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## Abnormal Pap tests and human papillomavirus infections among HIV infected and uninfected women who have sex with women

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## Abstract

**Objective**—To estimate the frequency of abnormal Pap and human papillomavirus (HPV) positivity among HIV seropositive and seronegative women who have sex with women (WSW).

**Methods**—Pap and HPV DNA PCR tests were obtained every six months from women in a U.S. cohort of HIV seropositive and seronegative women. WSW were women reporting no male and at least one female sex partner over five years. WSW were frequency matched 1:5 to women reporting sex only with men (WSM) and assessed using multivariable generalized estimating equation logistic regression models.

**Results**—Paps at study entry were abnormal in 12 (21%) of 49 HIV seropositive WSW, 151 (64%) of 245 HIV seropositive WSM, 3 (9%) of 24 HIV seronegative WSW, and 16 (11%) of 120 seronegative WSM. HPV was found at entry in 18 (42%) HIV seropositive WSW, 109 (52%) HIV seropositive WSM, 6 (27%) HIV seronegative WSW and 13 (13%) HIV seronegative WSM. After controlling for HIV serostatus and CD4 count, WSW had marginally lower odds than WSM of Pap abnormality (O.R. 0.59, 95% C.I. 0.33, 1.03) and of HPV (O.R. 0.53, 95% C.I. 0.32, 0.89).

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After controlling for partner gender, HIV seropositivity and lower CD4 count were associated with any HPV, oncogenic HPV, any abnormal Pap result, and HSIL or worse (P < 0.0001 for all).

**Conclusion**—While risks for abnormal Pap and HPV are modestly lower in WSW than WSM, both are common in HIV seropositive women regardless of sexual preference. WSW and WSM should be screened similarly.

#### Keywords

Human papillomavirus; HIV in women; women who have sex with women

#### Introduction

Women who have sex with women (WSW) have lower rates of Pap testing and initiate screening later than women who have sex with men (WSM) (1–5). Several reasons for this have been suggested. WSW who do not obtain Pap tests perceive fewer benefits from cancer prevention than those who are screened (6). WSW do not need contraception and have fewer encounters with reproductive health providers (6, 7). Many report adverse experiences with clinicians who assumed heterosexuality that created a barrier to screening (6, 7). Safe sex messages emphasizing risk reduction through condom use create a perception that female-female sex carries a lower risk for transmitting sexually transmitted infections, including human papillomavirus (HPV) (7). Providers may underestimate risk for WSW and fail to recommend screening (1). WSW also are less likely than heterosexual women to have health insurance and health care access (8).

Women with the human immunodeficiency virus (HIV) face higher rates of HPV infection and higher risk for Pap abnormalities, cervical precancer, and cancer (9–11). As a result, guidelines advise screening with two Pap tests in the first year after HIV diagnosis followed by annual screening for HIV seropositive women (12). These intervals are shorter than those recommended for the general population (13). How HIV coinfection affects risk for HPV and abnormal Pap testing among WSW and whether HIV seropositive WSW need more or less intensive screening than HIV seronegative WSW or WSM is unclear. We set out to determine rates of HPV and abnormal Pap test results among HIV seropositive WSW and compared that both to seronegative WSW and to WSM.

#### **Materials and Methods**

This study was based in the Women's Interagency HIV Study (WIHS), an observational multicenter cohort study of HIV seropositive women and at-risk HIV-uninfected comparison women. Enrollment began October 1, 1994 at 6 study consortia and over time enrolled 3,766 women (2791 HIV seropositive, 975 seronegative), including an expansion during 2001–2002 (14, 15). Institutional review boards at all sites approved the study, and all participants gave written informed consent for participation. Follow-up is ongoing, but this analysis includes information obtained between October 1, 1994 and October 5, 2010.

Every six months, WIHS participants had a physical examination that included a conventional single-slide cervical Pap smear and cervicovaginal lavage using 10 ml of saline, an aliquot of which was used for HPV testing using previously described protocols (9). Briefly, polymerase chain reaction (PCR) amplification using MY09/MY11 consensus primers was followed by hybridization with consensus and HPV type-specific probes. Adequate specimens were those with successful amplification of the human  $\beta$ -globin gene;  $\beta$ -globin negative specimens were excluded, and rates were calculated based on the number of  $\beta$ -globin positive results. Results were classified hierarchically for carcinogenic HPV types as defined by the International Association for Research on Cancer, including HPV16,

any alpha-9 type (HPV16, 31, 33, 52, or 58), any alpha-7 type (HPV18, 39, or 45) or for any carcinogenic type (alpha-7 and -9 types and types 35, 51, 56, 59, and 68), for any type, and negative for HPV. HPV results were not used in patient management and were available for study visits 1–23.

We defined the baseline visit for any individual as the first visit with an adequate (i.e.,  $\beta$ globin positive) HPV result, regardless of the time of entry to WIHS or chronological date. We excluded women with no adequate HPV tests, as well as those who reported hysterectomy prior to their baseline visit. Women were not censored at the time of cervical disease treatment, since they remained at risk for new or recurrent cervical HPV infection and cervical abnormalities; however, those who reported hysterectomy during follow-up were censored at the visit prior to that procedure.

Conventional single slide Pap smears were interpreted centrally at Qiagen (New York, NY, formerly Kyto, Kyto Meridien, or Dianon). Results were reported according to the 1991 Bethesda system for classification of cervicovaginal cytology and were classified as negative for squamous abnormality, atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), and cancer (16). Pap results were considered abnormal if they were ASCUS or a more severe squamous lesion.

WSW were defined as participants who reported no sex with male partners in the previous five years but did report sex with a female partner in the same period. Comparison women reported having had sex with men (WSM) and no female partners during the previous five years and all prior study visits. Sex was defined as vaginal or anal penetration or oral sex with either a male or female partner. One woman who reported no sex with any partner throughout the study was excluded. All available WSW were frequency matched to the WSM in a 1:5 ratio according to baseline age (<30, 30–34, 35–39, 40–44, >45), recruitment period (1994/95, 2001/02), and HIV serostatus CD4 stratum (HIV–, CD4>500, CD4:200–500, CD4<200).

Contingency tables were used in preliminary data analysis to compare baseline demographic and medical variables according to HIV serostatus and sexual orientation. Pearson's chisquare tests were used for standard contingency table analysis. For data stratified by both HIV serostatus and sexual preference, stratified Wilcoxon and Cochran-Mantel-Haenszel tests were applied to continuous and categorical variables respectively. The generalized estimating equation (GEE) method with a logit link was used in univariate and multivariable models incorporating data from repeated visits. WSW and frequency-matched WSM were both included in certain GEE models. The covariates in the GEE models of WSW and WSM included the matching variables (age, year of recruitment, HIV serostatus and CD4 stratum), race/ethnicity, smoking, sex with a female partner during the past 6 months, and highly active antiretroviral (HAART) use during the past 6 months. All statistical tests were twosided.

#### Results

Among all WIHS participants, 99 (61 HIV seropositive, 38 seronegative) were WSW. Eight women (3 HIV seropositive, 5 seronegative) were excluded because they had had a hysterectomy prior to enrollment, and 18 women (9 HIV seropositive, 9 seronegative) were excluded because they reported having a male sexual partner during follow-up. Overall, 73 (49 HIV seropositive, 24 seronegative) WSW were included in this analysis.

The median age of WSW at baseline was 37 years (38 years for HIV seropositive and 35 years for seronegative women, P = 0.18). The median duration of follow-up was 8.1 years

(8.0 years for HIV seropositive and 8.3 years for seronegative women). As shown in Table 1, no differences in other demographic and medical variables except smoking were apparent between WSW and women in the matched sample of WSM. The low rate of HAART use at baseline reflects the standard of care in 1994–5, when most subjects were enrolled.

Table 2 shows Pap results at baseline according to HIV serostatus and partner gender; differences did not achieve significance. However, during follow-up, WSW contributed 1123 Pap tests (754 from HIV seropositive and 369 from HIV seronegative women). Of these a cumulative total of 152 (20.2%) were abnormal in HIV seropositive WSW and 23 (6.2%) in seronegative WSW (P = 0.002). Most abnormal Pap tests were ASCUS or LSIL. Pap tests were reported as HSIL or worse in 4 HIV seropositive women and 1 seronegative woman (P = 0.9). A finding of atypical glandular cells on Pap testing was uncommon and did not differ by partner gender.

Pap test results in WSW and WSM were compared across multiple visits using multivariate GEE logistic regression models (see Table 3). These analyses showed that the odds of an abnormal Pap test were lower in WSW than in WSM. Other significant risk factors were HIV serostatus and CD4 count, cigarette smoking, and younger age. No other variable studied was associated with Pap test abnormality. In a separate model using HSIL as the outcome, HIV seropositivity with a CD4 count below 200/cmm was a significant risk factor (O.R. vs HIV seronegative women 7.3, 95% C.I. 2.2, 24, P for trend = 0.004).

Fifteen HPV results were inadequate among WSW. Results of 623 adequate HPV tests were available from WSW across study visits (455 from HIV seropositive women and 168 from seronegative women). Of these, HPV of any type was found in 156 (34.3%) Paps from HIV seropositive WSW and 21 (12.5%) Paps from seronegative women (P = 0.0003). Carcinogenic HPV was identified in 66 (14.5%) HIV seropositive women and 8 (4.8%) seronegative women (P = 0.06). Consistent with this, multivariate GEE models (Table 4) found that HPV was strongly associated with HIV serostatus and CD4 count. Furthermore, WSW had approximately half the risk of HPV positivity of WSM (P = 0.02). HPV risk was lower among Hispanic women than black women and was higher among former and current smokers than among nonsmokers. In a separate GEE model, after adjusting for the other variables listed in Table 4, carcinogenic HPV detection was associated with HIV seropositivity and CD4 count, but in contrast to findings for any HPV, the difference did not reach statistical significance.

#### Discussion

HIV was associated with increased odds for abnormal Pap tests among WSW in this study. More than one fifth of HIV seropositive WSW had an abnormal Pap at enrollment, and across all visits the odds of an abnormal Pap test increased monotonically with more severe immunodeficiency as measured by HIV seropositivity and lower CD4+ cell count. Most abnormalities were ASCUS or LSIL, reflecting infection with HPV. HIV seropositive WSW also had a higher risk of detection of any HPV and of carcinogenic HPV than seronegative WSW.

We compared findings in WSW and WSM, controlling for HIV serostatus, CD4+ count, and other covariates in multivariable models. Although odds were modestly lower in WSW than WSM for finding Pap abnormalities, including HSIL, and for detecting HPV, including carcinogenic HPV, differences were sometimes marginally significant and were much smaller than those associated with HIV status. These data suggest that WSW with HIV should be screened for cervical cancer using the same guidelines as those for the general population of HIV infected women (9).

Since our study included HIV seronegative women, our results also can be generalized to WSW who are not infected with HIV: they should be screened for cervical cancer like WSM according to recently published guidelines for the general population advocating three to five year screening intervals (13). Outreach in the lesbian community may be important to communicate this message.

This study is limited by several factors. Relatively small numbers of WSW limit our ability to assess type-specific infection risks among WSW. We did not ask women if they identified themselves as lesbian, and results may differ for such women. We did not obtain information about whether women we identified as WSW had exposure to male partners more than five years prior to enrollment in WIHS, although this is common in studies of lesbian women, and women who have never had a male partner may have lower risks for HPV infection and abnormal Pap. We did not gather information on lifetime number of partners or on specific sexual practices, which also may modify risk for HPV and abnormal Pap tests. Women in our study had regular Pap testing, and WSW with less frequent screening may face a higher risk for Pap abnormalities.

In summary, the high rates of abnormal Pap and HPV infection identified in HIV seropositive WSW suggest that these women should be managed according to established screening protocols for immunocompromised women. Further research is needed to assess the risk for cervical cancer precursors in HIV seropositive WSW and their outcomes after treatment.

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# Table 1

Demographic and clinical characteristics at baseline for human immunodeficiency virus (HIV) seropositive and seronegative women reporting sex with women (WSW) and a matched subset of women reporting sex with men (WSM). N (%)

		HIVL		HIV_	
Characteristic		-			P-value <sup>I</sup>
	WSW (N = 49)	WSM (N = 245)	WSW $(N = 24)$	WSM (N = 120)	
Recruits <sup>2</sup>					1.00
1994/95	42 (85.7)	210 (85.7)	16 (66.7)	80 (66.7)	
2001/02	7 (14.3)	35 (14.3)	8 (33.3)	40 (33.3)	
Age group <sup>2</sup>					1.00
<30	3 (6.1)	15 (6.1)	3 (12.5)	15 (12.5)	
30–34	11 (22.5)	55 (22.5)	9 (37.5)	45 (37.5)	
35–39	19 (38.8)	95 (38.8)	5 (20.8)	25 (20.8)	
40-44	7 (14.3)	35 (14.3)	5 (20.8)	25 (20.8)	
>=45	9 (18.4)	45 (18.4)	2 (8.3)	10 (8.3)	
Race/Ethnicity					0.07
White	13 (26.5)	38 (15.5)	6 (25.0)	14 (11.7)	
Hispanic	9 (18.4)	67 (27.4)	9 (37.5)	33 (27.5)	
Black	26 (53.1)	131 (53.5)	9 (37.5)	66 (55.0)	
Others	1 (2.0)	9 (3.7)	0 (0)	7 (5.8)	
Smoking					0.02
Never smoked	8 (16.3)	79 (32.4)	5 (20.8)	37 (31.1)	
Former smoker	11 (22.5)	40 (16.4)	1 (4.2)	16 (13.5)	
Current smoker	30 (61.2)	125 (51.2)	18 (75.0)	66 (55.5)	
Sex with female in past 6 months					$0.17^{3}$
No	13 (26.5)	245 (100.0)	3 (12.5)	120 (100.0)	
Yes	36 (73.5)	0(0)	21 (87.5)	0 (0)	
CD4 Coun <sup>(2</sup> (cells/cmm)					$1.00^{3}$
>500	15 (31.3)	75 (31.3)			
200–500	18 (37.5)	90 (37.5)			

Choundedia	IH	HIV+	IH	HIV-	
Cliaracteristic	WSW (N = 49)	WSM (N = 245)	WSW (N = 24)	WSM (N = 120)	P-value <sup>4</sup>
<200	15 (31.3)	75 (31.3)			
HIV DNA level					$0.07^{3}$
<=4000	21 (44.7)	76 (31.5)			
4001-20,000	4 (8.5)	52 (21.6)			
20,001-100,000	13 (27.7)	50 (20.8)			
>100,000	9 (19.2)	63 (26.1)			
HAART <sup>4</sup> use past 6 months					$1.00^{3}$
No	45 (91.8)	225 (91.8)			
Yes	4 (8.2)	20 (8.2)			

 $^{I}$ Stratified test p-value for comparing WSWs and WSMs.

<sup>2</sup>Matched variables.

 $^{\mathcal{J}}$ Comparison is within WSWs.

<sup>4</sup>Highly active antiretroviral therapy

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# Table 2

Results of cervical cytology and human papillomavirus (HPV) testing at baseline according to human immunodeficiency virus (HIV) serostatus and partner gender. N (%)

	HIV ser	HIV seropositive	HIV ser	HIV seronegative	ç
Characteristic	$WSW^I (N = 49)$	$WSM^{2}$ (N = 245)	WSW (N = 24)	WSM (N = 120)	P-value <sup>3</sup>
Cytology					0.17
Negative	37 (78.7)	144 (62.1)	21 (91.3)	104 (88.1)	
ASCUS <sup>4</sup>	6 (12.8)	48 (20.7)	2 (8.7)	13 (11.0)	
LSIL <sup>5</sup>	3 (6.4)	37 (16.0)	0 (0)	0 (0)	
HSIL <sup>6</sup>	1 (2.1)	3 (1.3)	0 (0)	1 (0.9)	
AGC <sup>7</sup>					0.43
No	46 (97.9)	226 (97.0)	23 (100.0)	114 (96.6)	
Yes	1 (2.1)	7 (3.0)	0 (0)	4 (3.4)	
Any HPV					0.15
No	25 (58.1)	88 (40.6)	16 (72.7)	85 (80.2)	
Yes	18 (41.9)	129 (59.5)	6 (27.3)	21 (19.8)	
Carcinogenic HPV					0.13
No	34 (79.1)	149 (68.7)	21 (95.5)	97 (91.5)	
Yes	9 (20.9)	68 (31.3)	1 (4.6)	9 (8.5)	

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<sup>2</sup>Women who have sex with men

 $^3$ Stratified test p-value for comparing WSWs and WSMs.

<sup>4</sup>Atypical squamous cells of undetermined significance

5 Low grade squamous intraepithelial lesion

6High grade squamous intraepithelial lesion

7Atypical glandular cells

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Table 3

Odd ratios for factors associated with abnormal Pap test results.

			<b>95</b> %	95% CI	
Characteristic		<b>Odds Ratio</b>			P-value
			LCL <sup>I</sup>	UCL <sup>2</sup>	
Dominan consider	WSM <sup>3</sup> (ref)	1			
raturel genuer	WSW <sup>4</sup>	0.49	0.27	0.87	0.02
	HIV-(ref)	1			<.00016
	CD4>500	2.52	1.67	3.80	<.0001
HIV <sup>2</sup> Serostatus and CD4 count (cens/cmm)	CD4:200–500	5.02	3.54	7.14	<.0001
	CD4<200	17.33	11.49	26.13	<.0001
	<30 (ref)	1			
	30–34	0.52	0.25	1.06	0.07
Age (years)	35–39	0.45	0.22	0.91	0.03
	40-44	0.34	0.17	0.68	0.002
	>=45	0.32	0.16	0.67	0.002
D consistences collected	1994/95 (ref)	1			
	2001/02	1.10	0.76	1.59	0.63
	Black (ref)	1			
D and (D the state)	White	1.13	0.71	1.81	0.60
NACC/LUMINCH	Hispanic	0.93	0.64	1.36	0.71
	Others	1.12	0.47	2.68	0.80
	Never smoked (ref)	1			
Smoking	Former smoker	0.96	0.62	1.50	0.86
	Current smoker	1.81	1.28	2.57	0.001
Cov with formals in nost 6 months	No (ref)	1			
Dea with remark in past o months	Yes	1.34	0.76	2.37	0.31
	No (ref)	1			
HAAR1' use past 6 months	Yes	1.25	26.0	1.62	0.09

I Lower confidence limit

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<sup>5</sup>Human immunodeficiency <sup>6</sup>P-value by test for trend

 $^{7}\mathrm{Highly}$  active antiretroviral therapy

<sup>4</sup>Women who have sex with women

 $^{\mathcal{J}}$ Women who have sex with men

<sup>2</sup>Upper confidence limit

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# Table 4

Odd ratios for factors associated with the finding of any human papillomavirus. Abbreviations as in Table 3.

			950	05% CI	
Characteristic		Odds Ratio (OR)			P-value
			LCL	UCL	
Dortroor condor	WSM (ref)	1			
	MSW	0.48	0.29	0.80	0.005
	HIV- (ref)	1			<.0001 <sup>1</sup>
HIV servetatus and CDA count (cells/cmm)	CD4>500	3.13	2.20	4.46	<.0001
	CD4:200–500	5.06	3.75	6.84	<.0001
	CD4<200	9.01	6.65	12.21	<.0001
	<30 (ref)	1			
	30–34	0.48	0.28	0.80	0.01
Age (years)	35–39	0.49	0.29	0.82	0.01
	40-44	0.52	0.31	0.89	0.02
	>=45	0.50	0.29	0.86	0.01
D	1994/95 (ref)	1			
Rectututient conort	2001/02	0.93	0.69	1.26	0.64
	Black (ref)	1			
D and (D that is it is	White	0.78	0.57	1.06	0.11
Nace/Edimenty	Hispanic	0.74	0.58	0.95	0.02
	Others	1.14	0.67	1.91	0.63
	Never smoked (ref)	1			
Smoking	Former smoker	0.67	0.50	0.88	0.005
	Current smoker	1.25	0.99	1.58	0.06
Oor with formals in most 6 months	No (ref)	1			
sex with ternate in past o months	Yes	1.26	0.73	2.19	0.41
UA ADT nee most 6 months	No (ref)	1			
SUMPLY O SEA OWNER THE SEA OWNER	Yes	0.88	0.74	1.04	0.13