Fahey MT, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. Am J Epidemiol. 1995;141(7):680-9.

39. Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, Matchar DB. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med. 2000;132(10):810-9.

40. Mitchell MF, Schottenfeld D, Tortolero-Luna G, Cantor SB, Richards-Kortum R. Colposcopy for the diagnosis of squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol. 1998;91(4):626-31.

41. O'Sullivan JP, Ismail SM, Barnes WS, Deery AR, Gradwell E, Harvey JA, et al. Interobserver variation in the diagnosis and grading of dyskaryosis in cervical smears: specialist cytopathologists compared with non-specialists. J Clin Pathol 1994;47:515–8.

42. Sherman ME, Schiffman MH, Lorincz AT, Manos MM, Scott DR, Kuman RJ, et al. Toward objective quality assurance in cervical cytopathology. Correlation of cytopathologic diagnoses with detection of high-risk human papillomavirus types. Am J Clin Pathol 1994;102:182–7.

43. Goldberg GL, Vermund SH, Schiffman MH, Ritter DB, Spitzer C, Burk RD. Comparison of Cytobrush and cervicovaginal lavage sampling methods for the

detection of genital human papillomavirus. Am J Obstet Gynecol. 1989;161(6 Pt 1):1669-72.

44. Martin-Hirsch P, Lilford R, Jarvis G, Kitchener HC. Efficacy of cervical-smear collection devices: a systematic review and meta-analysis. Lancet. 1999;354(9192):1763-70.

45. Bauman BJ. Use of a cervical brush for Papanicolaou smear collection. A meta-analysis. J Nurse Midwifery. 1993;38(5):267-75.

46. Zhou XH. Correcting for verification bias in studies of a diagnostic test's accuracy. Stat Methods Med Res. 1998;7(4):337-53.

47. Karnon J, Peters J, Platt J, Chilcott J, McGoogan E, Brewer N. Liquid-based cytology in cervical screening: an updated rapid and systematic review and economic analysis. Health Technol Assess. 2004;8(20):iii, 1-78.

48. Mattosinho de Castro Ferraz Mda G, Nicolau SM, Stavale JN, Focchi J, Castelo A, Dores GB, Mielzynska-Lohnas I, Lorincz A, Rodrigues de Lima G. Cervical biopsy-based comparison of a new liquid-based thin-layer preparation with conventional Pap smears. Diagn Cytopathol. 2004;30(4):220-6.

49. Bernstein SJ, Sanchez-Ramos L, Ndubisi B. Liquid-based cervical cytologic smear study and conventional Papanicolaou smears: a metaanalysis of prospective studies comparing cytologic diagnosis and sample adequacy. Am J Obstet Gynecol. 2001;185(2):308-17.

50. Taylor S, Kuhn L, Dupree W, Denny L, De Souza M, Wright TC Jr. Direct comparison of liquid-based and conventional cytology in a South African screening trial. Int J Cancer. 2006;118(4):957-62.

51. Massad LS, Schneider MF, Watts DH, Strickler HD, Melnick S, Palefsky J, Anastos K, Levine AM, Minkoff H. HPV testing for triage of HIV-infected women with papanicolaou smears read as atypical squamous cells of uncertain significance. J Womens Health (Larchmt). 2004;13(2):147-53.

52. Goldie SJ, Freedberg KA, Weinstein MC, Wright TC, Kuntz KM. Cost effectiveness of human papillomavirus testing to augment cervical cancer screening in women infected with the human immunodeficiency virus. Am J Med. 2001;111(2):140-9.

53. Goldie SJ, Weinstein MC, Kuntz KM, Freedberg KA. The costs, clinical benefits, and cost-effectiveness of screening for cervical cancer in HIV-infected women. Ann Intern Med. 1999;130(2):97-107.

54. Moscicki AB, Durako SJ, Ma Y, Darragh T, Vermund SH. Utility of cervicography in HIV-infected and uninfected adolescents. J Adolesc Health. 2003;32(3):204-13.

55. Parham G, Sahasrabuddhe VV, Mwanahamuntu M, Stringer E, Hicks M, Vermund S. Cervical Cancer Screening in HIV-infected women in Zambia. Poster presentation. 9th International Conference on Malignancies in AIDS and Other Immunodeficiencies (ICMAOI). National Cancer Institute, Bethesda, MD, Sept 26-27, 2005.

Table 1: Characteristics of included studies

ID	Author/ reference, year	Country/ Location	Study population and inclusion criteria	Exclusion criteria	Study size	Age distribution
1	Bradbeer 1987	United Kingdom/ London	Private colposcopy practice	NR	11 HIV+ , 9 with results	Range 23-36
2	Bryne et al. 1989	United Kingdom / London	STI clinic population in tertiary hospital, recalled for gynecology exam after HIV diagnosis	NR	19 HIV+, 8 with results	Range 19-48, 26 mean
3	Maiman et al. 1991	United States/ New York	HIV treatment setting	NR	32 HIV+	NR
4	Adachi et al. 1993	United States/ New York	Women enrolled in a prospective study of HIV, HPV and cervicovaginal abnormalities	Refusal to undergo colposcopy	38 HIV+	NR

Table 1 (contd.)

ID	Author/ reference, year	Country/ Location	Study population and inclusion criteria	Exclusion criteria	Study size	Age distribution
5	Boardman et al. 1994	United States/ Rhode Island	Women attending colposcopy clinic	NR	678 total, 41 HIV+	NR
6	Fink et al. 1994	United States/ New York	Urban county hospital ambulatory clinic for HIV care	Previous Treatment for cervical pathology	51 HIV+	Range 21-46 years
7	Fruchter et al. 1994	United States/ New York	Colposcopy service clinic in public hospital, abnormal pap smears	Prior treatment, HPV/ASCUS on pap smears	482 total, 41 HIV+	Range 20-50 years
8	Johnstone et al. 1994	United Kingdom/ Edinburgh	Population based, case-control study design: HIV+ pregnant women, Injection drug users, and neighborhood controls	NR	278 total, 98 HIV+	24.1 mean, SD +/- 4.6

Table 1 (contd.)

ID	Author/ reference, year	Country/ Location	Study population and inclusion criteria	Exclusion criteria	Study size	Age distribution
9	Korn et al. 1994	United States/ San Francisco	HIV-infected women attending gynecology clinics	Pregnancy	137 total, 52 HIV+	32.6 mean, SD +/- 6.8
10	Wright et al. 1994	United States/ New York	HIV/AIDS, methadone and STI clinics	NR	755 total, 398 HIV+,	>= 18 years
11	Del Priore et al. 1995	United States/ Illinois	Colposcopy practice of single consultant	NR	81 total, 24 HIV+	Mean 32.6 SD +/- 7
12	Robinson et al. 1997	United States/ Louisiana	Newly diagnosed HIV+ prenatal clinics	Previously diagnosed with HIV	32 HIV+	Range 16-37, mean 26
13	Maiman et al. 1998	United States/ New York	Patient care program clinics	NR	248 HIV+	NR
14	Spinillo et al. 1998	Italy/ Pavia	Self-referred/clinician referred patients in vaginitis clinic	NR	1193 total, 202 HIV+	Range 18-40 years

Table 1 (contd.)

ID	Author/ reference, year	Country/ Location	Study population and inclusion criteria	Exclusion criteria	Study size	Age distribution
15	Petry et al. 1999	Germany / Hannover	Patients consulted at clinical immunology division	NR	138 HIV+	Range 19-61 , 32.5 mean
16	Torrisi et al. 2000	Italy/Padua	HIV+ women attending Ob-Gyn clinics, along with other HIV-	NR	332 total, 104 HIV+	Range 24-76, 35 median
17	Branca et al. 2001	Italy/ DIANAIDS study: multiple sites	Patients enrolled in clinical trial for surveillance of HPV in HIV+ women	NR	58 total, 37 HIV+	Range 17-45 years
18	Cohn et al. 2001	United States/ 6 cities	Receiving primary care in clinics	Cervical cancer, prior treatment, current bleeding diathesis, pregnant	103 HIV+	>= 18 years

Table 1 (contd.)

ID	Author/ reference, year	Country/ Location	Study population and inclusion criteria	Exclusion criteria	Study size	Age distribution
19	Robinson et al. 2003	United States/ substudy of ACTG#293/ multiple sites	Enrolled in ACTG / AIDS Clinical trials	Large lesions, previous excisional / ablative therapy for cervical dysplasia,	246 HIV+	NR

Note: *NR = Not reported, HIV= Human Immunodeficiency virus, AIDS= Acquired Immunodeficiency Syndrome, HPV= Human Papillomavirus, STI: Sexually Transmitted Infections, ACTG: Adult AIDS Clinical Trials Group*

Table 2: Characteristics of study and subject characteristics in the included studies

ID	Author/reference, year	Prev. ≥ CIN1	Prev. ≥ CIN2	Collection device for Pap smear	QC of histo.	Blinding of tests	Conc. HIV-ve controls
1	Bradbeer 1987	0.78	0.33	NR	NR	NR	No
2	Bryne et al. 1989	0.75	0.50	Spatula (cervical scrape)	NR	NR	No
3	Maiman et al. 1991	0.41	0.00	NR	Yes	No	No
4	Adachi et al. 1993	0.64	0.28	Ayre's spatula, cotton swab	NR	NR	Yes
5	Boardman et al. 1994	0.74	0.00	Ayre's spatula, endocervical brush	NR	NR	Yes
6	Fink et al. 1994	0.76	0.08	Ayre's spatula, cotton swab	No	NR	Yes
7	Fruchter et al. 1994	1.00	0.39	Ayre's spatula, cotton swab	No	No	Yes
8	Johnstone et al. 1994	0.63	0.45	NR	NR	NR	Yes
9	Korn et al. 1994	0.48	0.12	Ayre's spatula, endocervical brush	NR	NR	Yes

Table 2 (contd.)

ID	Author/reference, year	Prev. ≥ CIN1	Prev. ≥ CIN2	Collection device for Pap smear	QC of histo.	Blinding of tests	Conc. HIV-ve controls
10	Wright et al. 1994	0.32	0.06	NR	Yes	Yes	Yes
11	Del Priore et al. 1995	0.77	0.12	Ayre's spatula, endocervical brush		No	Yes
12	Robinson et al. 1997	0.31	0.09	NR	Yes	NR	No
13	Maiman et al. 1998	0.76	0.12	NR	Yes	Yes	Yes
14	Spinillo et al. 1998	0.32	0.18	Ayre's spatula, endocervical brush	No	Yes	Yes
15	Petry et al. 1999	0.12	0.12	Dacron swab, endocervical brush	NR	No	No
16	Torrisi et al. 2000	0.85	0.32	Ayre's spatula, endocervical brush	NR	NR	Yes
17	Branca et al. 2001	0.78	0.22	Ayre's spatula, endocervical brush	Yes	Yes	Yes
18	Cohn et al. 2001	0.54	0.12	Dacron swab, endocervical brush	Yes	Yes	No
19	Robinson et al. 2003	0.70	0.09	NR	Yes	Yes	No

Table 3: Reported true and false positive and negative results, and measures of accuracy (sensitivity, specificity and diagnostic odds ratio) at the ≥ CIN 1 threshold.

ID No	Author, Year	*N*	TP	FP	FN	TN	Sens	Spec	DOR
1**	Bradbeer 1987	9	5	2	2	0	0.71	0.00	--
2	Bryne et al. 1989	8	5	1	1	1	0.83	0.50	5.00
3**	Maiman et al. 1991	32	3	0	10	19	0.23	1.00	--
4**	Adachi et al. 1993	25	16	8	0	1	1.00	0.11	--
5**	Boardman et al. 1994	--	--	--	--	--	--	--	--
6	Fink et al. 1994	51	18	4	21	8	0.46	0.67	1.71
7**	Fruchter et al. 1994	41	41	0	0	0	1.00	--	--
8	Johnstone et al. 1994	40	24	3	1	12	0.96	0.80	96.00
9	Korn et al. 1994	73	22	6	13	32	0.63	0.84	9.03
10	Wright et al. 1994	392	101	15	23	253	0.81	0.94	74.07
11	Del Priore et al. 1995	52	23	1	17	11	0.58	0.92	14.88
12**	Robinson et al. 1997	32	0	0	10	22	0.00	1.00	--
13	Maiman et al. 1998	248	67	9	122	50	0.35	0.85	3.05
14	Spinillo et al. 1998	202	47	4	17	134	0.73	0.97	92.62
15	Petry et al. 1999	138	16	50	1	71	0.94	0.59	22.72

Table 3 (contd.)

ID No	Author, Year	*N*	TP	FP	FN	TN	Sens	Spec	DOR
16	Torrisi et al. 2000	53	43	5	2	3	0.96	0.38	12.90
17	Branca et al. 2001	37	26	2	3	6	0.90	0.75	26.00
18	Cohn et al. 2001	103	29	7	27	40	0.52	0.85	6.14
19	Robinson et al. 2003	246	147	49	24	26	0.86	0.35	3.25
SUMMARY ESTIMATES (13 studies included)		1708	604	155	281	668	0.68 (0.64-0.71)	0.81 (0.77-0.83)	12.67 (5.24-30.42)

Note: **: excluded studies

Table 4: Reported true and false positive and negative results, and measures of accuracy (sensitivity, specificity and diagnostic odds ratio) at the ≥ CIN 2 threshold.

ID No	Author, Year	*N*	TP	FP	FN	TN	Sens	Spec	DOR
1	Bradbeer 1987	9	1	1	2	5	0.33	0.83	2.50
2**	Bryne et al. 1989	8	4	0	0	4	1.00	1.00	--
3**	Maiman et al. 1991	32	0	0	0	32	--	1.00	--
4**	Adachi et al. 1993	25	6	0	1	18	0.86	1.00	--
5**	Boardman et al. 1994	41	0	0	0	41	--	1.00	--
6	Fink et al. 1994	51	1	2	3	45	0.25	0.96	7.50
7	Fruchter et al. 1994	41	7	10	9	15	0.44	0.60	1.17
8	Johnstone et al. 1994	40	14	4	4	18	0.78	0.82	15.75
9**	Korn et al. 1994	73	4	0	5	64	0.44	1.00	--
10	Wright et al. 1994	392	4	5	19	364	0.17	0.99	15.33
11	Del Priore et al. 1995	52	2	1	4	45	0.33	0.98	22.50
12**	Robinson et al. 1997	32	0	0	3	29	0.00	1.00	--
13	Maiman et al. 1998	248	9	11	20	208	0.31	0.95	8.51
14	Spinillo et al. 1998	202	18	4	19	161	0.49	0.98	38.13
15	Petry et al. 1999	138	11	3	6	118	0.65	0.98	72.11

Table 4 (contd.)

ID No	Author, Year	*N*	TP	FP	FN	TN	Sens	Spec	DOR
16**	Torrisi et al. 2000	53	11	0	6	36	0.65	1.00	--
17	Branca et al. 2001	37	4	6	4	23	0.50	0.79	3.83
18	Cohn et al. 2001	103	4	1	8	90	0.33	0.99	45.00
19	Robinson et al. 2003	246	4	11	19	212	0.17	0.95	4.06
SUMMARY ESTIMATES (12 studies included)		1823	104	59	132	1528	0.40 (0.33-0.47)	0.96 (0.94-0.97)	10.57 (4.97-22.50)

Note: **: excluded studies

Table 5: Variation in the accuracy parameters of cytology (≥CIN 1 threshold) by study characteristics

Covariate	Sensitivity (95% CI)	Specificity (95% CI)	DOR (95% CI)
True disease prevalence (≥ CIN1)			
>=50%	0.64 (0.60-0.67)	0.66 (0.60-0.72)	5.84 (3.07-11.11)
< 50%	0.77 (0.72-0.83)	0.86 (0.84-0.89)	37.1 (11.86-116.01)
Sampling device			
Ayre's Spatula	0.68 (0.62-0.73)	0.89 (0.84-0.92)	11.39 (4.24-30.56)
Cotton/Dacron Swab	0.56 (0.47-0.66)	0.66 (0.59-0.73)	5.31 (1.53-18.52)
Cytobrush	0.72 (0.66-0.77)	0.80 (0.75-0.84)	17.38 (7.23-41.76)
Not documented	0.66 (0.62-0.70)	0.82 (0.778-0.85)	14.25 (2.21-91.86)
QC of histological outcomes			
Yes	0.65 (0.61-0.69)	0.82 (0.78-0.86)	9.84 (2.37-40.86)
No / not reported	0.73 (0.67-0.78)	0.78 (0.74-0.83)	15.51 (5.04-47.70)
Blinding of test results			
Yes	0.66 (0.62-0.70)	0.85 (0.82-0.88)	14.28 (3.68-55.45)
No / Not reported	0.73 (0.66-0.79)	0.66 (0.59-0.73)	10.20 (3.92-26.52)
Concurrent HIV-negative controls			
Yes	0.63 (0.59-0.67)	0.91 (0.89-0.93)	16.76 (5.32-52.76)
No/ Not reported	0.79 (0.73-0.84)	0.56 (0.50-0.63)	4.99 (2.49-10.00)

Table 6: Variation in the accuracy parameters of cytology (≥CIN 2 threshold) by study characteristics

Covariate	Sensitivity (95% CI)	Specificity (95% CI)	DOR (95% CI)
True disease prevalence (≥ CIN2)			
>=25%	0.59 (0.42-0.75)	0.72 (0.58-0.83)	3.67 (0.58-24.57)
< 25%	0.36 (0.28-0.44)	0.97 (0.95-0.97)	14.41 (6.95-29.86)
Sampling device			
Ayre's Spatula	0.43 (0.32-0.55)	0.94 (0.91-0.96)	9.61 (2.31-10.11)
Cotton/Dacron Swab	0.46 (0.32-0.62)	0.94 (0.91-0.97)	12.49 (1.23-127.18)
Cytobrush	0.49 (0.37-0.60)	0.97 (0.95-0.98)	25.94 (8.85-76.01)
Not documented	0.33 (0.24-0.44)	0.96 (0.95-0.97)	8.37 (4.55-15.40)
QC of histological outcomes			
Yes	0.26 (0.18-0.36)	0.96 (0.95-0.97)	8.15 (4.09-16.28)
No / not reported	0.53 (0.43-0.63)	0.94 (0.92-0.96)	12.10 (3.18-46.07)
Blinding of test results			
Yes	0.33 (0.25-0.41)	0.96 (0.95-0.97)	11.36 (5.10-25.31)
No / Not reported	0.56 (0.43-0.69)	0.92 (0.88-0.95)	9.43 (2.02-44.13)
Concurrent HIV-negative controls			
Yes	0.42 (0.34-0.50)	0.95 (0.94-0.97)	9.07 (3.81-21.58)
No/ Not reported	0.36 (0.24-0.50)	0.96 (0.94-0.98)	14.99 (2.58-87.23)

Figure 1: Forest plots of the sensitivity, specificity and diagnostic odds ratio of cytology among HIV-infected women defined at the ≥ CIN 1 threshold.

Figure 2a: Pooled Sensitivity at ≥ CIN 1

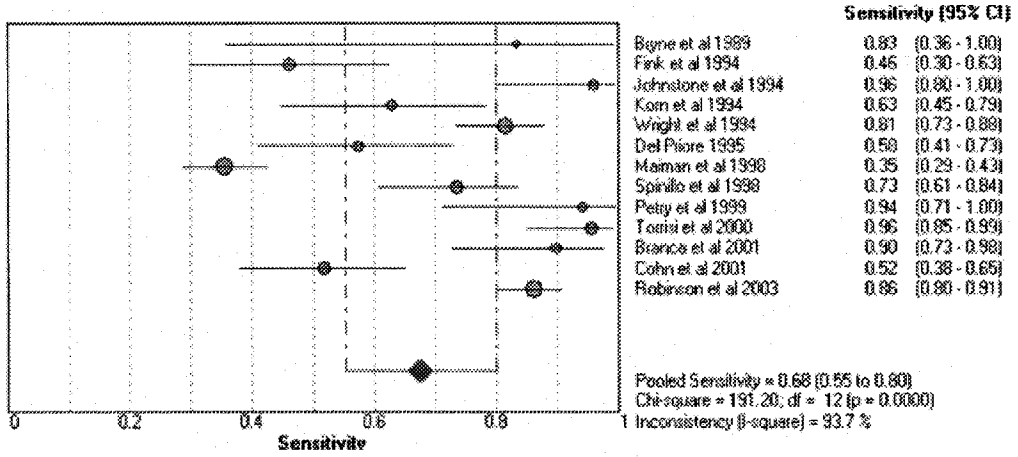


Figure 2b: Pooled Specificity at ≥ CIN 1

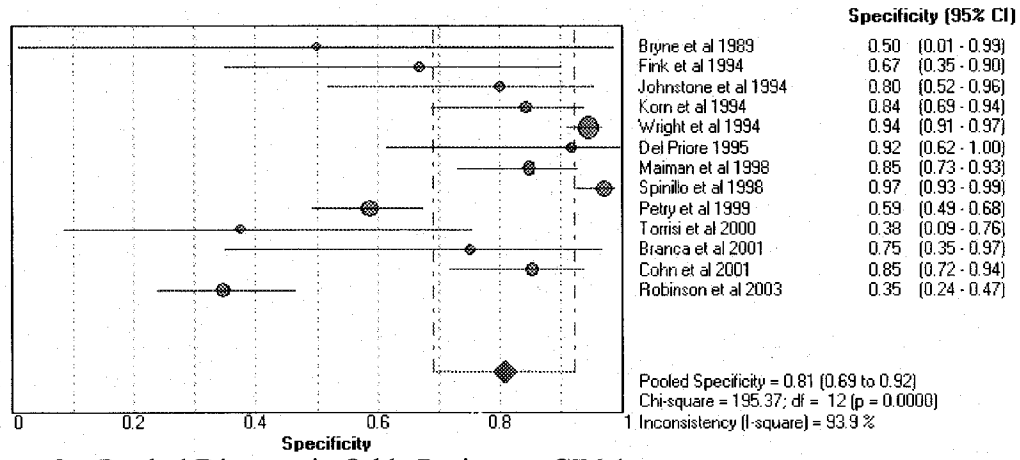


Figure 2c: Pooled Diagnostic Odds Ratio at ≥ CIN 1

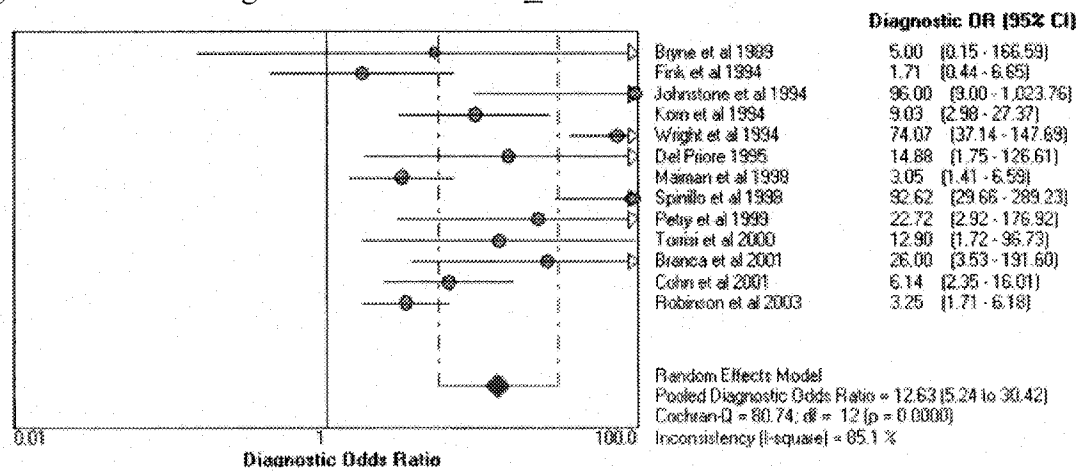


Figure 2: Forest plots of the sensitivity, specificity and diagnostic odds ratio of cytology among HIV-infected women defined at the ≥ CIN 2 threshold.

Figure 1a: Pooled Sensitivity at ≥ CIN 2

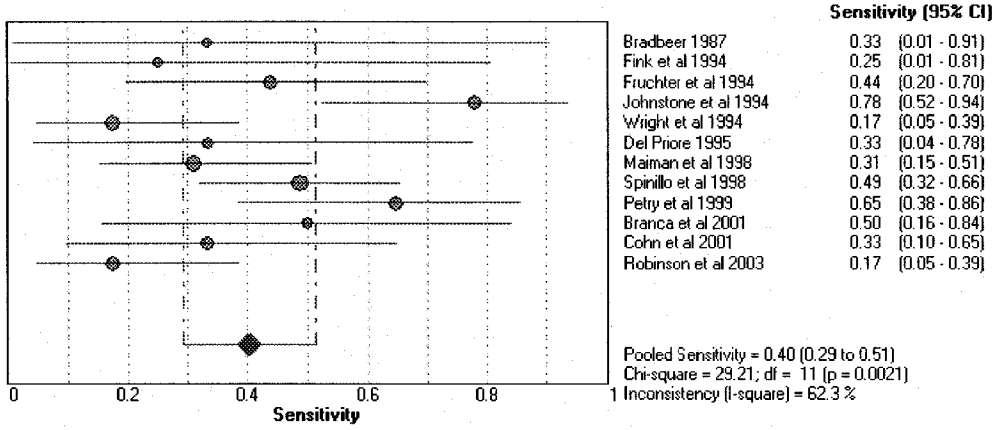


Figure 1b: Pooled Specificity at ≥ CIN 2

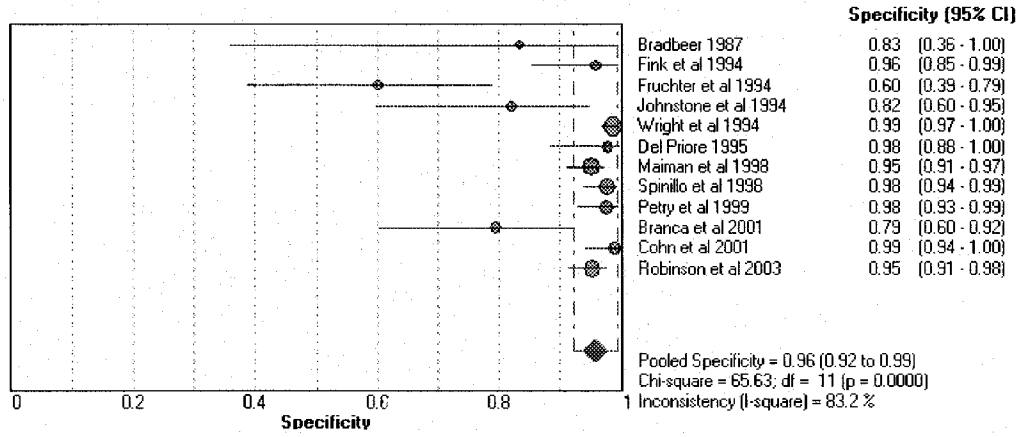


Figure 1c: Pooled Diagnostic Odds Ratio at ≥ CIN 2

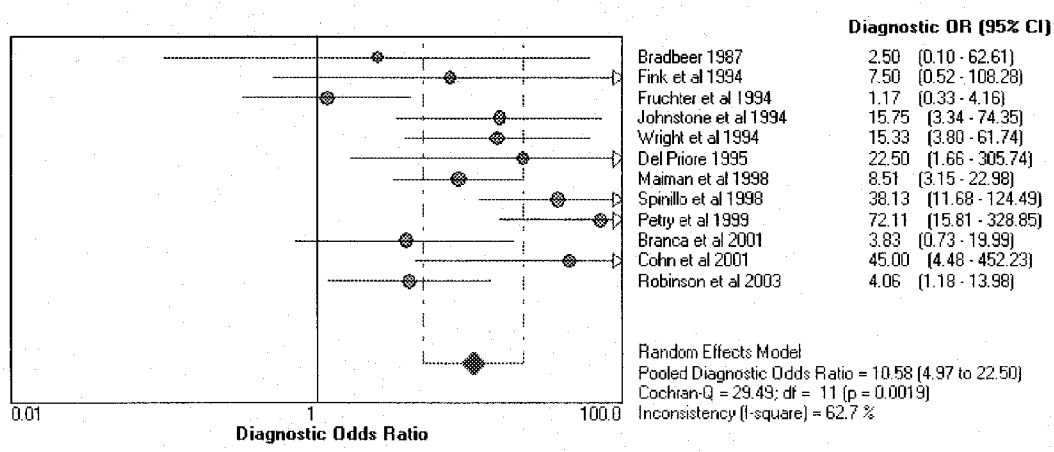


Figure 3: Summary Receiver Operating Characteristic Curve for pooled estimates of sensitivity and specificity at the ≥ CIN1 threshold.

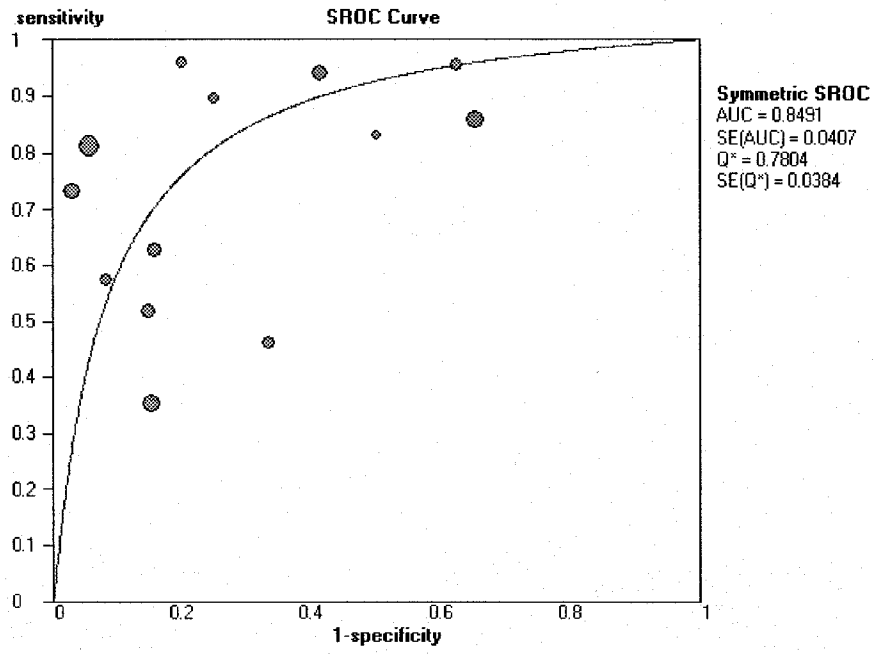
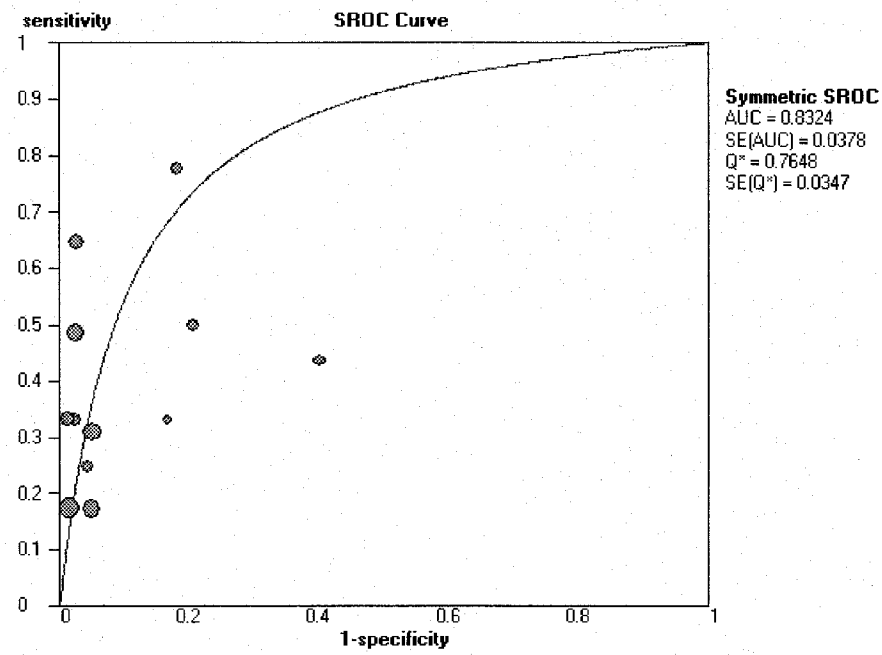


Figure 4: Summary Receiver Operating Characteristic Curve for pooled estimates of sensitivity and specificity at the ≥ CIN2 threshold.



VISUAL INSPECTION WITH ACETIC ACID COMPARED TO CYTOLOGY TO SCREEN FOR CERVICAL CANCER IN HIV-INFECTED WOMEN IN ZAMBIA

by

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ABSTRACT

Background: Visual inspection with acetic acid (VIA)-based cervical cancer screening has been proposed as an alternative to cytology for use in resource-limited settings, yet no data s available for the test performance of VIA among HIV-infected women, We compared the accuracy of VIA against cytology for the detection of cervical intraepithelial neoplasia (CIN) among HIV-infected women in Lusaka, Zambia.

Methods: We screened 150 non-pregnant women, using VIA and concurrent liquid cytology using Thin-Prep Pap test. We performed colposcopy on all participants and histopathology was obtained when indicated. Sensitivity and specificity, positive and negative predictive values, and efficiency of VIA and liquid cytology were compared for significant differences using a colposcopic-histology diagnosis that served as the 'gold standard'.

Results: The mean age of the screened women was 36.3 years (range 23-49) and their mean CD4+ count was 209/μL (range 7-942). The VIA positivity rate was 46% while that of high grade squamous intraepithelial lesions (SIL) or suspected cancer on cytology was 52.7%. VIA had a higher specificity (62.1% vs. 58.6%) than cytology but a lower sensitivity (73.5% vs. 91.2%) and a lower overall efficiency (64.7%, vs. 66%) ($p>0.5$ for all).

Conclusion: Although it had a lower sensitivity than cytology in our study, the comparable specificity of VIA and the significantly improved efficiency of the

combination makes VIA a useful adjunct test to cytology. Reduced loss to follow-up may improve overall program effectiveness of programs incorporating VIA. "See-and-treat" protocols involving VIA-based screening need evaluation in clinical trials for HIV-infected women in resource limited settings.

Key words: HIV, Cervical Cancer, Screening, VIA, Cytology, Sensitivity and Specificity, Diagnostic Accuracy, Zambia

INTRODUCTION

Cancer of the uterine cervix is caused by oncogenic types of human papillomavirus (HPV) (1) and represents the leading cause of cancer-related morbidity and mortality among women living in resource-constrained settings of the developing world.(2, 3) Each year, an estimated 490,000 women are newly diagnosed and 274,000 women die from this disease worldwide. (4) About 83% of the new cases and 85% of deaths occur in developing countries where access to screening services is limited or minimal.(4,5) In these same settings, the HIV/AIDS pandemic has overwhelmed many health care systems and affects women in the reproductive age group.(6) HIV-infected women are at increased risk for the development, progression and recurrence of HPV-induced cervical neoplasia.(7-10) Zambia, one of the sub-Saharan African nations hardest hit by the AIDS pandemic, has especially high HIV seroprevalence rates (16-25%) as well as one of the highest age-adjusted prevalence of invasive cervical cancer in the world (61.1/100,000). (4,11) The recently increasing availability of antiretroviral therapy in sub-Saharan Africa and elsewhere has the potential for prolonging the life of HIV-infected women long enough for malignancies like cervical cancer to progress.(12-14) Screening women living

with HIV for early detection and treatment of cervical intraepithelial neoplasia (CIN) can prevent excess morbidity and mortality due to cervical cancer.

Given the complexity of implementing and sustaining cytology-based cervical cancer screening programs in resource-constrained settings, there is interest in assessing the utility of non-cytological methods. (15) Visual Inspection with Acetic Acid (VIA) has emerged as a low-cost, low-technology alternative to cervical cytology for use in these settings. (16-18) This clinical test can be taught to nurses or lay health workers and is independent of any laboratory infrastructure. It also provides immediate test results thereby allowing linking screening to diagnosis or treatment in the same clinic visit and thereby reducing loss to follow-up. Test results are dependent on the proficiency of the provider. If practitioner skills are optimized, the significantly lower costs associated with a VIA-based screening program may make it an attractive option. Recent research has focused on comparison of VIA and other alternative screening methods. (16-21) However, estimates of test performance of VIA in HIV-infected women are as yet unknown. We sought to evaluate the accuracy of VIA among HIV-infected women at the University Teaching Hospital in Lusaka, Zambia.

METHODS

Settings and participants: This study was approved by the Research Ethics Committee of the University of Zambia and the Institutional Review Board of the University of Alabama at Birmingham. We offered recruitment to 150 consecutive HIV-infected women attending the HIV-care clinic at the University Teaching Hospital in Lusaka. After explanation of the study and clinical procedures, all 150 women provided written

informed consent administered in English, Bemba, or Nyanja. Eligibility criteria included prior documented evidence of HIV-infection (prior test records of two positive rapid HIV 1/2 tests: Determine® and Capillus ®) and being physically and mentally capable to follow the study procedures. We excluded women who were pregnant (menstrual history), had a history of previous diagnosis or treatment of cervical neoplasia, or had undergone hysterectomy. After a clinical and pelvic examination, women showing signs of sexually transmitted infections (ulceration, discharge) were counseled and treated using guidelines for syndromic management and asked to return to the clinic after 2 weeks for study screening and enrollment. (22)

Study design: We used a cross-sectional study design in which cytology sample and VIA were carried out on all recruited women in the same clinic visit. Assuming that cytology had a sensitivity of about 60% (extrapolated from other studies in the developing world), we studied 150 HIV-infected women to provide us 80% power at a two-tailed α=0.05 to detect a 15% difference between the sensitivity of VIA and cytology, assuming a CIN disease prevalence of 20%. The reference investigation ('gold standard') applied was a combination of colposcopy and histology, wherein colposcopy was performed on all women but histology was limited to (i) women who had abnormal lesions on colposcopy or (ii) women in whom abnormal lesions on VIA and cytology warranted histological confirmation in the same or follow-up visit. Histology gave the definitive diagnosis when both histology and colposcopy were done. Histology was performed in the same or subsequent clinic visits from directed cervical biopsy specimens, endocervical curettages (ECC), or loop electrosurgical excision procedures (LEEP) as clinically indicated.

Clinical tests: In the clinical protocol, after administering the consent and confirming the eligibility, basic sociodemographic data were collected using a structured questionnaire. A blood sample was collected if no CD4+ cell count was documented in the previous three weeks. A trained gynecologist conducted a physical and pelvic examination in all women. A trained nurse collected specimens for Pap smears from the ectocervix and endocervix using a plastic Ayres spatula and cytobrush, respectively. The spatula and cytobrush were then vigorously rinsed in vials containing PreservCyt ® solution that were stored at room temperature for up to 2 weeks before being batch-transported to the US for cytological analysis. After collecting the cervical specimens, the nurse performed VIA by swabbing the cervix with dilute 3% acetic acid through the vaginal specula and observing the changes on the cervix surface with the aid of a 100-watt incandescent gooseneck lamp one minute after application. The test results were recorded in quadrants on the patient case report forms, summarized as "VIA positive" or "VIA negative" as per published guidelines developed for use in resource limited settings. (23). Nurses performing VIA and cytology had undergone an intensive 2-week initial training that included both theoretical and hands-on components. A single gynecologist performed colposcopic examination in all women without knowledge of VIA results. Colposcopic findings were again recorded in quadrants. If indicated by colposcopy, tissue confirmation by biopsy, LEEP, or ECC was obtained in the same (or subsequent, if preferred by the woman) clinic visits for histopathological analysis. Repeat colposcopy and screening after 6 and 12 months were advised, as indicated, and women were provided later ablative (if histologically indicated) or surgical treatment (hysterectomy) (n=1) if necessary for cervical cancer.

Laboratory tests: Upon arrival in the cytology lab in the US, the specimen vials (pre-labeled with a unique identifying number) were logged and processed with a ThinPrep® 3000 processor. (24-26) All slides were stained using the ThinPrep® Imaging System TP-3000 stain protocol. Slides had coverslips applied on the "Sakura Tissue-Tek® GLAS Automated Glass Coverslipper" and allowed to dry prior to review. All ThinPrep® samples were screened and diagnosed by a certified senior cytotechnologist according to the 2001 Bethesda System guidelines. (27) All abnormal slides and 10% of negatives were subsequently reviewed by a board certified cytopathologist. We performed HPV typing using the Roche Linear Array PCR from the residual fluid in the specimen (see chapter 3 of this thesis). (28) The histopathological specimens were analyzed in Zambia by a senior consulting histopathologist (VM) and analyzed according to the CIN system. (29) The cytotechnician, cytopathologist, and histopathologist were all blinded to the clinical profile and colposcopic findings to ensure unbiased reporting.

Statistical methods: Data entry was done both on site and repeated in the US and statistical analysis was done using SPSS14.0 for Windows™ (SPSS Inc., Chicago, IL) and Intercooled Stata 8.0 for Windows™ (Stata Corporation, College Station, TX). Both VIA and cytology results were dichotomized for comparison purposes (Figure 1). A positive cytology test included results positive for high-grade squamous intraepithelial lesions (HSIL) and squamous cell carcinoma (SCC), while negative results included low-grade SIL (LSIL), atypical squamous cells of undetermined significance (ASCUS) and normal cytology. The results of colposcopy and histopathology were classified as per the CIN system as normal, CIN1, CIN 2, CIN 3, and Invasive Cervical Cancer (ICC). The final composite diagnosis was done post-hoc and was based on reconciliation between the

colposcopic and histopathologic diagnoses. The final composite diagnosis classified the outcome as 'disease present' or 'disease absent' at the clinically relevant ≥CIN2 threshold (that included CIN 2, CIN 3 and ICC). The sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively), and efficiency were computed by 2 X 2 contingency tables (Table 2). The same measures for the paired combinations of test results (VIA and cytology in series combination) were calculated (Table 2). Exact 95% confidence intervals were computed for each measure using recommended methods for binomial proportions (30, 31). Chi-square tests were used to compare differences in point estimates of the measures of performance (calculated as proportions) between tests. Two-tailed p-values were computed for statistical significance.

RESULTS

Recruitment took place between July and September 2004. None of the 150 HIV-infected women approached declined to participate. The mean age was 36.3 years (range 23-49) and their mean CD4+ cell count was 208.5/μL (SD:± 177.5, range 7-942). The results of the screening tests and the reference investigations are summarized in a flow diagram (Figure 1). Other socio-demographic and clinical characteristics are summarized with the test results of both VIA and cytology (Table 1). No significant associations in women's characteristics were noted with positive VIA or cytology with two exceptions: women over 30 years of age and those with CD4+ counts <200/μL were more likely to have a positive cytology result (p=0.04 and p=0.03, respectively).

Screening Test results: Of the 150 results on cytology, 10 (6.7%) had no abnormality and 140 (93.3%) had a squamous cell abnormality. ASCUS was reported in 26 (17.3%), LSIL

in 35 (23.3%), HSIL in 49 (32.7%), and 30 (20.0%) had SCC on cytology. Dichotomizing cytology results at the ≥HSIL threshold resulted in 79/150 (52.6%) being classified as cytology positive, while 69 (46%) women were detected as VIA-positive (Table 3).

Reference Diagnosis: The results on the final composite colpo-histological reference included 88 (58.7%) women deemed within normal limits, 28 (18.7%) women with evidence of CIN 1, 5 (3.3%) women with evidence of CIN 2, 28 (18.7%) women with CIN 3, and 1 (0.7%) woman diagnosed with Invasive cervical cancer. Based on these results, 34 women (22.7%, 95% CI: 16.2-30.2) were classified as 'disease' present. ("truly diseased") (Table 3, Figure 1) No woman had complications from the screening tests or the reference investigations (colposcopy and/or histology).

Estimates of screening test accuracy: (Table 4, 5) The individual sensitivity of VIA (73.5%, 95% C.I. 55.6%-87.1%) was marginally lower than that of cytology ≥ HSIL (91.2%, 95% C.I. 76.3%-98.1%) (p=0.11, Table 4), whereas the specificity of the two tests was not statistically different. (62.1% versus 58.6%, p=0.6). The efficiency of VIA was lower than that of cytology (64.7% versus 66%), but the efficiency of the combination (76%) increased significantly compared to VIA and cytology individually (p=0.04 and 0.07, respectively). The point estimate of PPV of VIA (36.2%) was insignificantly lower than that of cytology (39.2% and 67.1%, respectively). However, the PPV of the combination (48%) was higher than the PPV of the individual tests (no p-values significant). The NPV of the combination (90%) was slightly higher than that of VIA (88.9%) but lower than that of cytology (95.7%) although the comparisons were statistically not significant. (p=0.8 and p=0.2 respectively)

DISCUSSION

Accounting for the small size of our study, our results suggest that VIA had a higher specificity (62.1%) than cytology (58.6%) but a lower sensitivity (73.5%) than cytology (91.2%) among HIV-infected women. Conventional Pap smears would be the preferred approach to screening HIV-infected women in developing countries if patient follow-up rates were excellent and if cytology were as inexpensive as VIA. To the contrary, however, the linking of screening and treatment in the same clinic visit using a low-cost test like VIA minimizes the necessity of referral visits, decreases direct and indirect costs associated with screening, and reduces rates of loss to follow-up. We speculate that this will actually result in better programmatic outcomes when using VIA independently to screen HIV-infected women in resource limited settings. VIA should be evaluated in a randomized prevention clinical trial for its real-world effectiveness compared to a cytology-based program for HIV-infected women in resource-limited settings.

Although VIA is attractive being a one-step, single visit, low-cost test, it also suffers from some inherent disadvantages. Acetowhitening is common in non-pathologic situations when increased nuclear protein is present, e.g., squamous metaplasia, congenital transformation zone, healing and regenerating epithelium associated with inflammation, leukoplakia (hyperkeratosis), and condylomata. This is a potential source of a falsely positive VIA result that will result in unnecessary treatment in the single visit approach. Careful training and clinical experience can reduce the false positive rate. (32) In addition, lack of proper lighting and clinical inexperience may result in false-negative characterizations. (18,32) A theoretical limitation of VIA is the poor image resolution

when observation is unmagnified, i.e., through the naked eye, although previous studies of VIA with magnification have not shown to improve test accuracy (33). A theoretical limitation of VIA is the poor image resolution when observation is unmagnified, i.e., through the naked eye, although previous studies of VIA with magnification have not shown to improve test accuracy (33). We are currently exploring the use of a low-cost digital camera enhancement to VIA in our screening program in Zambia to overcome the problems with low resolution and poor illumination. While this is reminiscent of cervicography, the photo is available immediately, can be re-taken if necessary and can be magnified on-site for making appropriate treatment decisions

In HIV-negative (or unknown status) women, large studies in various settings suggest that the performance of VIA is very comparable to that of cervical cytology, with sensitivity ranging from 67-79% versus 47-62%, and specificity ranging from 49-86% compared 60-95% respectively for conventional cytology. (15) Since its conceptualization in 1982 (34), VIA has been proposed as a screening tool suitable for resource-limited settings since it is independent of any laboratory infrastructure and may actually be offer the chance to treat the lesions in the same visit, even without histopathogical confirmation. (35-36) Histopathological confirmation may be ideal, (37) yet when its costs serve as an obstacle to care rather than facilitating the needed intervention, then VIA might be a bridging method until full laboratory services and infrastructure can be developed.

Our study results demonstrate that VIA performed better than cytology in its capacity to differentiate true negative results, i.e. had a higher specificity among HIV-infected women. However, its capacity to detect true positives, i.e. sensitivity was slightly lower

than cytology. Our study results are discordant in this respect with other published estimates from studies conducted in the general population, probably due to the unique properties of the cervix in the HIV-infected women of our study. We observed a high proportion of women with increased vascularity of the cervix that probably reduced the 'uptake' of acetic acid in epithelial cells and results in lower or slower acetowhitening. Another important determinant affecting sensitivity is the amount of illumination available to facilitate the correct interpretation of the acetowhitening in VIA screening as is discussed above.

Combining VIA with cytology in a two-stage screening algorithm might help improve the performance and detection rates of preinvasive neoplasia, while restricting the number of false positive treatments and/or referrals. (38) Cost-effectiveness modeling has suggested the value of this combination (39-42) and large field studies are currently underway to demonstrate its real-world utility. (16, 43) The data from this study provide the preliminary evidence of the usefulness of this strategy in HIV-infected women. The series combination of VIA with cytology in our study substantially improved the efficiency of the tests considered individually. The potential utility of these results lie in development of an algorithm that includes VIA as an adjunct test to cytological screening programs in setting where such programs exist, or as a primary screening test in settings where there is limited or no availability of cytology based screening programs. VIA can be used to triage patients who can then be screened (cost-effectively) with cytology. Conversely, re-screening cytology positive subjects with VIA to determine need for further colposcopic diagnosis may provide optimal utilization of scarce manpower and

technological resources and is particularly attractive from a program implementation standpoint.

A significant limitation of the current study was that the cytological analysis was conducted in the US, due to lack of availability of cytotechnologists in Zambia. This is not an unusual scenario in many developing country settings. In fact, the results of this study point to the fact that in spite of being a low-cost, low-technology test, VIA turned out to be comparable in its accuracy to a highly sensitive cytological technique like Thin-Prep Pap cytology. (24-26)

VIA can be taught to local health care providers and can be implemented independent of sophisticated infrastructure and highly skilled manpower. The role of continuous training and re-training of providers (e.g., nurses, village health workers, clinical officers, health technicians, or midwives) is another crucial determinant of success of any VIA-based screening program. (32) Specifically for HIV-infected women, an additional dimension is that of the associated stigma and discrimination, frequently preventing access to health care services. (44) The simplicity of VIA can be exploited to integrate its use in routine preventive and clinical services for HIV-infected women or women at high-risk of acquiring HIV. VIA can be potentially integrated into routine voluntary counseling and testing (VCT) services, STI care using syndromic management protocols, treatment settings along with provision of highly active antiretroviral therapy, as well as counseling and clinic visits in pre-natal or post-natal settings. (45) Finally, although a prophylactic HPV vaccine is on the horizon, the importance of screening and monitoring for cervical precancerous lesions will not diminish, thereby justifying the role of implementing low-cost protocols involving VIA. (46)

This study provides the first reported estimates of accuracy of VIA-based cervical cancer screening in HIV-infected women in a resource constrained setting. These preliminary findings need replication in other settings and further studies are required to develop appropriate, cost-effective prevention intervention protocols using low-cost, low-technology approaches like VIA, that could be used independently or as adjuncts to existing cytology-based screening protocols for cervical cancer prevention among HIV-infected women.

REFERENCES

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189:12-9.
2. Drain PK, Holmes KK, Hughes JP, Koutsky LA. Determinants of cervical cancer rates in developing countries. *Int J Cancer.* 2002;100(2):199-205.
3. Sankaranarayanan R, Ferlay J. Worldwide burden of gynaecological cancer: The size of the problem. *Best Pract Res Clin Obstet Gynaecol.* 2006 ;20(2):207-25.
4. GLOBOCAN: Cancer incidence and mortality worldwide database. Lyon, France: International Agency for Research on Cancer. Available online: http://www-dep.iarc.fr/globocan/database.htm. (Accessed March 10, 2006)
5. Sankaranarayanan R, Budukh AM, Rajkumar R. Effective screening programmes for cervical cancer in low- and middle-income developing countries. *Bull World Health Organ.* 2001;79(10):954-62.

6. Report of the Global HIV/AIDS Epidemic, 2005, UNAIDS. Available online at http://www.unaids.org (Accessed March 10, 2006)
7. Sun XW, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright TC Jr. Human papillomavirus infection in women infected with the human immunodeficiency virus. *N Engl J Med* 1997; 337: 1343-9.
8. Vermund SH, Kelley KF, Klein RS, Feingold AR, Schreiber K, Munk G, Burk RD. High risk of human papillomavirus infection and cervical squamous intraepithelial lesions among women with symptomatic human immunodeficiency virus infection. *Am J Obstet Gynecol.* 1991;165(2):392-400
9. Mandelblatt JS, Kanetsky P, Eggert L, Gold K. Is HIV infection a cofactor for cervical squamous cell neoplasia? *Cancer Epidemiol Biomarkers Prev.* 1999;8(1):97-106.
10. Tate DR, Anderson RJ. Recrudescence of cervical dysplasia among women who are infected with the human immunodeficiency virus: a case-control analysis. *Am J Obstet Gynecol* 2002; 186: 880-2.
11. HIV/AIDS in Zambia: The Epidemic and its impact: AVERT web publication: Available online: http://www.avert.org/aids-zambia.htm (Accessed March 10, 2006)
12. The President's Emergency Plan for AIDS Relief: Five-Year Strategy – (6/27/05). USAID: PEPFAR. Available online at: http://www.state.gov/documents/organization/48746.pdf (Accessed March 10, 2006)

13. The Global Fund for AIDS, Tuberculosis and Malaria. Available online at http://www.theglobalfund.org/en/about/publications/brochure (Accessed March 10, 2006)

14. Parham G, Sahasrabuddhe VV, Mwanahamuntu M, Stringer E, Hicks M, Vermund S. Cervical Cancer Screening in HIV-infected women in Zambia. Poster presentation. 9th International Conference on Malignancies in AIDS and Other Immunodeficiencies (ICMAOI). National Cancer Institute, Bethesda, MD, Sept 26-27, 2005.

15. Sankaranarayanan R, Gaffikin L, Jacob M, Sellors J, Robles S. A critical assessment of screening methods for cervical neoplasia. *Int J Gynaecol Obstet.* 2005;89 Suppl 2:S4-S12.

16. Sankaranarayanan R, Nene BM, Dinshaw KA, Mahe C, Jayant K, Shastri SS, Malvi SG, Chinoy R, Kelkar R, Budukh AM, Keskar V, Rajeshwarker R, Muwonge R, Kane S, Parkin DM, Chauhan MK, Desai S, Fontaniere B, Frappart L, Kothari A, Lucas E, Panse N; Osmanabad District Cervical Screening Study Group. A cluster randomized controlled trial of visual, cytology and human papillomavirus screening for cancer of the cervix in rural India. *Int J Cancer.* 2005;116(4):617-23.

17. Wright TC Jr, Denny L, Kuhn L, Goldie S. Use of visual screening methods for cervical cancer screening. *Obstet Gynecol Clin North Am.* 2002;29(4):701-34.

18. Gaffikin L, Lauterbach M, Blumenthal PD. Performance of visual inspection with acetic acid for cervical cancer screening: a qualitative summary of evidence to date. *Obstet Gynecol Surv.* 2003;58(8):543-50.

19. Denny L, Kuhn L, Pollack A, Wright TC Jr. Direct visual inspection for cervical cancer screening: an analysis of factors influencing test performance. *Cancer.* 2002;94(6):1699-707.

20. Sellors J, Lewis K, Kidula N, Muhombe K, Tsu V, Herdman C. Screening and management of precancerous lesions to prevent cervical cancer in low-resource settings. *Asian Pac J Cancer Prev.* 2003;4(3):277-80.

21. Sankaranarayanan R, Shastri SS, Basu P, Mahe C, Mandal R, Amin G, Roy C, Muwonge R, Goswami S, Das P, Chinoy R, Frappart L, Patil S, Choudhury D,Mukherjee T, Dinshaw K. The role of low-level magnification in visual inspection with acetic acid for the early detection of cervical neoplasia. *Cancer Detect Prev.* 2004;28(5):345-51.

22. World Health Organization. Guidelines for the management of sexually transmitted infections. Geneva, Switzerland. Available online at: http://www.who.int/reproductive-health/publications/rhr_01_10_mngt_stis (Accessed March 10, 2006)

23. A practical manual on visual screening for cervical neoplasia. Edited by R. Sankaranarayanan and Ramani S. Wesley 2003. IARC Press, Lyon, France. Available online at: http://screening.iarc.fr/viavili.php (Accessed March 10, 2006)

24. Abulafia O, Pezzullo JC, Sherer DM: Performance of the ThinPrep liquid-based cervical cytology in comparison with conventionally prepared Papanicolaou smears: A quantitative survey. *Gynecol Oncol* 2003;90(1):137-144.

25. Biscotti C, Dawson A, Dziura B, Galup L, Darragh T, Rahemtulla A, Wills-Frank L: Assisted Primary Screening Using the Automated ThinPrep Imaging System. *Am J Clin Pathol* 2005;123(2):281-7.

26. Dawson AE. The Changing Face of Cervical Screening. Challenges for the Future. *Diagn Cytopathol* 2005;33(2):63-64.

27. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr, Young N; Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*. 2002;287(16):2114-9.

28. Roche Linear Array PCR Information material. (Available online: http://www.roche-diagnostics.cz/download/mol/virologicke/hpv/LINEAR%20ARRAY%20HPV%20Genotypizacni%20Test.pdf.) (Accessed March 10, 2006)

29. Richart RM. Cervical intraepithelial neoplasia. *Pathol Annu* 1973;8:301–328

30. McKenzie, D., Vida, S., Mackinnon, A.J., Onghena, P. and Clarke, D. (1997) Accurate confidence intervals for measures of test performance. *Psychiatry Research*, 69, 207-209.

31. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med.* 1998;17(8):857-72.

32. Blumenthal PD, Lauterbach M, Sellors JW, Sankaranarayanan R. Training for cervical cancer prevention programs in low-resource settings: focus on visual inspection with acetic acid and cryotherapy. *Int J Gynaecol Obstet.* 2005;89 Suppl 2:S30-7.

33. Sankaranarayanan R, Shastri SS, Basu P, Mahe C, Mandal R, Amin G, Roy C, Muwonge R, Goswami S, Das P, Chinoy R, Frappart L, Patil S, Choudhury D, Mukherjee T, Dinshaw K. The role of low-level magnification in visual inspection with acetic acid for the early detection of cervical neoplasia. *Cancer Detect Prev.* 2004;28(5):345-51.

34. Ottaviano M and P La Torre. 1982. Examination of the cervix with the naked eye using acetic acid test. *Am J Obst Gyn* 143(2): 139–142.

35. Gaffikin L, Blumenthal PD, Emerson M, Limpaphayom K; Royal Thai College of Obstetricians and Gynaecologists (RTCOG)/JHPIEGO Corporation Cervical Cancer Prevention Group. Safety, acceptability, and feasibility of a single-visit approach to cervical-cancer prevention in rural Thailand: a demonstration project. *Lancet.* 2003;361(9360):814-20.

36. Denny L, Kuhn L, De Souza M, Pollack AE, Dupree W, Wright TC Jr. Screen-and-treat approaches for cervical cancer prevention in low-resource settings: a randomized controlled trial. *JAMA.* 2005;294(17):2173-81.

37. Suba EJ, Murphy SK, Donnelly AD, Furia LM, Huynh ML, Raab SS. Systems analysis of real-world obstacles to successful cervical cancer prevention in developing countries. *Am J Public Health.* 2006;96(3):480-7.

38. Denny L, Kuhn L, Risi L, Richart RM, Pollack A, Lorincz A, Kostecki F, Wright TC Jr. Two-stage cervical cancer screening: an alternative for resource-poor settings. *Am J Obstet Gynecol.* 2000;183(2):383-8.

39. Goldie SJ, Kuhn L, Denny L, Pollack A, Wright TC. Policy analysis of cervical cancer screening strategies in low-resource settings: clinical benefits and cost-effectiveness. *JAMA.* 2001;285(24):3107-15.

40. Mandelblatt JS, Lawrence WF, Gaffikin L, Limpahayom KK, Lumbiganon P, Warakamin S, King J, Yi B, Ringers P, Blumenthal PD. Costs and benefits of different strategies to screen for cervical cancer in less-developed countries. *J Natl Cancer Inst.* 2002;94(19):1469-83.

41. Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, Gordillo-Tobar A, Levin C, Mahe C, Wright TC; Alliance for Cervical Cancer Prevention Cost Working Group. Cost-effectiveness of cervical-cancer screening in five developing countries. *N Engl J Med.* 2005;353(20):2158-68.

42. Legood R, Gray AM, Mahe C, Wolstenholme J, Jayant K, Nene BM, Shastri SS, Malvi SG, Muwonge R, Budukh AM, Sankaranarayanan R. Screening for cervical cancer in India: How much will it cost? A trial based analysis of the cost per case detected. *Int J Cancer.* 2005;117(6):981-7.

43. Sankaranarayanan R, Basu P, Wesley RS, Mahe C, Keita N, Mbalawa CC, Sharma R, Dolo A, Shastri SS, Nacoulma M, Nayama M, Somanathan T, Lucas E, Muwonge R, Frappart L, Parkin DM; IARC Multicentre Study Group on Cervical Cancer Early Detection. Accuracy of visual screening for cervical neoplasia: Results from an IARC multicentre study in India and Africa. *Int J Cancer.* 2004;110(6):907-13.

44. Duffy L. Suffering, shame, and silence: the stigma of HIV/AIDS. *J Assoc Nurses AIDS Care.* 2005;16(1):13-20.

45. Program Capacity Assessment Tool: Integrating Cervical Cancer Prevention into Reproductive Health Services. PATH. Reproductive Health Reports #4. PATH (July 2001).

46. Mahdavi A, Monk BJ. Vaccines against human papillomavirus and cervical cancer: promises and challenges. *Oncologist*. 2005;10(7):528-38

Table 1: Sociodemographic and clinical characteristics of participants stratified by VIA and cytology test results

Characteristic	N	VIA positive (n=69)		*Cytology positive (n=79)*	
		N	p-value	N	p-value
Age (in years)	150				
<= 30	29	12		10	
> 30	121	57	0.68	69	*0.04*
Education	150				
No/some school education	79	31		38	
High school graduate & above	71	38	0.10	41	0.26
Marital status	105				
Never married /non-cohabiting[1]	94	44		51	
Married / cohabiting	56	25	0.87	28	0.73
Family Income	150				
< $100/month	87	42		42	
>= $100/month	56	45	0.73	35	0.12
Age at first intercourse	149				
< 18 years	57	25		30	
>= 18 years	92	43	0.74	48	0.96
Lifetime number of sexual partners	145				
1-5 partners	120	54		62	
>= 6 partners	25	11	0.93	13	0.98

Table 1 (continued)

Characteristic	N	VIA positive (n=69)		*Cytology positive (n=79)*	
Condom use	147				
Non-consistent/never	110	50		58	
Consistent	37	18	0.85	20	0.89
Parity	141				
Nulliparous	21	8		9	
1 birth or more	120	55	0.67	66	0.35
CD4+ cell count [3]	145				
<200/ μL	91	39		55	
>=200/ μL	54	28	0.31	22	*0.03*
Access to antiretroviral therapy [3]	147				
Never taken antiretroviral therapy	34	13		15	
Currently taking antiretroviral therapy	113	54	0.43	63	0.25

Note:

[1] 'non-cohabiting' includes separated/divorced/widowed

[2]: as verified clinically and treated using syndromic management

[3]: as recorded from accompanying clinical record form of each participant

Table 2: Definitions of measures of test performance, with the abbreviations

Measure	*Formula*	Definitions
Sensitivity or True Positivity Rate (TPR)	TP/ (TP+FN)	Proportion of positive test results among diseased subjects
Specificity or True Negative Rate (TNR)	TN / (TN+FP)	Proportions of negative test results among non-diseased subjects
Positive Predictive Value (PPV)	TP / (TP + FP)	Proportion diseased among subjects with a positive test result
Negative Predictive Value (NPV)	TN / (TN + FN)	Proportion on-diseased among subjects with a negative test result
Efficiency	TP+TN / (TP+FN+FP+TN)	Proportion of subjects correctly identified by a test

Note: TP, FP, FN, and TN represent the numbers of true positive, false positives, false negatives and true negatives, respectively.

Table 3: Results of tests: VIA and Cytology, individually and in combination, against the reference diagnosis

		Reference Diagnosis	
Test	Result (Total)	Diseased N=34	Non-diseased N=116
VIA	Positive (n=69)	25	44
	Negative (n=81)	9	72
Cytology	Positive (n=79)	31	48
	Negative (n=71)	3	68
VIA + Cytology	Positive (n=50)	24	26
	Negative (n=100)	10	90

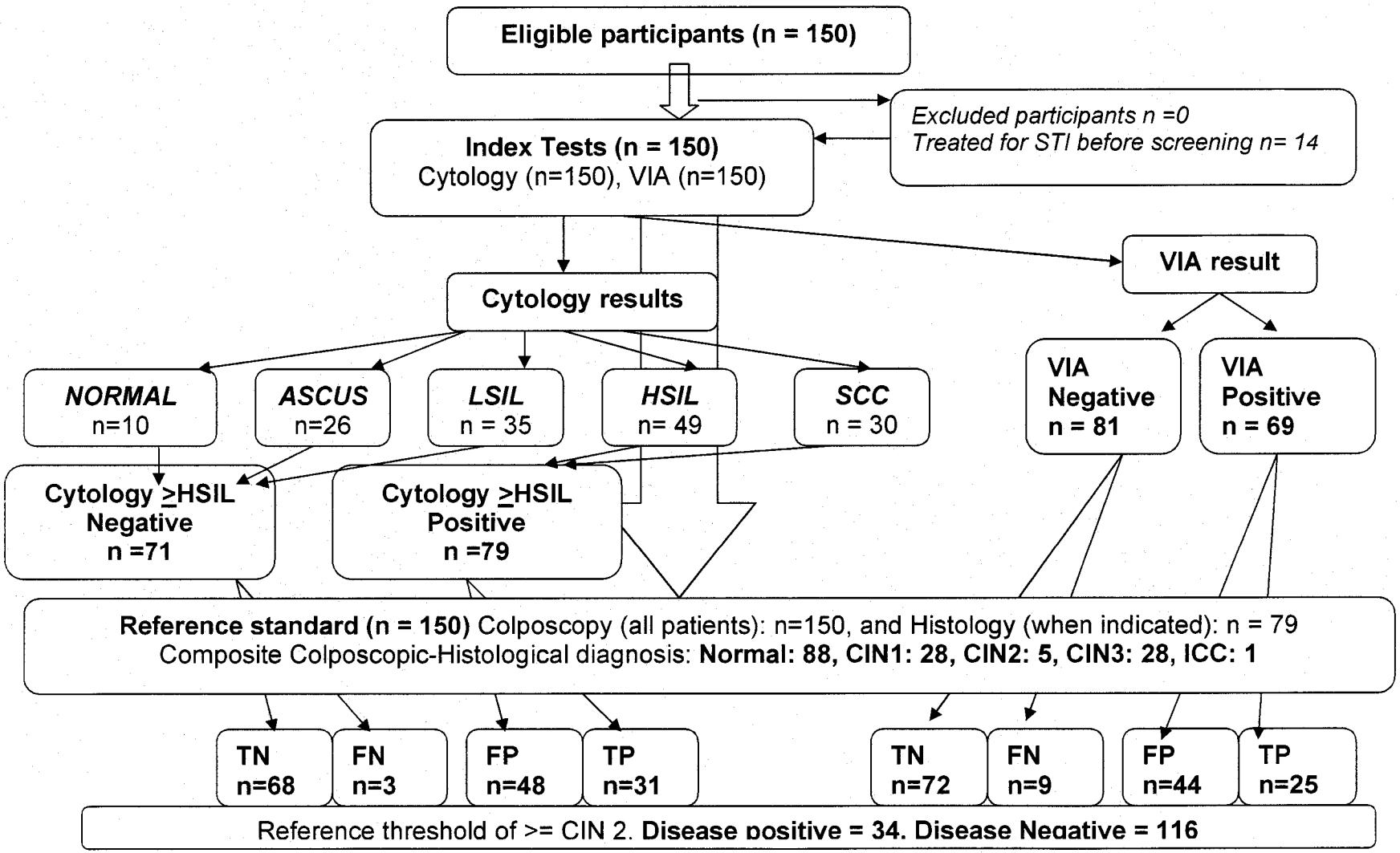
Table 4: Point estimates (and 95% CI) of the measures of test performance of VIA, Cytology and their combination against the composite reference standard.

	Sensitivity	Specificity	PPV	NPV	*Efficiency*
VIA	73.5% (55.6%-87.1%)	62.1% (52.6%-70.9%)	36.2% (25.0%-48.7%)	88.9% (80.0%-94.8%)	64.7% (56.4%-72.3%)
Cytology	91.2% (76.3%-98.1%)	58.6% (49.1%-67.7%)	39.2% (28.4%-50.9%)	95.8% (88.1%-99.1%)	66.0% (57.8%-73.5%)
VIA + Cytology	70.6% (52.5%-84.9%)	77.6% (68.9%-84.8%)	48.0% (33.7%-62.6%)	90.0% (82.4%-95.1%)	76.0% (68.3%-82.6%)

Table 5: Comparison of test measures against each other and the resultant p-values (at 0.05 level of significance)

	Sensitivity	Specificity	PPV	NPV	*Efficiency*
VIA vs. Cytology	p=0.11	p=0.59	p=0.71	p=0.11	p=0.89
VIA vs. VIA-Cytology combination	p=0.99	p=0.01	p=0.19	p=0.80	*p=0.04*
Cytology vs. VIA-Cytology combination	p=0.06	*p=0.002*	p=0.32	p=0.16	p=0.07

Figure 1: Flow-diagram of the clinical screening protocol



HIGH-PREVALENCE OF GENITAL HPV INFECTION AND MULTIPLE HPV GENOTYPES IN HIV-INFECTED WOMEN IN LUSAKA, ZAMBIA

by

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ABSTRACT

Background: New HPV vaccines may reduce cervical cancer risk in women living with HIV if circulating HPV is dominated by types 16 and 18. Among HIV-infected women, we measured the prevalence of genital HPV, cervical cytological abnormalities, and HIV-disease status.

Methods: We collected cervical samples from 150 non-pregnant, HIV-infected women from an HIV clinic in Lusaka, Zambia and analyzed specimens with liquid-based cytology and PCR-based HPV-DNA typing. PGMY09/11 biotinylated primers amplified a 450 base pair fragment of the L1 ORF of each HPV type.

Results: Prevalence of HPV infection was 97.8% (146 of 150) for any type and 85.3% (128 of 150) for high-risk types. Participants were infected with a median of four types (range 0-13 different viruses). HPV 52 was most prevalent (38% of participants), followed by types 61 and 62 (26% each), type 58 (23%), type 53 (20%), type 84 (19%), and types 35 and 81 (18% each). Types 16 (17.3%) and 18 (13.3%) were ranked 4th and 7th in frequency among the detected oncogenic HPV types. Women were more likely to have multiple high-risk HPV types when they had more severe cytological abnormalities (p for trend: 0.001) or lower CD4+ cell counts (p for trend = 0.02).

Conclusion: Nearly all HIV-infected Zambian women were co-infected with HPV, most with multiple high-risk HPV types. New HPV 16 and 18-based vaccines may

prove less effective in this population. Cervical cancer screening should be a routine part of HIV-related care.

Key words: Human immunodeficiency virus, human papillomavirus, cervical cancer, screening, HPV vaccine, Zambia

INTRODUCTION

Human papillomavirus (HPV) infection is the principal cause of invasive cervical cancer.(1-4) Women infected with the human immunodeficiency virus (HIV) are at heightened risk for the development of HPV-associated cervical neoplasia. (5-10) With the increasing availability of affordable antiretroviral therapy (ART) in resource-limited settings, women living with HIV are expected to live longer, permitting malignancies like cervical cancer to manifest and progress. (11-12) Prophylactic HPV vaccines offer hope for primary prevention and substantial reduction of cervical cancer-associated morbidity and mortality. (13-14, 42-43) However, it is unknown to what extent new HPV vaccines will be helpful in the developing world due to a lack of data on the diversity of circulating HPV types and ignorance as to the extent of type-specific cross-reacting immunity. Vaccine immunogenicity among HIV-infected women has not been reported. HPV vaccines may become available in limited supply (due to high cost) in sub-Saharan Africa in the near future. We conducted a cross sectional study in Lusaka, Zambia, to assess cervical HPV types and their association with cytological abnormalities and the immunosuppressed state of the HIV-infected women.

METHODS

Participants and setting: HIV-infected women attending the University Teaching Hospital in Lusaka, Zambia (the nation's principal tertiary care center) for HIV/AIDS care and treatment between July and September 2004 were invited to participate. All patients provided written, informed consent and none refused participation. Pregnant and menstruating women and those having a history of hysterectomy were excluded. Enrolled patients (n = 150) underwent a complete physical and gynecological evaluation, including cervical samples for cytological analysis and HPV DNA PCR. The samples were collected using a cervical spatula for the ectocervix and cytobrush for the endocervix and stored in vials containing PreservCyt ® transport medium (Cytyc Corporation, Marlborough, MA). All samples were stored at room temperature (37°C) for <2 weeks before being batch transported for analysis. A trained nurse performed visual inspection with acetic acid (VIA) followed by a colposcopic examination by a physician. A CD4+ cell count obtained unless a result was available within 3 weeks of the visit date from our NIAID-certified laboratory. ART was taken by 113 (75.3%) of the women at baseline. The study was approved by the Research Ethics Committee of the University of Zambia and the Institutional Review Board of the University of Alabama at Birmingham.

Cytology: Cytology specimen vials were pre-labeled with a unique identifying number; slides were stained using the ThinPrep® Imaging System TP-3000 protocol. Cells were protected in a Sakura Tissue-Tek® GLAS Automated Glass Coverslipper and allowed to dry. All ThinPrep® samples were screened by a certified senior cytotechnologist. All abnormal slides and 10% of negatives were subsequently

reviewed by a board certified cytopathologist. The cytotechnicians and cytopathologist were fully blinded to the clinical profile, the colposcopic impressions, and all other findings. The results were classified according to the 2001 Bethesda System guidelines as normal, Atypical Squamous Cells of Undetermined significance (ASCUS), Low grade SIL (LSIL), High Grade SIL (HSIL), and suspicious for Squamous Cell carcinoma (SCC).

HPV detection and typing: HPV typing on residual PreservCyt® samples was based on four processes using the Roche Linear Array ® PCR assay (Roche Molecular Systems, Pleasanton, CA) specifications:

i. *DNA isolation and specimen preparation:* HPV DNA was released by lysing cervical cell specimens under denaturation conditions at elevated temperatures. Lysis was performed in the presence of proteinase K, chaotropic agents, and detergent. DNA was isolated and purified using a vacuum and column, and the DNA was then eluted. The β-globin gene was isolated concurrently.

ii. *PCR amplification of target DNA using HPV specific complementary primers:* Consensus primers that amplify a 450-base pair fragment of the L1 Open Reading Frame (ORF) region of genital HPV were used. Cellular controls used primers that amplify a 268-base pair fragment of the human β-globin gene. Polymerase chain reaction was run on a Perkin-Elmer 9600/9700 thermo-cycler™ were run per the manufacturer's protocol and the target DNA was amplified.

iii. *Hybridization of the amplified products to oligonucleotide probes specific to the targets*: The linear array detection technique with a membrane strip that has target-specific oligonucleotides (i.e., PGMY09 and PGMY11 biotinylated primers) immobilized in a specific sequence on the strip was used. The amplified product were hybridized to target-specific probes prior to colorimetry

iv. *Detection of the probe-bound amplified products by visual colorimetric determination.* HPV types in the amplified product were identified by comparison to a type-specific template by visual colorimetric determination.

The Roche PCR-linear array test for HPV allows the simultaneous distinction of 37 specific HPV types, including 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39 and CP6108. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 (MM9), 82 (MM4) are designated as "high-risk" for the development of invasive cervical cancer, while types 26, 53 and 66 are designated as being "probably high-risk".(2) Types 6, 11, 40, 42, 54, 61, 64, 70, 72, 81 and CP 6108 are designated as "low-risk", while the risk for types 55, 62, 64, 67, 69, 71, 83(MM7), 84 (MM8), and IS 39 is as yet undefined in epidemiological studies.(2) CD4+ cell counts were trichotomized as <200/μL, 200-499/μL, and ≥500/μL.

Data management and statistical analyses: Double data entry was performed and statistical analyses were done using SPSS14.0 for Windows™ (SPSS Inc., Chicago, IL) and Intercooled Stata 8.0 for Windows™ (Stata Corporation, College Station, TX). Mantel-Haenszel Chi-square test of trend was used to analyze the association

between HPV type prevalence and cytology and CD4+ cell count. Two-way analysis of variance was used for comparison of mean number of HPV types among cytology groups and among CD4+ cell count categories.

RESULTS

The mean age of the 150 women was 36.2 years (range: 23 to 49). HPV DNA was detected in 146 (97.3%) of the 150 patients. All negative and positive controls for PCR amplification, HPV DNA detection, and genotyping yielded the appropriate results, as did β-globin gene analyses. The total number of HPV genotypes in these 150 women was 656 (Fig.1). HPV 52 was the most prevalent type, present in 57 out of 150 (38%) of the samples. The probe for HPV type 52 is a mixed probe with types 33, 35 and 58, therefore on post-hoc analysis, it was noted that 45 (30%) of samples also had presence of types 33, 35, and 58, and only 12 (8%) samples has HPV type 52 present exclusively. The next most frequent types were 61 (26%), 62 (26%), 58 (23.3%), 53 (20.0%), 84 (18.7%), 35 (18%), and 81 (18%). Genotypes 16 (17.3%) and 18 (13.3%) were ranked 4^{th} and 7^{th} among high risk (oncogenic) types, respectively. We found no HPV infections in four (2.7%) women, a single genotype in 15 (10.0%) women, and multiple genotypes ($\geq$2) in 131 (87.3%) women (median = 4, mean = 4.37 (S.D. $\pm$0.45), range = 0-13 types per woman).

Of the 656 types detected, 290 (44.2%) were high-risk, 60 (9.1%) were probable high-risk, 167 (25.5%) were low-risk, 139 (21.2%) were of undefined risk. None of the women carried a high-risk or a probably high-risk type exclusively (i.e., there was

always a low risk / undefined risk type present concurrently in all specimens). Two women (1.3%) carried only low-risk genotypes and five women (3.3%) carried only HPV types of undefined risk. There was a significant trend for an increase in the proportion of HPV positivity for any type of HPV and for high-risk types of HPV with increasing severity of cytology (two-tailed p-value via Chi-square for trend: 0.004 and <0.001 respectively; Table 2). The mean number of all HPV types and high-risk HPV types increased with increasing severity of cytology (p=0.05 and p=0.002, respectively). We examined the association between CD4+ cell counts and HPV types in 145 women (96.7%) for whom a CD4+ cell count was obtained. Mean CD4+ cell count was 210/μL and the median CD4+ count was 167/μL. Increasing proportions of women harbored both "any HPV type" and "high-risk HPV type" with decreasing CD4+ cell counts (test for trend, both p = 0.01; Table 4). The mean numbers of both "any" and "high-risk" HPV type increased significantly with declining CD4+ cell counts (both p = 0.02).

DISCUSSION

Using DNA PCR, this study found one of the highest reported HPV rates ever seen (97.3%). Other studies of HIV-infected women using PCR report between 54-98% HPV prevalence in Brazil and the U.S. (10,27-29) Our high HPV prevalence reflects both the very high sensitivity of the PGMY 09/11 primer system and the extraordinary risk that Zambian women with HIV face. High sensitivity of PCR permits detection of HPV DNA in samples with a low (or even non-viable) HPV viral load; some of these might be judged HPV negative (viral DNA absent) with

alternative primer sets or methods. The PCR methods used in the present study have been validated in diverse patient populations (21, 22, 26). The assay is reproducible and accurate when used with different clinical samples from the same patient or in follow-up samples (21, 30). Direct comparison between PCR and other HPV assays has shown a high level of agreement, including among women who harbor multiple HPV genotypes (30-32). In the present study, all negative and positive controls yielded the appropriate results, suggesting our results to be internally valid. Since our patients came for HIV care and many were placed on ART, results may not apply to less ill (i.e. relatively immunocompetent) women. Our results were nonetheless similar to studies elsewhere in sub-Saharan Africa, i.e., Kenya, Zimbabwe, and Mozambique. (26, 33, 34) Our women were in public sector care at a hospital in Lusaka that provides both primary and referral care and were selected consecutively among new or follow-up patients seeking HIV-related care.

The results of the HPV genotyping show a peculiar distribution. Most remarkably, low-risk and undefined-risk HPV types (mainly HPV 61, 62, 84, 81) were considerably higher in their prevalence than HPV 16 and 18, which are the most commonly reported types worldwide (36). Even other high-risk types, HPV 58, 52 (with 33, 35, 58 confections), 35, 45, and 53 (probable high-risk) were more common than types 16 and 18. A US study (28) reported that HPV 53, 58, and 61 were the most prevalent types in HIV-infected women, types that were also more common in our population. In contrast, other studies (29,35) have reported that types 16 and 18 were the most prevalent types in HIV-infected women in other settings, just as is found in HIV-uninfected women. (28). There may be considerable differences in the

spectrum and prevalence of HPV genotypes in HIV-infected women from different geographic origins, due perhaps to differing behavioral and socioeconomic characteristics or to male factors.

The majority of participants (87.3%) in our study carried multiple genotypes. Comparable studies in HIV-infected women from Brazil and the U.S. have reported 12-79% of study participants to have multiple HPV types (10, 27-29). This may reflect frequent exposure of these patients to multiple HPV types due to sexual contact or may reflect an HIV-impaired immune system that fails to clear HPV, increasing its persistence. (37) If HPV replication is more efficient in an immunodeficient host, higher viral replication will make persistence more likely since HPV clearance is reduced in HIV-infected women (38-41).

Our study did not attempt to correlate the HIV-1 viral load with the change in the relative composition of HPV-types because validated viral load assays (i.e., that had passed international proficiency tests) were not available in Zambia at the time of the study. Nonetheless, our observation that the increased prevalence of infection with multiple HPV types with decreasing CD4+ cell counts suggests the increasing risk of women for cervical disease with advancing HIV-disease state. We speculate that the relative composition of HPV types may differ among HIV-infected women as their immunocompetence is affected either by advancing disease or immune restoration from antiretroviral therapy. (3, 6)

A Brazilian study (27) reported a higher number of HPV types with CD4+ cell count >350/μL whereas our study found more types in women with lower CD4+ cell counts. Most HIV-infected Brazilian women had been receiving ART for > 1 year,

resulting in higher CD4+ cell counts without immunological success in clearing HPV infections. In contrast, the majority of our study population had been receiving ART for < 2 months such that our mean CD4+ cell count was 210/ μL and median was 167/μL.

Numbers of HPV types/woman were higher with the increasing grade of cytological results. The majority of patients with ASCUS, LSIL and HSIL harbored multiple HPV types, suggesting that increasing severity is due to immunosuppression-associated, persistent HPV infection.

With HPV vaccines awaiting final licensure and may make their way into the markets in the very near future, we are concerned that without cross-type immunity, the impact of the current vaccines in this high-risk population may not be as good as that seen in published clinical trials among HIV-uninfected women.(42,43) High cost of the vaccine and the limited valency (HPV 16 and 18 are the only two high risk types targeted by the vaccine) suggests that new HPV vaccine constructs ma be needed for women in developing nations for the primary prevention and eventual control of cervical cancer.

REFERENCES

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a

necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189(1):12-9.

2. Munoz N, Bosch F, Diaz M, De Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJF, Meijer CJLM. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N EngL J Med* 2003;348:518-527.
3. Broker TR, Jin G, Croom-Rivers A, Bragg SM, Richardson M, Chow LT, Vermund SH, Alvarez RD, Pappas PG, Squires KE, Hoesley CJ. Viral latency--the papillomavirus model. *Dev Biol (Basel.* 2001;106:443-51;
4. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004;324(1):17-27.
5. Vermund SH, Kelley KF, Klein RS, Feingold AR, Schreiber K, Munk G, Burk RD. High risk of human papillomavirus infection and cervical squamous intraepithelial lesions among women with symptomatic human immunodeficiency virus infection. *Am J Obstet Gynecol.* 1991;165(2):392-400
6. Sun XW, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright TC Jr. Human papillomavirus infection in women infected with the human immunodeficiency virus. *N Engl J Med* 1997; 337: 1343-9.
7. Fruchter RG, Maiman M, Arrastia CD, Matthews R, Gates EJ, Holcomb K. Is HIV infection a risk factor for advanced cervical cancer? *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; 18: 241-5.

8. Heard I, Palefsky JM, Kazatchkine MD. The impact of HIV antiviral therapy on human papillomavirus (HPV) infections and HPV-related diseases. *Antivir Ther*. 2004 ;9(1):13-22.

9. Tate DR, Anderson RJ. Recrudescence of cervical dysplasia among women who are infected with the human immunodeficiency virus: a case-control analysis. *Am J Obstet Gynecol* 2002; 186: 880-2.

10. T.V. Ellerbrock, M.A. Chiasson, T.J. Bush, X.W. Sun, D. Sawo, K. Brudney et al., Incidence of cervical squamous intraepithelial lesions in HIV-infected women. *JAMA* 2000, 283: 1031–7.

11. Report of the Global HIV/AIDS Epidemic, 2005, UNAIDS. Available online at http://www.unaids.org. Accessed on March 10, 2006

12. The President's Emergency Plan for AIDS Relief: Five-Year Strategy. USAID publication. Available online at http://www.state.gov/documents/organization/48746.pdf Accessed on March 10, 2006

13. Franco EL, Harper DM. Vaccination against human papillomavirus infection: a new paradigm in cervical cancer control. *Vaccine*. 2005;23(17-18):2388-94.

14. Goldie SJ, Kohli M, Grima D, Weinstein MC, Wright TC, Bosch FX, Franco E. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. *J Natl Cancer Inst.* 2004;96(8):604-15.

15. HIV/AIDS in Zambia: The Epidemic and its impact: AVERT web publication. Available online: http://www.avert.org/aids-zambia.htm. Accessed on March 10, 2006

16. Hubbard RA. Human papillomavirus testing methods. *Arch Pathol Lab Med* 2003;127(8):940-5.

17. HC2 [product insert]. Hybrid Capture 2 DNA Test, L0838 vol. Gaithersburg, Md; Digene Corp; 2002:49

18. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. *J Natl Cancer Inst* 2000;92:397–402.

19. Schiffman M, Herrero R, Hildesheim A. et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. *JAMA* 2000;283:87–93

20. Lorincz AT, Richart RM. Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. *Arch Pathol Lab Med.* 2003;127(8):959-68.

21. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, Schiffman MH, Scott DR, Apple RJ. Improved amplification of genital human papillomaviruses. *J Clin Microbiol.* 2000;38(1):357-61.

22. Coutlee F, Gravitt P, Kornegay J. et al. Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. *J Clin Microbiol* 2002;40:902–907

23. Jacobs MV, Walboomers JM, Snijders PJ, Voorhorst FJ, Verheijen RH, Fransen-Daalmeijer N, Meijer CJ. Distribution of 37 mucosotropic HPV types

in women with cytologically normal cervical smears: the age-related patterns for high-risk and low-risk types. *Int J Cancer.* 2000;87(2):221-7.

24. Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter SchoggetJ, Lindeman J, ter Harmsel B, Burger M, Quint W. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *J Clin Microbiol.* 1999;37(8):2508-17.
25. Kleter B, van Doorn LJ, ter Schegget J, Schrauwen L, van Krimpen K, BurgerM, ter Harmsel B, Quint W. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. *Am J Pathol.* 1998;153(6):1731-9.
26. De Vuyst H, Steyaert S, Van Renterghem L, Claeys P, Muchiri L, Sitati S, Vansteelandt S, Quint W, Kleter B, Van Marck E, Temmerman M. Distribution of human papillomavirus in a family planning population in Nairobi, Kenya. *Sex Transm Dis.* 2003;30(2):137-42.
27. Levi JE, Kleter B, Quint WG, Fink MC, Canto CL, Matsubara R, Linhares I, Segurado A, Vanderborght B, Neto JE, Van Doorn LJ. High prevalence of human papillomavirus (HPV) infections and high frequency of multiple HPV genotypes in human immunodeficiency virus-infected women in Brazil. *J Clin Microbiol.* 2002;40(9):3341-5.
28. Palefsky, J. M., H. Minkoff, L. A. Kalish, A. Levine, H. S. Sacks, P. Garcia, M. Young, S. Melnick, P. Miotti, R. Burk.. Cervicovaginal human

papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. *J. Natl. Cancer Inst.* 1999;1:226-36

29. Goncalves MA, Massad E, Burattini MN, Villa LL. Relationship between human papillomavirus (HPV) genotyping and genital neoplasia in HIV-positive patients of Santos City, Sao Paulo, Brazil. *Int J STD AIDS.* 1999;10(12):803-7.
30. Molijn A, Kleter B, Quint W, van Doorn LJ. Molecular diagnosis of human papillomavirus (HPV) infections. *J Clin Virol.* 2005;32 Suppl 1:S43-51.
31. Vernon SD, Unger ER, Williams D. Comparison of human papillomavirus detection and typing by cycle sequencing, line blotting, and hybrid capture. *J Clin Microbiol* 2000;38:651–655
32. van Doorn LJ, Quint W, Kleter B, Molijn A, Colau B, Martin MT, Kravang-In, Torrez-Martinez N, Peyton CL, Wheeler CM. Genotyping of human papillomavirus in liquid cytology cervical specimens by the PGMY line blot assay and the SPF(10) line probe assay. *J Clin Microbiol.* 2002;40(3):979-83.
33. Womack SD, Chirenje ZM, Gaffikin L, Blumenthal PD, McGrath JA, Chipato T, Ngwalle S, Munjoma M, Shah KV. HPV-based cervical cancer screening in a population at high risk for HIV infection. *Int J Cancer.* 2000;85(2):206-10.
34. Castellsague X, Menendez C, Loscertales MP, Kornegay JR, dos Santos F, Gomez-Olive FX, Lloveras B, Abarca N, Vaz N, Barreto A, Bosch FX, Alonso P. Human papillomavirus genotypes in rural Mozambique. *Lancet.* 2001;358(9291):1429-30.

35. Bollen LJ, Chuachoowong R, Kilmarx PH, Mock PA, Culnane M, Skunodom N, Chaowanachan T, Jetswang B, Neeyapun K, Asavapiriyanont S, Roongpisuthipong A, Wright TC, Tappero JW. Human Papillomavirus (HPV) Detection Among Human Immunodeficiency Virus-Infected Pregnant Thai Women: Implications for Future HPV Immunization. *Sex Transm Dis.* 2006; 33 (e-Pub ahead of print)

36. Clifford GM, Gallus S, Herrero R, Munoz N, Snijders PJ, Vaccarella S, Anh PT, Ferreccio C, Hieu NT, Matos E, Molano M, Rajkumar R, Ronco G, de Sanjose S, Shin HR, Sukvirach S, Thomas JO, Tunsakul S, Meijer CJ, Franceschi S; IARC HPV Prevalence Surveys Study Group. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet.* 2005;366(9490):991-8.

37. Brown, D. R., J. M. Schroeder, J. T. Bryan, M. H. Stoler, K. H. Fife.. Detection of multiple human papillomavirus types in condylomata acuminata lesions from otherwise healthy and immunosuppressed patients. *J. Clin. Microbiol.* 1999 37:3316-3322.

38. Ahdieh L, Munoz A, Vlahov D, Trimble CL, Timpson LA, Shah K. Cervical neoplasia and repeated positivity of human papillomavirus infection in human immunodeficiency virus-seropositive and -seronegative women.*Am J Epidemiol.* 2000;151(12):1148-57.

39. Ahdieh-Grant L, Li R, Levine AM, Massad LS, Strickler HD, Minkoff H, Moxley M, Palefsky J, Sacks H, Burk RD, Gange SJ. Highly active

antiretroviral therapy and cervical squamous intraepithelial lesions in human immunodeficiency virus-positive women. *J Natl Cancer Inst.* 2004;96(14):1070-6.

40. Lillo FB, Ferrari D, Veglia F, Origoni M, Grasso MA, Lodini S, Mastrorilli E, Taccagni G, Lazzarin A, Uberti-Foppa C. Human papillomavirus infection and associated cervical disease in human immunodeficiency virus-infected women: effect of highly active antiretroviral therapy. *J Infect Dis.* 2001;184(5):547-51.
41. Delmas MC, Larsen C, van Benthem B, Hamers FF, Bergeron C, Poveda JD, Anzen B, van den Hoek A, Meier F, Pena JM, Savonius H, Sperandeo D, Suligoi B, Vernazza P, Brunet JB. Cervical squamous intraepithelial lesions in HIV-infected women: prevalence, incidence and regression. European Study Group on Natural History of HIV Infection in Women. *AIDS* 2000; 14(12):1775-84.
42. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, Chiacchierini LM, Jansen KU; Proof of Principle Study Investigators. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002;347(21):1645-51.
43. Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, Zahaf T, Innis B, Naud P, De Carvalho NS, Roteli-Martins CM, Teixeira J, Blatter MM, Korn AP, Quint W, Dubin G; GlaxoSmithKline HPV Vaccine Study Group. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of

infection with human papillomavirus types 16 and 18 in young women: a randomized controlled trial. *Lancet* 2004;364(9447):1757-65.

Table 1: Cytology and human papillomavirus (HPV) types, grouped by their carcinogenic risk

HPV types	Frequency (%of)	*Cytology results*				
		Normal (n=10)	ASCUS[a] (n=26)	LSIL[b] (n=35)	HSIL[c] (n=49)	SCC[d] (n=30)
Any High risk types	128 (85.3%)	3	17	32	46	30
HPV 16	26 (17.3)	0	6	2	7	11
HPV 18	20 (13.3)	2	3	4	5	6
HPV 31	21 (14.0)	0	2	3	10	6
HPV 33	12 (8.0)	0	0	4	4	4
HPV 35	27 (18.0)	2	4	6	6	9
HPV 39	19 (12.7)	1	3	4	8	3
HPV 45	26 (17.3)	0	3	6	12	5
HPV 51	22 (14.7)	0	2	5	9	6
HPV 52[e]	12 (8.0)	0	3	5	3	1
HPV 52[f]	57 (38.0)	2	8	14	19	14
HPV 56	19 (12.7)	1	1	6	7	4
HPV 58	35 (23.3)	1	2	7	15	10
HPV 59	12 (8.0)	0	1	6	1	4
HPV 68	20 (13.3)	1	3	5	7	4
HPV 73	12 (8.0)	0	2	5	3	2
HPV 82	7 (4.7)	0	1	1	5	0
Any Probable HR types	52 (34.7%)	1	8	14	18	11
HPV 26	9 (6.0)	0	0	2	4	3
HPV 53	30 (20.0)	1	6	6	10	7
HPV 66	21 (14.0)	0	2	10	6	3
Any Low-risk types	90/150 (60%)	4	16	24	30	16
HPV 6	7 (4.7)	0	0	3	3	1

Table 1 (contd.)

HPV types	Frequency (%of)	*Cytology results*				
		Normal (n=10)	ASCUS[a] (n=26)	LSIL[b] (n=35)	HSIL[c] (n=49)	SCC[d] (n=30)
Any low risk types (cont.)						
HPV 11	1 (0.7)	1	0	0	0	0
HPV 40	9 (6.0)	1	1	3	3	1
HPV 42	17 (11.3)	1	3	5	4	4
HPV 54	13 (8.7)	1	4	3	2	3
HPV 61	39 (26.0)	1	7	12	14	5
HPV 70	19 (12.7)	1	4	4	5	5
HPV 72	14 (9.3)	0	3	4	3	4
HPV 81	27 (18.0)	1	2	10	11	3
CP 6108[g]	21 (14.0)	1	4	6	5	5
Any HPV of undefined risk	90/150 (60%)	5	14	24	30	17
HPV 55	14 (9.3)	0	5	3	3	3
HPV 62	39 (26.0)	2	5	7	18	7
HPV 64	0 (0.0)	0	0	0	0	0
HPV 67	9 (6.0)	0	1	3	4	1
HPV 69	2 (1.3)	0	0	1	0	1
HPV 71	23 (15.3)	2	4	3	11	3
HPV 83	16 (10.7)	1	3	6	4	2
HPV 84	28 (18.7)	2	2	9	8	7
IS 39[h]	8 (5.3)	0	1	3	3	1

Note: [a] ASCUS: Atypical Squamous Cells of Undetermined Significance, [b] LSIL: Low-grade Squamous Intraepithelial Lesions, [c] HSIL: High-grade Squamous Intraepithelial Lesions, [d] SCC: Squamous Cell Carcinoma, [e] and [f]: Note: HPV 52 cross reacts with HPV 33, 35, and 58. Thus, [e] is HPV 52 alone and [f] is HPV 52 and HPV type 33, 35, and/or 58 co-infection.

Table 2: Proportion of HPV type positivity with increasing severity of cytological results

Cytology result	*n (% of 150)*	*Any HPV type*	*High-risk HPV types*
Normal	10 (6.7)	8 (80.0%)	3 (30.0%)
ASCUS	26 (17.3)	25 (96.2%)	17(65.4%)
LSIL	35(23.3)	34 (97.1%)	32(91.4%)
HSIL	49 (32.6)	49 (100%)	46 (93.9%)
SCC	30 (20.0)	30 (100%)	30 (100%)
X^2 for trend[a]		8.2	32.5
p-value[b]		*0.004*	*<0.001*

Note: [a]Mantel-Haenszel Chi-square test of trend, [b]2 tailed p-value

Table 3: Numbers of HPV types (mean for "any" HPV type and for "high-risk" HPV type) according to categories of cytology results

Cytology	*Mean number of "any" HPV types, 95% CI*[a]	*Mean number of "high-risk" HPV types, 95 %CI*[a]
Normal	2.40 (0.11-4.69)	0.800 (-0.358-1.958)
ASCUS	3.58 (2.67-4.48)	1.385 (0.790-1.979)
LSIL	4.91 (3.93-5.90)	1.971 (1.543-2.400)
HSIL	4.55 (3.83-5.28)	2.082 (1.736-2.428)
SCC	4.80 (3.65-5.95)	2.500 (1.974-3.026)
Overall	*4.37 (3.92-4.82)*	*1.933 (1.709-2.158)*
p-value[b]	*0.05*	*0.002*

Note: [a] 95% confidence interval, two-tailed, [b] Two-tailed p-value by analysis of variance

Table 4: Proportion of HPV type positivity with decreasing immunocompromised state (reflected by decreasing CD4+ cell counts)

CD4+ cell count/μL	*N (% of 145)*	*Any HPV types*	*High-risk HPV types*
>=500	11 (7.5)	10 (90.9%)	8 (72.7%)
200-499	43 (29.6)	40 (93%)	33 (76.7%)
<200	91 (62.7)	91 (100%)	83 (91.2%)
X^2 for trend[a]		6.580	6.011
p-value[b]		*0.013*	*0.014*

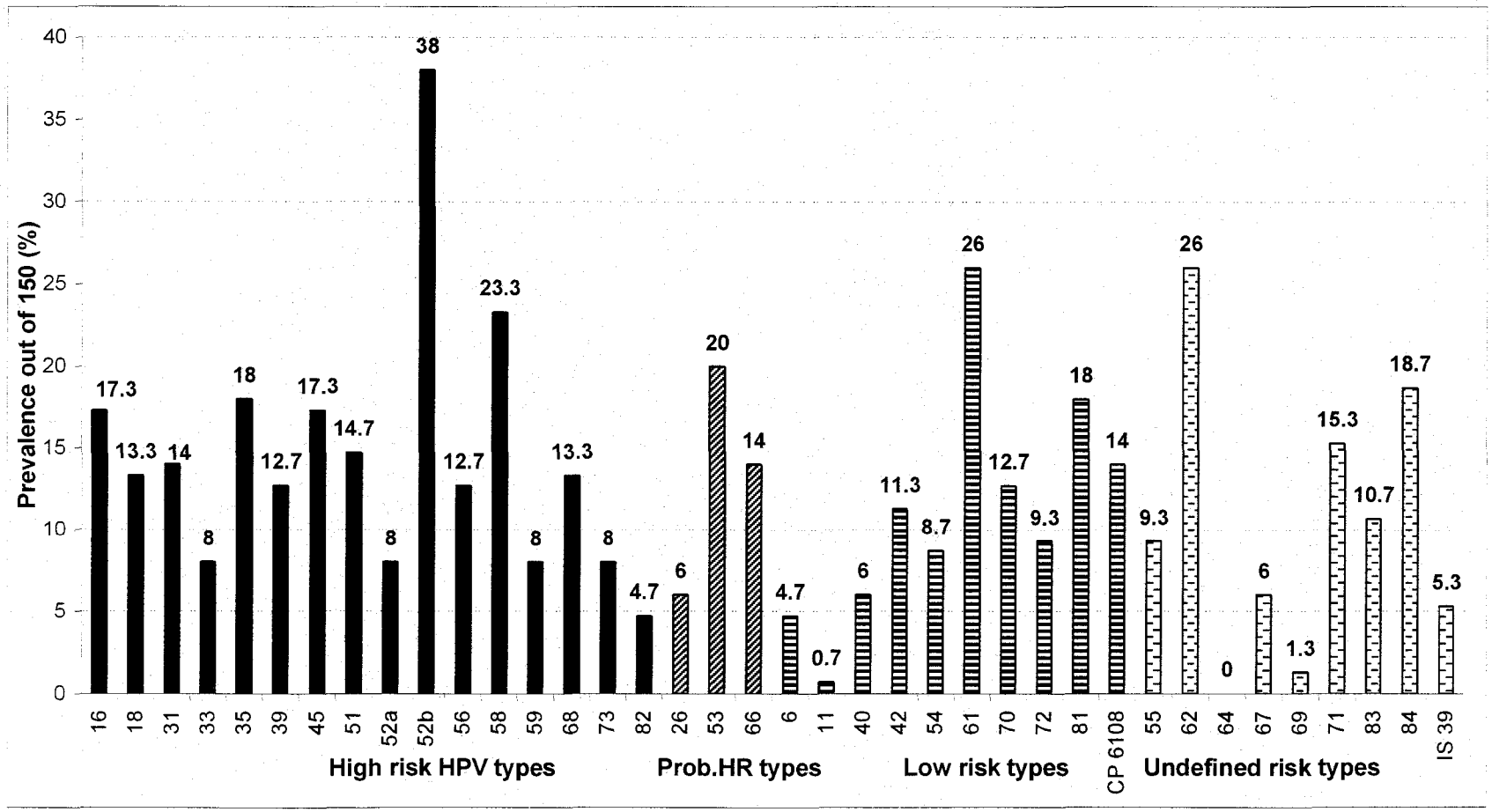
Note: [a]Mantel-Haenszel Chi-square test of trend, [b]2 tailed p-value

Table 5: Mean numbers of HPV types (any type and high-risk HPV type) according to categories of CD4+ cell counts:

CD4+ cell count	*Mean number of any HPV type, 95% CI*[a]	*Mean number of high-risk HPV type, 95 %CI*[a]
>=500	4.86 (4.25-5.46)	2.165 (1.865-2.465)
200-499	3.70 (2.93-4.46)	1.628 (1.225-2.031)
<200	3.09 (1.70-4.48)	1.182 (0.595-1.769)
Overall	*4.38 (3.92-4.84)*	*1.931 (1.702-2.160)*
p-value[b]	*0.021*	*0.020*

Note: [a] 95% confidence interval, two-tailed, [b] Two-tailed p-value by analysis of variance

Figure 1: Distribution of HPV genotypes (n=656) detected by PCR in 150 HIV-infected women. The percentage indicates the proportion of the participants in which a particular HPV genotype was detected.



Note: HPV 52 cross reacts with HPV 33, 35, and 58. Thus, 52a is HPV 52 alone and 52b is HPV 52 and HPV type 33/35/58 co-infection.

CONCLUSION

This dissertation has highlighted the critical importance of cervical cancer screening among HIV-infected women living in resource limited settings. As antiretroviral therapy for HIV/AIDS becomes widely available, HIV-infected women may in fact live long enough, only to be at additional risk for development of malignancies like cervical cancer. It is thus critical to screen these women with increased periodicity, just as women living in western, industrialized settings.

Standards of care for cervical cancer screening have relied on the microscopic detection of cellular preneoplastic changes through cytology-based screening (Pap smears). Cervical cancer incidence has plummeted wherever cytology-based screening programs have been implemented effectively. However, it has been difficult to establish and sustain such programs in resource-limited settings like Zambia due to lack of resources and manpower, coupled with the lack of awareness and education.

The first manuscript of this dissertation used meta-analytic techniques to review and report results of studies conducted worldwide to measure the accuracy of cervical cytology to detect CIN in HIV-infected women. Pooled estimates of sensitivity and specificity for cervical cytology have been reported in this study at two clinically relevant thresholds ($\geq$CIN1 and $\geq$ CIN 2). These estimates support the contention that cervical cytology is not a very accurate test among HIV-infected women, even in the most controlled settings in the industrialized world. This highlights the critical need to

undertake research on alternative screening tests and protocols, especially for high-risk HIV-infected women.

Although VIA has gained recognition as a promising alternative to cervical cytology for screening in resource limited settings, none of the studies report the measures of accuracy of VIA among HIV-infected women. The second manuscript of this dissertation reports the first estimates of accuracy of VIA compared to cytology for screening HIV-infected women in a resource limited setting. We report that VIA had a slightly higher specificity than cytology (62.1% vs. 58.6%), although there was a loss of sensitivity (73.5% vs. 91.2%), although none of these differences were statistically significant at our sample size of 150 women. Combining VIA and cytology in two-stage series combination algorithm achieved greatest test efficiency. These results suggest that VIA has the potential to serve as a useful adjunct or alternative to cytology for HIV-infected women in settings like Zambia, where access to health care is limited and resources to screen for cervical cancer are very scarce. The findings of this study need to be replicated in additional settings and confirmed in randomized prevention clinical trials.

The third manuscript analyzes the prevalence and types of genital HPV in the women participating in our cervical cancer screening study in Zambia. Fully 146 of 150 women had human papillomavirus (HPV) detected on a cervical swab tested by polymerase chain reaction (PCR), 87.3% of whom harbored multiple types. HPV prevalence was found to be correlated with increasing severity of cytological lesions and degree of immunosuppression. The relative prevalence of various HPV types was different from that reported elsewhere, e.g., types 16 and 18 were far less common than expected. Prophylactic vaccines against HPV may be available for use in resource limited settings

in the next few years, but unless cross-type immunity is demonstrated, their utility may be limited in areas or populations where types 16 and 18 are uncommon. More importantly, the findings of this research highlight the fact that it is important to study the natural history of HPV-mediated cervical disease in HIV-infected women, especially since HPV infections are transient and highly correlated with the level of immunosuppression.

The studies described in this dissertation have several strengths. The systematic review described uses a very sensitive electronic search algorithm reinforced by additional measures to ensure completeness of data. We report the pooled estimates of sensitivity, specificity and efficiency, and also perform sub-group meta analysis to elucidate the impact of various patient and study characteristics on the outcomes. The cross-sectional study in Zambia used a composite colposcopic-histological 'gold standard' on all participants thereby minimizing verification bias. We have compared VIA performed under field settings to an extremely sophisticated cytological assessment in the US. The usefulness and relative accuracy estimates of VIA has been reinforced in such a rigorous comparison, pointing to its utility in the real-world setting. Finally, the PCR assay allowed us to detect the presence of 37 different types of HPV in this high-risk population, which are not yet reported from a similar population in sub-Saharan Africa. The availability of cytology results and CD4+ cell counts also allowed us to compare their association with their cervical disease state and immunological status. The findings of this study also need to be replicated in other settings to confirm the relatively lower preponderance of HPV types 16 and 18.

Among the limitations to this research, the number of studies included in the meta-analysis at both thresholds were small, mainly due to the nature of previous studies that were either not designed to calculate accuracy of cytology (but provided the results nevertheless) or due to the fact that we omitted studies which had zero cell values for TP, FP, FN, or TN. In our Zambian cross sectional study, we were unable to compare VIA against conventional cytology performed in Zambia, largely because of the lack of trained cytotechnologists to read the Pap smears. However this situation is not at all uncommon in settings like Zambia, and in fact point to the increased need for alternative screening tools like VIA. Finally in the study on HPV typing, we have used an investigational assay (Roche Linear array PCR) that has not yet received FDA approval. However, the primer set used for the PCR has been extensively validated worldwide, thus we are confident that our findings could be reproducible elsewhere.

In conclusion, the global literature suggests that cytology is suboptimally sensitive in HIV-infected women for identifying CIN. VIA is a highly acceptable screening tool for HIV-infected women in Zambia. While its sensitivity was slightly lower than for cytology, the fact that one can provide VIA screening and treatment on the same visit suggests that it may actually result in more salutary outcomes. HPV is almost universally found using PCR in HIV-infected women, but types 16 and 18 are not common in this Zambian population, suggesting the need for studies of the utility of newly available HPV vaccines. Long-term cohort studies to better understand the natural history of HPV-induced cervical neoplastic disease and randomized prevention clinical trials of screening methods for HIV-infected women in resource-limited settings are needed.

APPENDIX A

APPROVALS FROM INSTITUTIONAL REVIEW BOARD AND RESEARCH ETHICS COMMITTEE

UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office of Human Research Protections (OHRP). The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56 and ICH GCP Guidelines. The Assurance became effective on November 24, 2003 and the approval period is for three years. The Assurance number is FWA00005960.

Principal Investigator: PARHAM, GROESBECK

Co-Investigator(s):

Protocol Number: F040406003

Protocol Title: *Cervical Cancer Screening Among HIV-Infected Women in Zambia*

The IRB reviewed and approved the above named project on 4/14/2004. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received FULL COMMITTEE review.

IRB Approval Date: 4/14/2004

Date IRB Approval Issued: 06.24.04

Identification Number: IRB00000196

Albert Oberman, M.D., M.P.H.
Albert Oberman, M.D., MPH
Vice Chair
Board Albert Oberman, M.D., M.P.H.

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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Assurance No. FWA00000338
IRB00001131 of IOR G0000774

Ref: 001-05-04
22 June, 2004

Dr Groesbeck Parham, MD
Department of Obstetrics and Gynaecology
University of Alabama at Birmingham
Alabama, USA

Dear Dr Parham,

RE: SUBMITTED RESEARCH PROPOSAL

The following research proposal was presented to the Research Ethics Committee Meeting on 9 June, 2004, where changes were recommended. We would like to acknowledge receipt of the corrected version. The proposal has now been approved. Congratulations!

Title of proposal: 'Cervical Cancer Screening among HIV-infected women in Zambia'

Conditions:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this committee every six months and a final copy of your report at the end of the study.

Please note that the HIPAA requirement does not apply in this country at present.

Yours sincerely,

Prof. J. T. Kirnashani, MB, ChB, PhD
CHAIRMAN
RESEARCH ETHICS COMMITTEE

Date of approval: 22 June, 2004 Date of Expiry: 21 June, 2005
Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a progress report (Progress Report Forms can be obtained from the Secretariat).

APPENDIX B

ELECTRONIC RETRIEVAL STRATEGY

Electronic retrieval strategy (Manuscript 1)

((Human Immunodeficiency Viruses) OR (HIV) OR (HIV infections) OR (HIV seropositivity) OR (HIV seroprevalence) OR (Acquired Immunodeficiency Syndrome) OR (AIDS) OR (HIV-Related Opportunistic Infections) OR (Opportunistic Infections, AIDS-Related)) AND ((Cervical Cancer) OR (Cervical Intraepithelial Neoplas*) OR (Cervical Intraepithelial Neoplasia, Grade III) OR (Neoplas*, Cervical Intraepithelial) OR (Intraepithelial Neoplas*, Cervical) OR (Neoplas*, Cervical Intraepithelial) OR (Carcinoma in Situ) OR (Cervix Dysplas*) OR (Dysplas*, Cervix) OR (Carcinoma*, squamous cell) OR (Squamous Cell Carcinoma*) OR (Carcinoma*, squamous) OR (Planocellular Carcinoma*) OR (Epidermoid Carcinoma*) OR (Condition*, Precancerous) OR (Precancerous Condition*) OR (Condition*, Preneoplastic) OR (Preneoplastic Condition) OR (Infection*, Tumor Virus) OR (Tumor Virus Infection*) OR (Human Papillomavirus*) OR (Papillomavirus*, Human) OR (Papilloma Virus*, Human) OR (Infection*, Papovaviridae) OR (Papovaviridae Infection*)) AND ((Mass Screening) OR (Screening*, Mass) OR (Screening*) OR (Sensitivity and Specificity) OR (Sensitivity) OR (Specificity) OR (Predictive Value of Tests) OR (ROC Curve) OR (Curve*, ROC) OR (ROC Analys*) OR (Analys*, ROC) OR (Receiver Operating Characteristic*) OR (Characteristic*, Receiver Operating) OR (Surveillance) OR (Epidemiolog*) OR (Epidemiologic* method*) OR (Epidemiologic* Stud*) OR (Stud*, Epidemiologic*) OR (Case-Control Stud*) OR (Stud*, Case-Control) OR (Retrospective Stud*) OR (Stud*, Retrospective) OR (Cohort Stud*) OR (Stud*, Cohort) OR (Follow-Up Stud*) OR (Stud*, Follow-Up) OR (Longitudinal Stud*) OR (Stud*, Longitudinal) OR (Prospective Stud*) OR (Stud*, Prospective) OR (Cross-Sectional Stud*) OR (Stud*, Cross-Sectional) OR (Seroepidemiologic* Studies) OR (Stud*, Seroepidemiologic*) OR (Controlled Trial, Randomized) OR (Trial, Randomized Controlled) OR (Randomized Controlled Trials) OR (Comparative Stud*) OR (Risk Factor*) OR (Cytolog*) OR (Preparation Techni*, Histocytologic) OR (Histocytologic* preparation techni*) OR (Techni*, Cytohistologic* Preparation) OR (Vaginal Smears) OR (Papanicolaou Smear) OR (Smear*, Vaginal) OR (Smear*, Papanicolaou) OR (Papanicolaou Test) OR (Test, Papanicolaou) OR (Colposcop*) OR (diagnos*[ti]) OR (imaging[ti]) OR (pathology[ti]) OR (histopatholog*[ti]) OR (biopsy[ti]) OR (etiology[ti]) OR (detect*[ti]) OR (pathohistology[ti]) OR (colposcop* [ti]) OR (visual*[ti])) AND (human) AND (female)

APPENDIX C

STATISTICAL METHODS AND FORMULAE FOR META-ANALYSIS OF SCREENING/DIAGNOSTIC TESTS

Statistical methods and formulae for Meta-Analysis of screening/diagnostic tests

Unlike controlled trials, in meta-analysis of diagnostic tests, each study is summarized by a pair of statistics (usually sensitivity and specificity) that measures the test's accuracy. Then the overall test accuracy indexes are calculated as the weighted average of these summary statistics. (1, 3) Meta-analysis for diagnostic studies are only performed when studies have recruited clinically similar patients and have used comparable experimental and reference tests. The following methods were used for this meta-analysis that was done using the MetaDiSc software.

i) summarizing data from each individual study,

ii) investigating the homogeneity of studies graphically and statistically,

iii) computing the pooled indexes and

1. **Summary statistics in individual studies**

The results of each individual study were presented in a 2 x 2 table (Table 1) showing the number of people who have been classified as positive and negative by the experimental test among the groups of participants with and without disease according the reference test.

	Reference Test (Colposcopic-histologic diagnosis)		
Screening test (cytology)	With Disease (≥ CIN1 / 2)	Without disease (≥ CIN1 / 2)	Total
Test positive	***a***	***b***	***P***
Test negative	***c***	***d***	***N***
	D	***ND***	***T***

a= number of participants with disease who test positive: True Positives (TP)

b= number of participants without disease who test positive: False Positives (FP)

c= number of participants with disease who test negative: False Negaives (FN)

d= number of participants without disease who test negative: True Negatives (TN)

P= total number of participants with a positive test result

N= total number of participants with a negative test result

D= total number of participants with disease

ND= total number of participants without disease

T = total number of participants screened

Accuracy was by *sensitivity* (proportion of positives among people with disease) and *specificity* (proportion of negatives among people without disease).

Sens = a / D

Spec = d / ND

Another measure of the test accuracy, useful in meta-analysis, is the diagnostic odds ratio (DOR)

DOR = [Sens/(1-Spec)] / [(1-Spec)/Sens]

Also, DOR = (a/b) / (c/d), or a*d / b*c

The DOR expresses how much greater the odds of having the disease are for the people with a positive test result than for the people with a negative test result. It is a single measure of diagnostic test performance that combines both sensitivity and specificity.

Standard errors and confidence intervals of individual indexes

The confidence intervals of sensitivity and specificity were calculated using the F distribution method to compute the exact confidence limits for the binomial proportion (x/n). (2)

$$LL = \left(1 + \frac{n-x+1}{xF_{2x,2(n-x+1),1-\alpha/2}}\right)^{-1} \qquad UL = \left(1 + \frac{n-x}{(x+1)F_{2(x+1),2(n-x),\alpha/2}}\right)^{-1}$$

The distribution of logarithm of the diagnostic odds ratio is also approximately normal, with standard error given [1] by

$$SE(\ln DOR) = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$$

Thus the confidence interval of the DOR is

$$DOR\, e^{\pm z_{\alpha/2} SE(\ln DOR)}$$

2. Investigating the homogeneity of studies graphically and statistically,

The degree of variability among study results were evaluated graphically by plotting the sensitivity and specificity from each study on a forest plot. Some divergence was to be expected by chance, but variation in other factors may influence the observed heterogeneity.

The homogeneity of the sensitivities and specificities can also be tested as belows: (The subscripts i to designate an individual study and T for overall index). (4)

$$G^2_{Sen} = 2\sum_i \left(a_i \ln \frac{a_i}{\frac{a_T \times D_i}{D_T}} + c_i \ln \frac{c_i}{\frac{c_T \times D_i}{D_T}} \right) \qquad a_T = \sum_i a_i \quad c_T = \sum_i c_i \quad D_T = \sum_i D_i$$

$$G^2_{Spe} = 2\sum_i \left(d_i \ln \frac{d_i}{\frac{d_T \times ND_i}{ND_T}} + b_i \ln \frac{b_i}{\frac{b_T \times ND_i}{ND_T}} \right) \qquad b_T = \sum_i b_i \quad d_T = \sum_i d_i \quad ND_T = \sum_i ND_i$$

The homogeneity of diagnostic odds ratios was tested using Cochran's Q test based upon inverse variance weights [1], which also has a chi-squared distribution with k-1 degrees of freedom.

$$Q = \sum_i w_i \left(\ln \theta_i - \ln \theta_T \right)^2 \qquad w_i = \frac{1}{SE\left(\ln \theta_i \right)^2}$$

where θ is the diagnostic odds ratio.

As meta-analyses often include small numbers of studies the power of both tests (G2 and Q) is low, so they are poor at detecting true heterogeneity among studies as significant. An alternative approach to quantify the effect of heterogeneity is the I^2 (Inconsistency) index which describes the percentage of total variation across studies that is due to heterogeneity rather than chance [5-7]. I^2 is calculated as follows:

$$I^2 = \frac{\chi^2 - (d.f.)}{\chi^2} \times 100$$

where χ^2 is the G^2 or Q statistic and *d.f.* its degrees of freedom.

C. Computing the pooled indexes

Since we computed two different diagnostic thresholds, sensitivity and specificity were pooled by:

$$Sen_T = \frac{\sum_i a_i}{\sum_i D_i} \qquad Spe_T = \frac{\sum_i d_i}{\sum_i ND_i}$$

These formulas correspond to weighted averages in which the weight of each study is its sample size.

The diagnostic odds ratios are pooled by the DerSimonian Laird method (random effects model) to incorporate variation among studies.This method computes a weighted by averaging the logs of the individual DORs.

$$\ln \theta_T^{DL} = \frac{\sum_i w_i^{DL} \ln \theta_i}{\sum_i w_i^{DL}}$$

The DerSimonian Laird weights are defined as:

$$w_i^{DL} = \frac{1}{SE(\ln \theta_i)^2 + \tau^2}$$

Where θ is the DOR and Q stands for the Cochran homogeneity statistic calculated using the Mantel-Haenszel overall estimate and w_i the inverse variance weights.

$$\tau^2 = \begin{cases} \dfrac{Q-(k-1)}{\sum_i w_i - \left(\dfrac{\sum_i w_i^2}{\sum_i w_i}\right)} & \text{if } Q > k-1 \\ 0 & \text{if } Q < k-1 \end{cases}$$

References:

1. Deeks JJ. Systematic reviews of evaluations of diagnostic and screening tests. In Egger M, Smith GD, Altman DG (eds). Systematic Reviews in Health Care. Meta-analysis in context. London: BMJ Books; 2001:248-282.

2. Leemis LM, Trivedi KS. A Comparison of Approximate Interval Estimators for the Bernoulli Parameter. *Am Stat* 1996; **50**:63-68.

3. Devillé WL, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, Bezemer PD. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002; **2**:9.

4. Agresti A. Analysis of ordinal categorical data. New York: John Wileys & Sons; 1984.

5. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327:557-560.

6. McCullagh P, Nelder JA. Generalized Linear Models. Boca Raton: Chapman & Hall; 1989.

7. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. Stat Med 2002; 21:1525-1537.

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I certify that I have read this document and examined the student regarding its content. In my opinion, this dissertation conforms to acceptable standards of scholarly presentation and is adequate in scope and quality, and the attainments of this student are such that he may be recommended for the degree of Doctor of Public Health.

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