Performance of HPV Testing vs. Cytology for Cervical Cancer Screening in Senegal

Katherine McLean

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Sarah Prager

Stephen Hawes

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Abstract

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Katherine McLean

Chair of the Supervisory Committee:

Sarah Prager, Assistant Professor

Obstetrics and Gynecology

Objective: To examine the performance of stand-alone and combined screening tools for the identification of cervical intraepithelial neoplasia grades 2 or 3, carcinoma in-situ, or invasive cervical cancer (CIN2+) in women in Senegal.

Methods: Since 1997, we collected HIV serology, high risk HPV (HR-HPV) PCR testing, and cytologic evaluation for 3,120 women presenting to one of three outpatient clinics in Dakar, Senegal. Cervical biopsy was performed on 1,100 patients, and these results were then extrapolated to the remaining study population.

Results: Overall, 10.7% of the patients tested positive for HIV. HR-HPV testing was positive in 29.9% of patients, and cytology was abnormal in 19.6% of patients. After extrapolation of biopsy results for HIV negative patients, the

sensitivity of HR-HPV testing alone for detecting CIN2+ was estimated to be 67.4%, with a specificity of 81.8%, and PPV of 10.0%. A single cytologic evaluation had a sensitivity of 77.9%, a specificity of 90.1%, and a PPV of 24.0%. In the HIV positive population, the sensitivity of HR-HPV testing was 93.3%, the specificity was 30.5%, and the PPV was 18.4%. The sensitivity of cytologic evaluation was 85.8%, the specificity was 55.5%, and the PPV was 35.5%. This could be compared to triaging HIV positive patients with HR-HPV testing, then performing cytology on those who tested positive, which would yield a sensitivity of 86.3%, a specificity of 47.4%, a PPV of 35.8%.

Conclusions: Our data suggests that particularly in HIV positive patients, triage using HR-HPV testing, followed by cytology in those who are positive, may be promising.

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Introduction

Cervical cancer is diagnosed in 473,000 women each year world-wide, and is responsible for approximately 253,500 deaths [1]. Over 80% of cervical cancers occur in developing nations [2], and cervical cancer is the leading cause of cancer mortality among women in Africa. Cervical cancer screening methods have led to a dramatic decrease in mortality in developed nations, while the poorest nations have lagged behind due to a lack of screening resources [3].

Since the 1940s, screening has traditionally consisted of cervical cytology followed by ablative procedures after dysplasia is identified. Previous studies have raised concerns regarding the sensitivity of a single cytologic evaluation for detection of high-grade cervical intraepithelial neoplasia [4,5]. Current screening methods based in cytology, including those in developing nations, have been shown to prevent at least 70% of cervical cancers [6], but this success has depended upon the ability to screen patients on a consistent, typically annual, basis. Such frequent testing is not usually available in most developing nations, allowing much higher rates of invasive cancer.

The discovery that infection with high-risk types of human papillomavirus (HPV) is required for development of cervical dysplasia and carcinoma [7],

however, has expanded the potential for additional screening tools. Randomized trials have demonstrated that while HPV testing has a higher sensitivity than cytology, it has a much lower specificity [5, 8-10]. This fact has prompted many questions, such as those regarding how we can best utilize, or even possibly combine, screening methods to achieve better success in developing nations where frequent screening is not yet realistic. There are also additional factors that need to be considered given their potential influence on the success of screening in developing nations. For example, the prevalence of human immunodeficiency virus (HIV) is much higher in developing countries, with the majority of infected women residing in Africa [3]. Many previous studies have shown that HIV infection leads to a longer duration of HPV infection and higher rates of cervical neoplasia [11-14], although the exact relationship between HIV and cervical cancer is not well understood. In this study, we sought to better understand the influence of HIV status on the sensitivity and specificity of a given screening regimen in high-risk populations.

Methods

Between 1998 and 2010, as part of ongoing research projects regarding HIV, HPV, and biomarkers for cervical cancer, we collected cervical cancer screening data on 3,120 women presenting to the outpatient infectious disease clinic at Fann University, an oncology clinic at the Dantec Hospital, and a community health clinic in the Pikine district in Dakar, Senegal. Subjects were interviewed to obtain demographic information, gynecologic examinations were completed, and blood samples were collected for HIV serology. As part of the study protocol, liquid-based cervical cytology samples, as well as HPV testing, were obtained on all participants during the gynecologic exams. Patients were excluded if they were pregnant or did not have an intact cervix. According to study protocols, all women with abnormal cytology, in addition to a subset of women with normal cytology, were sent for colposcopy and biopsy, for a total of 1,100 patients. Cytology slides and biopsies were prepared in Senegal and sent to the University of Washington in Seattle for evaluation. Approval for the study was obtained from both the University of Dakar and the University of Washington.

A two-test system was used to diagnose HIV, the first of which was a serum test for the presence of HIV-1 or HIV-2 antibodies, as previously described [15]. If antibodies were present, then the second test was used to distinguish between HIV-1 and HIV-2.

Between 1998 and June 2003, HPV detection and typing analyses were performed via a PCR-based reverse-line strip test method (Roche Molecular Systems, Alameda, CA) with probes for low-risk HPV types 6, 11, 40, 42, 53, 54, 57, 66, and 84 and high-risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, and 83, as previously described [16]. Beginning in June 2003, a PCR-based fluorescent Luminex assay was used to detect 38 HPV types (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, 81, 82, 83, 83, IS39, and CP6108), as previously described [17]. For the purpose of analysis, we classified HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 57, 58, 59, 62, 64, 66, 71, 73, 81, 83, 84, and IS39 as high risk (HR-HPV), based upon epidemiologic evidence of risk for invasive cervical cancer [7].

Cytology results were attained from ThinPrep smears for all subjects and were classified as negative, atypical cells of unknown significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial

lesion (HSIL), carcinoma in situ (CIS), invasive squamous cell carcinoma (ISCC), or adenocarcinoma. Biopsy material was placed into formalin for histopathologic examination by the study pathologist and were read as negative, reactive atypical changes, cervical intraepithelial neoplasia type 1 (CIN-1), cervical intraepithelial neoplasia type 2 (CIN-2), cervical intraepithelial neoplasia type 3 (CIN-3), CIS, adenocarcinoma, or ISCC. All samples were interpreted by the study pathologist without knowledge of clinical or other laboratory findings.

To model the potential performance of cytology and HR-HPV testing in the original screening population of 3,120 Senegalese women, we extrapolated the extent of cervical disease that would be expected in the screening population with a gold standard of histologic diagnosis using the observed relationships among same-day cytology, HR-HPV DNA detection, and histology among the 1,100 women from this screening population who actually underwent biopsy. This approach assumes that valid estimates will be obtained if women with similar cytology and HPV results who do and do not undergo biopsy have similar distributions of histology results. Test sensitivities, specificities, as well as positive and negative predictive values were then calculated for HR-HPV testing and cytologic evaluation as stand-alone tests, as well as in combination, for detection of CIN-2 or worse (CIN2+) disease.

Results

HIV testing was positive in 10.7% of patients. Among these patients, 73.0% were infected with HIV-1, 18.3% were infected with HIV-2, and 8.7% were infected with both virus types. In HIV positive patients, the mean age at study enrollment was 40.4 years, the mean age at coitarche was 17.8, and 55.8% had more than one lifetime sexual partner (see Table 1). In this group, the mean number of pregnancies was 4.8 and 2.2% were smokers. HR-HPV testing was positive in 75.1% of patients, and cytology was abnormal (ASCUS or worse) in 45.7% of patients (see Table 2). Among HIV negative patients, the mean patient age was 44.0 years at study enrollment and 18.4 at coitarche, and 35.0% had more than one lifetime sexual partner (see Table 1). The mean number of pregnancies was 6.4 and 0.7% of patients were smokers. HR-HPV testing was positive in 24.6% of HIV negative patients, and cytology was abnormal in 16.6% (see Table 2).

After extrapolation of biopsy results for HIV positive patients, the sensitivity of HR-HPV testing was 93.3%, with a specificity of 30.5%, and a PPV of 18.4% (see Tables 3,4,5). The sensitivity of cytologic evaluation was 85.8%, the specificity was 55.5%, and the PPV was 35.5%. This could be compared to

triaging HIV positive patients with HR-HPV testing, then performing cytology on those who tested positive, which would yield a sensitivity of 86.3%, a specificity of 47.4%, and a PPV of 35.8%

In contrast, in the HIV negative patients, the sensitivity of HR-HPV testing alone for detecting CIN2+ was 67.4%, with a specificity of 81.8%, and positive predictive value (PPV) of 10.0% (see Tables 3,4,5). A single cytologic evaluation had a sensitivity of 77.9%, a specificity of 90.1%, and a PPV of 24.0%. Triaging HIV negative patients with HR-HPV testing, then performing cytology on those who tested positive, was not as effective, yielding a sensitivity of 37.7%, a specificity of 96.3%, and a PPV of 46.5%

Discussion

Recent studies have attempted to clarify the best approach to screening in resource-poor settings, particularly where patients have had little or no previous screening. For example, based on their large scale project in India, Sankaranarayanan et al [18] concluded that "a single round of HPV testing was associated with a significant decline in the rate of advanced cervical cancers and associated deaths" and that "HPV testing was the most objective and reproducible of all cervical screening tests". The authors go on to say that testing should be reserved for patients over the age of 30 in order to minimize over-treatment of patients who would clear their HPV infection without intervention. Our study confirms previous findings that HPV testing is quite promising given its high level of sensitivity. These data certainly raise concerns, however, regarding the high prevalence of HR-HPV positivity among HIV infected women. This results in very low specificity when using a single HPV evaluation to assess for CIN2+ disease. The majority of these women do not have CIN2+ disease, despite their HR-HPV and HIV positivity. Obviously it is not practical, nor ethical from a patient safety perspective, to treat 75% of HIV positive patients with invasive procedures based on these results.

Cytologic evaluation may still be necessary in terms of helping to determine which of these patients are at highest risk for CIN2+ disease. This is where one of our greatest challenges still lies, especially given the controversy that exists regarding the efficacy of cytologic evaluation in HIV positive women. For example, Fink et al [19] concluded that cytology was of limited utility in this group of patients due to a high false positive rate and instead recommended consideration of routine colposcopy. Akinwuntan et al came to a similar conclusion based on PAP sensitivity of only 57% in HIV-positive women [20]. In contrast, we found a much higher sensitivity of PAP alone in HIV positive patients, at 77.9%, which is encouraging, especially since this is not the first study with such findings [21]. The American College of Obstetricians and Gynecologists currently recommends cytologic screening twice in the first year after diagnosis of HIV, then annual screening if the results remain normal [22]. While in the HIV negative population we did not find sufficient sensitivity for triage with HR-HPV testing, then utilizing cytology only in those who are positive, this could be a viable option in HIV positive patients. Although this study is limited by its relatively small HIV positive sample size, it does suggest that a combination of screening methods may still be most useful in this population.

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Table 1. Demographic and Behavioral Characteristics of Outpatient Clinic Participants Undergoing Cervical Cancer Screening in Dakar, Senegal 1998-2010

	HIV Positive (n = 334)	HIV Negative (n = 2,786)
Mean age at study enrollment (years)	40.4	44.0
Mean age at coitarche (years)	17.8	18.4
Lifetime sexual partnerships		
1 lifetime sexual partner	44.2%	65.0%
2-5 lifetime sexual partners	54.5%	34.0%
Mean number of pregnancies	4.8	6.4
Smokers	2.2%	0.7%
Marital Status		
Single	6.0%	2.3%
Monogamous marriages	29.2%	39.1%
Polygamous marriages	27.1%	46.5%
Separated	16.9%	6.2%
Education		
No formal education	54.1%	54.6%
Primary education only	32.6%	29.3%
Secondary education	11.5%	14.3%
Birth Control Method		
Condoms for contraception	3.0%	9.8%
Using no contraception	87.3%	79.1%

Table 2. Cervical Cancer Screening Results for Outpatient Clinic Participants in Dakar, Senegal 1998-2010

	HIV positive (n = 334)	HIV negative (n = 2,786)
HPV DNA Detection		
HR-HPV positive*	75.1%	24.6%
HPV 16 positive	14.9%	2.9%
HPV 18 positive	12.5%	1.9%
HPV 31 positive	9.5%	1.7%
Other HR-HPV positive	38.3%	18.1%
Cytology		
Abnormal cytology	45.7%	16.5%
ASCUS	7.2%	8.9%
LSIL	15.5%	3.1%
HSIL	10.4%	1.9%
Cytology concerning for CIS	4.2%	1.0%
Cytology concerning ICC	8.3%	1.4%

*We classified HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 57, 58, 59, 62, 64, 66, 71, 73, 81, 83, 84, and is39 as high risk (HR-HPV)

Table 3. Cervical Biopsy Results for 1,100 Outpatient Clinic Participants in Dakar, Senegal 1998-2010

	HIV positive (n = 188 biopsies)	HIV negative (n = 912 biopsies)
Inadequate	7 (3.7%)	33 (3.6%)
Necrotic	0	2 (0.2%)
Negative	84 (44.7%)	551 (60.4%)
Reactive NOS	12 (6.4%)	94 (10.3%)
Atypical NOS	15 (8.0%)	32 (3.5%)
CIN1	42 (22.3%)	90 (9.9%)
CIN2/3	17 (9.0%)	46 (5.0%)
Carcinoma in-situ	6 (3.2%)	34 (3.7%)
ISCC	5 (2.7%)	26 (2.9%)
Adenocarcinoma in-situ	0	2 (0.2%)
Adenocarcinoma	0	2 (0.2%)

Table 4. Cervical Screening and Biopsy Summary

PAP result	HR-HPV Result*	Screened (n = 3,120)		Biopsied (n = 1,100)		CIN2+ on Biopsy (proportion of biopsied with CIN-2+)	
		HIV-	HIV+	HIV-	HIV+	HIV-	HIV+
Normal	-	1709	56	297	30	7 (2.4%)	1 (3.3%)
	+	461	97	136	49	16 (11.8%)	2 (4.1%)
ASCUS	-	187	7	151	4	10 (6.5%)	0
	+	40	17	24	13	5 (22.2%)	3 (23.1%)
LSIL	-	40	4	29	2	0	0
	+	43	46	29	32	6 (20.7%)	5 (14.3%)
HSIL	-	21	4	18	2	2 (11.1%)	1 (50.0%)
	+	28	31	24	14	11 (45.8%)	3 (21.4%)
CIS	-	5	3	7	2	3 (42.9%)	0
	+	20	11	17	7	7 (41.2%)	6 (85.7%)
ISCC	-	8	1	6	1	4 (66.7%)	0
	+	26	27	22	16	19 (86.4%)	5 (31.3%)
Adenocarcinoma	-	1	0	0	0	0	0
	+	2	0	1	0	1 (100%)	0
Unsatisfactory	-	135	9	98	5	7 (7.1%)	0
	+	61	20	52	11	5 (9.6%)	3 (27.3%)

*We classified HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 57, 58, 59, 62, 64, 66, 71, 73, 81, 83, 84, and is39 as high risk (HR-HPV)

Table 5. Extrapolated Results for Sensitivity, Specificity, and PPV for Cervical Cancer Screening Methods

	HIV positive	HIV negative
	HPV testing alone	
Sensitivity	93.3%	67.4%
Specificity	30.5%	81.8%
PPV	18.4%	10.0%
	PAP alone	
Sensitivity	85.8%	77.9%
Specificity	55.5%	90.1%
PPV	35.5%	24.0%
	Triage with HPV test, then PAP if HPV+	
Sensitivity	86.3%	37.7%
Specificity	47.4%	96.3%
PPV	35.8%	46.5%