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Primary HPV and Molecular Cervical Cancer Screening in US Women Living With Human Immunodeficiency Virus

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Background. Primary human papillomavirus (HPV) screening (*PHS*) utilizes oncogenic human papillomavirus (oncHPV) testing as the initial cervical cancer screening method and typically, if positive, additional reflex-triage (eg, HPV16/18-genotyping, Pap testing). While US guidelines support *PHS* usage in the general population, *PHS* has been little studied in women living with HIV (WLWH).

Methods. We enrolled n = 865 WLWH (323 from the Women's Interagency HIV Study [WIHS] and 542 from WIHS-affiliated colposcopy clinics). All participants underwent Pap and oncHPV testing, including HPV16/18-genotyping. WIHS WLWH who tested oncHPV[+] or had cytologic atypical squamous cells of undetermined significance or worse (ASC-US+) underwent colposcopy, as did a random 21% of WLWH who were oncHPV[−]/Pap[−] (controls). Most participants additionally underwent p16/Ki-67 immunocytochemistry.

Results. Mean age was 46 years, median CD4 was 592 cells/μL, 95% used antiretroviral therapy. Seventy WLWH had histologically-determined cervical intraepithelial neoplasia grade 2 or greater (CIN-2+), of which 33 were defined as precancer (ie, [i] CIN-3+ or [ii] CIN-2 if concurrent with cytologic high grade squamous intraepithelial lesions [HSILs]). *PHS* had 87% sensitivity (Se) for precancer, 9% positive predictive value (PPV), and a 35% colposcopy referral rate (Colpo). "*PHS with reflex HPV16/18-genotyping and Pap testing*" had 84% Se, 16% PPV, 30% Colpo. PHS with only HPV16/18-genotyping had 24% Colpo. "*Concurrent oncHPV and Pap Testing" (Co-Testing)* had 91% Se, 12% PPV, 40% Colpo. *p16/Ki-67 immunochemistry* had the highest PPV, 20%, but 13% specimen inadequacy.

Conclusions. *PHS* with reflex HPV16/18-genotyping had fewer unnecessary colposcopies and (if confirmed) could be a potential alternative to Co-Testing in WLWH.

Keywords. HIV; human papillomavirus (HPV); cervical cancer screening; p16/Ki-67; primary HPV screening.

Women living with human immunodeficiency virus (HIV) [WLWH] have an elevated incidence of cervical precancer and cancer as compared to the general population [1, 2]. Although the risk of cervical cancer in WLWH in the United States (US) may be decreasing [3, 4], the prevalence of abnormal Pap tests (ie, atypical squamous cells of undetermined significance or more severe dysplasia [ASC-US+]) in these women remains as high as 25%–35% at each screening visit [5]. Furthermore, most of these abnormal Pap tests do not reflect clinically relevant disease, namely, a histologic diagnosis of cervical intraepithelial neoplasia grade 2 or more severe dysplasia (CIN-2+) based on a colposcopically-directed biopsy [5].

To address this and other concerns, oncogenic human papillomavirus (oncHPV) DNA testing has been increasingly incorporated into cervical cancer screening guidelines for WLWH. Despite the very high prevalence of oncHPV in WLWH, reflex oncHPV testing following a Pap negative for intraepithelial lesion or malignancy (Pap[−]) was shown to have a strong negative predictive value (NPV), with low 3- to 5-year incidence of CIN-2+, regardless of CD4 count [6]. US guidelines therefore extended the screening interval to every 3 years in WLWH who tested oncHPV[−]/Pap[−] [7]. Conversely, the risk of precancer was high in WLWH who tested oncHPV[+] despite a normal Pap and several-fold greater still if HPV16 was specifically

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detected [8]. Additional studies demonstrated high sensitivity (Se) and NPV of oncHPV testing in the triage of borderline Pap test results (ie, ASC-US) [9, 10]. Collectively, these findings helped lead to the adoption of an oncHPV "Co-Testing" strategy, involving concurrent Pap and oncHPV tests, as an option for cervical cancer screening in WLWH [7].

Most recently, the US Preventive Services Task Force (USPSTF) approved Primary HPV Screening (*PHS*) for cervical cancer screening in the general population [11]. *PHS* utilizes an oncHPV assay for initial cervical cancer screening rather than a Pap test, and USPSTF guidelines recommend that oncHPV[−] women not be retested for 5 years [11]. While national guideline committees differ somewhat in their recommendations [12], for oncHPV[+] women they typically recommend reflex HPV16/18-genotyping and, if genotyping is negative, a third test (eg, reflex Pap testing) [12, 13]. *PHS* is of particular interest, since recent data indicate that this approach may reduce unnecessary colposcopies, relative to Co-Testing [6]. That is, while Co-Testing had moderately higher sensitivity, some studies found it resulted in more frequent colposcopy without identifying meaningfully more clinically relevant disease than *PHS* [14].

The current investigation is, to our knowledge, the first formal study of *PHS* for cervical cancer screening in WLWH. We also examined another promising screening approach, dual immunocytochemical staining for Ki-67 (a proliferation marker) and p16 (a cyclin-dependent kinase inhibitor that can accumulate when there is overexpression of the HPV oncoprotein E7). While there is increasing evidence that p16/Ki-67 testing may have a high positive predictive value (PPV) for cervical precancer/cancer, data are limited for WLWH [15, 16]. The use of direct comparisons between assays in a single study (such as this) is important in order to avoid concerns regarding variations in study design, methods, and patient populations.

METHODS

Study Participants and Sample Collection

Women's Interagency HIV Study (WIHS)

The WIHS is an ongoing geographically and ethnically diverse cohort study of HIV-seropositive (HIV[+]) and HIV-seronegative (HIV[−]) women enrolled through similar clinical and outreach sources at each of 10 clinical consortia. The initial 6 sites (Bronx, Brooklyn, Chicago, Los Angeles, San Francisco, and Washington, DC) conducted enrollment during 1994–1995 (n = 2059 HIV[+], n = 569 HIV[−] women), and 2001–2002 (n = 737 HIV[+], n = 406 HIV[−]) [17]. Clinical sites at University of North Carolina (UNC), Emory University, University of Alabama (UAB), and University of Miami were subsequently added, with enrollment conducted during 2013–2015 (n = 610 HIV[+], n = 235 HIV[−]). The core clinical, specimen collection, and enrollment methods have purposely been kept consistent, as previously reported [18]. Briefly, interviewer-administered questionnaires are completed at each semiannual visit, during which participants receive a pelvic examination, including a Pap test and a cervicovaginal lavage (CVL) for HPV DNA testing. Pap tests are interpreted centrally. Colposcopy in WIHS is recommended for a cytologic diagnosis of ASC-US+.

Cervical Cancer Screening Study (CCSS)

WIHS Enrollees. WLWH newly enrolled in WIHS, and HIV[+] colposcopy patients at WIHS-affiliated colposcopy clinics, were (serially) enrolled in CCSS, using the following criteria: (i) ≥18 years of age; (ii) able and willing to give informed consent; (iii) HIV[+]; (iv) CD4+ cell count data within the past 12 months; (v) an intact cervix (no history of hysterectomy), (vi) able to complete the interview questionnaire. Briefly, enrollment of n = 323 WIHS WLWH into the CCSS occurred soon after WIHS enrollment at 3 of the new southern WIHS sites (Emory, UNC, and UAB) during 2013–2015. Each participant agreed to collection of 2 liquid-based cytology (LBC) test specimens (PreservCyt/ThinPrep; Hologic), obtained prior to any other cervicovaginal specimens, using a plastic Ayre spatula and an endocervical brush (ie, instead of a standard Pap without LBC as used during other WIHS visits). As shown in Figure 1, all WIHS WLWH in CCSS with ASC-US+ or oncHPV[+] test, as well as a random 21% with a normal Pap and negative oncHPV test (controls), were referred to colposcopy and had additional molecular assay testing. All other WIHS women in CCSS with a normal Pap and negative oncHPV test had standard WIHS follow-up.

WIHS-Associated Colposcopy Clinics. An additional n = 542 WLWH were serially enrolled at presentation to WIHS-affiliated colposcopy clinics (2014–2016) at Montefiore Medical Center (MMC), Jacobi Medical Center (JMC), and Bronx Lebanon Hospital (BLH) in Bronx, NY, and in San Francisco, CA at Zuckerberg General Hospital (SFZGH) and the University of California San Francisco (UCSF). We included all HIV[+] colposcopy patients who met the above enrollment criteria, including those who presented for follow-up of an abnormal Pap test, repeat colposcopic follow-up [eg, for persistent squamous intraepithelial lesions (SIL) or CIN), or for follow-up of treatment (as WLWH are at high risk of treatment failure and rapid disease recurrence). All colposcopy clinic enrollees in the CCSS then underwent their colposcopy. A separate written informed consent for CCSS was signed by both WIHS and WIHS-affiliated colposcopy patient participants. All study methods were reviewed and approved by the appropriate local institutional review boards.

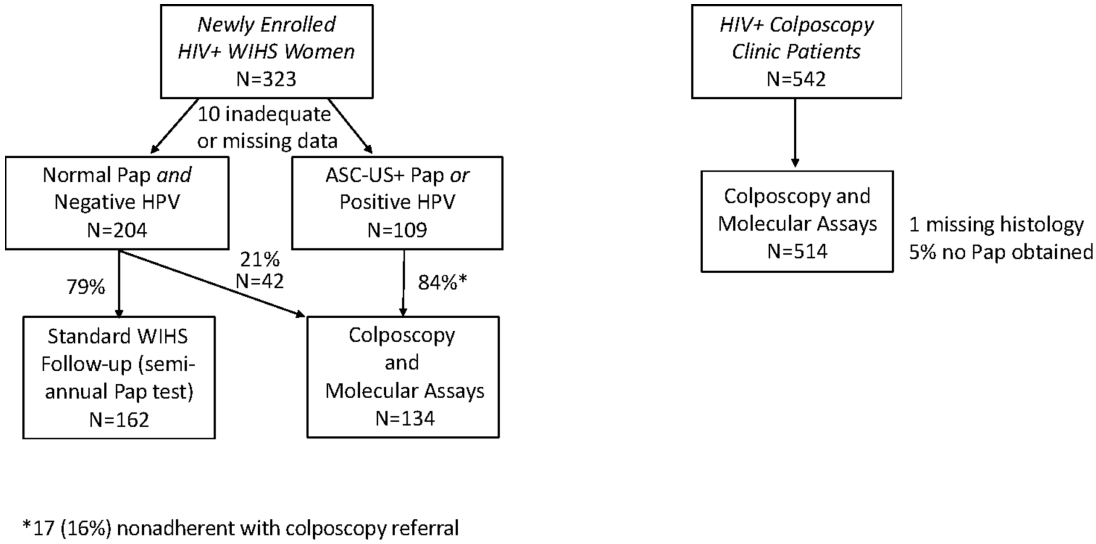

Figure 1. Patient Enrollment. All women in the CCSS had to meet the following enrollment inclusion criteria whether recruited from the WIHS or WIHS-affiliated colposcopy clinics: (i) ≥18 years of age; (ii) able and willing to give informed consent; (iii) HIV[+]; (iv) CD4+ cell count data within the past 12 months; (v) an intact cervix (no history of hysterectomy), (vi) able to complete the interview. The enrollment of n = 323 WIHS women living with HIV (WLWH) into the CCSS occurred soon after WIHS enrollment (2013–2015) at three new southern WIHS sites. As shown, all WIHS WLWH in CCSS with ASC-US+ or oncHPV[+] test, as well as a random 21% with a normal Pap and negative oncHPV test (controls), were referred to colposcopy. All other WIHS women in CCSS with a normal Pap and negative oncHPV test had standard WIHS follow-up (see Methods). An additional n = 542 WLWH were serially enrolled in CCSS at the time of their presentation to WIHS-affiliated colposcopy clinics. This included WLWH who presented for follow-up of an abnormal Pap test, repeat colposcopic follow-up (eg, for persistent SIL or CIN), or WLWH returning for follow-up after CIN treatment (as WLWH are at high risk of treatment failure/disease recurrence). To minimize the possibility that sampling order might affect the results, with every other participant we alternated whether the first versus the second liquid Pap specimen collected was the one tested in each given assay. Each vial held 20 cc. The full volume of one vial was sent for Pap and HPV testing, and used to prepare a standard monolayer cervical Pap slide, and then immediately sent for Cobas HPV DNA testing, including HPV16/18-genotyping. All residual material from that vial (approximately 10 cc) was then stored at –20°C. The second 20 cc vial was vortexed and divided into two 10 cc aliquots stored at –20°C until requested for testing. One of these 10 cc vials was stored for p16/Ki-67 immuno-cytochemistry testing by the manufacturer (CINTech+, MTM/Roche Laboratories AG), and shipped in batches on dry ice in intervals from 2–12 months after collection (depending on the collection date and the laboratory schedule). Abbreviations: ASC-US, atypical squamous cells of undetermined significance; CCSS, Cervical Cancer Screening Study; CIN, cervical intraepithelial neoplasia; HIV, human immunodeficiency virus; HPV, human papillomavirus; oncHPV, oncogenic HPV; SIL, squamous intraepithelial lesion; WIHS, Women's Interagency HIV Study; WLHS, women living with human immunodeficiency virus.

Colposcopy, Pathology, and Specimen Processing

Colposcopists obtained at least 2 biopsies from visible acetowhite lesions, even when the colposcopic impression was low-grade disease or metaplasia, consistent with recent clinical guidelines and data from our group and others demonstrating that this approach improves the accuracy of diagnosis [19, 20].

Pap specimen processing and cytologic and histologic review was centralized at the UAB Clinical Pathology Laboratory. Upon arrival at UAB each of the two liquid Pap (PreservCyt) specimens was gently vortexed to evenly distribute exfoliated cells and then aliquoted for each assay. To minimize the possibility that sampling order might affect the results, with every other participant, we alternated whether the first versus the second liquid Pap specimen collected was the one tested in each given assay (as we have done in prior studies) [21, 22]. See details in Figure 1.

All Pap tests were screened by two cytotechnologists, and all specimens that were read as ASC-US+ by at least one cytotechnologist were additionally screened by 2 independent UAB cytopathologists and, if necessary, any discrepant results were reviewed by a third. Histologic specimens were likewise screened by 2 pathologists, with adjudication by a third if necessary. On occasions when the adjudicator's findings did not correspond with either of the prior reviews, each of the UAB pathologists conferred to determine a final (joint) diagnosis.

Consistent with prior WIHS and other cohort studies, precancer was defined as either [i] CIN-3+ or [ii] CIN-2 if concurrent with a cytologic diagnosis of HSIL [23–25]. This definition was considered preferable to p16 immunohistochemistry, since cytology (HSIL) has high specificity, whereas a large comprehensive study recently showed that p16 immunohistologic staining is sensitive but has low specificity [26].

Laboratory Testing

In addition to a monolayer Pap test (PreservCyt/ThinPrep, Hologic) each sample was tested for oncHPV (including type-specific HPV16 and 18 results) in a CLIA-certified laboratory, using the FDA-approved Cobas test (Roche Diagnostics). This assay detects 14 oncHPV types and provides separate HPV16 and 18 results. p16/Ki-67 (CINTech+, MTM/Roche Laboratories AG) immunocytochemistry was conducted by MTM/Roche itself using masked specimens in the 79% of WLWH with available unopened liquid Pap specimens. Approximately 8% of PreservCyt specimens had a bloody appearance. However, only

3% of Pap and 2% of HPV DNA test results were inadequate, and only 0.25% of Pap slides were found to contain blood cells. A prior study of p16/Ki-67 testing in WLWH reported high rates of inadequate specimens (ie, >20%) [27], and it was 13% in the current study based on the stored specimens tested. See details in Figure 1.

Statistical Methods

Relative Sensitivity (Se) and Specificity (Sp)

The primary endpoint was precancer, (ie, [i] CIN-3+ or [ii] CIN-2 if concurrent with cytologic HSIL). A log binomial model that incorporated a generalized estimating equation (GEE) was used to take into account correlations between multiple different test results in the same subject. A log link was used to allow direct estimation of the relative Se and false positive rate (FPR). Note that: *1 – FPR = Sp*. A vector Z was included to account for covariates such as CD4 count and adherence to antiretroviral therapy (ART). Model (1) was:

$$\log \mathrm{P}\,(\text{test is positive}) = \beta_0 + \beta_1 \text{Precancer}\ \ (\text{present}) + \beta_2 \text{Test2} + \beta_3 \text{Precancer} * \text{Test2} + \beta_4 \text{z}$$

Model Description

The anti-log of β_0 (ie, e^{β_0}) is the probability that test 1 is positive, given there is no disease = FPR for test 1. Then $e^{\beta_0+\beta_2}$ is the probability that test 2 is positive when there is no precancer = FPR test 2. Controlling for covariates, the adjusted FPR for test 1 = $e^{\beta_0+\beta_4 z}$ and for test 2 = $e^{\beta_0+\beta_2+\beta_4 z}$. β_1 reflects the presence of disease. Therefore, the adjusted Se of test 1 is the probability that test 1 is positive given there is disease = $e^{\beta_0+\beta_1+\beta_4 z}$ and the adjusted Se of test 2 = $e^{\beta_0+\beta_1+\beta_2+\beta_3+\beta_4 z}$, where β_3 allows the relative Se and relative FPR to have different values. The relative Se is then $e^{\beta_2+\beta_3}$, and the relative FPR is e^{β_2}.

Note that multiple molecular assays can be included and compared in a single model. All confidence intervals (CIs) were estimated based on the robust variance estimator derived from the GEE model.

A related model was used to estimate the positive predictive value (PPV) and negative predictive value (NPV), as described in the footnote to Table 3.

Colposcopy Rates

Overall *colposcopy rates = % immediate colposcopy referral + % colposcopy referral at 1-year* (repeat screening); estimated using a combination of cross-sectional CCSS and longitudinal WIHS cohort data, as described in the footnote to Table 4.

Risk of Precancer in oncHPV[–] WIHS Women

Life-table methods were utilized to estimate the cumulative incidence of precancer in all HIV[+] and HIV[–] women in the main WIHS cohort who (i) were oncHPV[–] at baseline and (ii) enrolled during 1994–95 and 2001–02 (to provide long-term follow-up data). The 95% CIs were calculated based on the life-table estimator under a normal approximation assumption, as in prior studies [6, 8].

RESULTS

The mean age of CCSS study participants at enrollment was 46 years and did not differ significantly between new WIHS enrollees and women recruited from colposcopy clinics. Most of the participants were non-Hispanic African-American women. The median CD4 cell count was 592 cells/μL (interquartile range [IQR] 367–846) and 95% of all participants reported using highly active antiretroviral therapy (HAART) at baseline (Table 1). Of the 865 CCSS WLWH, 557 had a normal Pap test (252 WIHS enrollees and 305 colposcopy patients), while 63 WIHS women and 192 colposcopy patients had ASC-US+. The *PHS* FDA-approved assay used, Cobas, provided 3 endpoints, which we categorized hierarchically by oncogenicity (ie, HPV16 > HPV18 > other oncHPV; as shown in Table 1). Among the WIHS enrollees, 91 (29%) were oncHPV[+] (17 HPV16+, 5 HPV18+, 69 other oncHPV+ types; categorized hierarchically). In the colposcopy group, 213 (42%) were oncHPV[+] (32 HPV16+, 26 HPV18+, 155 other oncHPV types).

Colposcopy was conducted in all 542 colposcopy clinic patients; albeit one histologic result was missing and in 28 (5%) patients the colposcopist neglected to obtain a Pap specimen. Colposcopy was also conducted in 92 (84%) of 109 WIHS CCSS participants with ASC-US+ or oncHPV, as well as 42 (21%) randomly selected WIHS CCSS participants with a normal Pap and negative oncHPV results (controls). The diagnoses of all women who underwent colposcopy are shown in Table 2. The majority of colposcopies were normal. A total of 70 colposcopically-obtained biopsies were read as CIN-2+ of which 33 (47%) were precancer (ie, histologic CIN-2 with concomitant cytologic HSIL or histologic CIN3+).

Tables 3 and 4 show the sensitivity (Se), specificity (Sp), positive (PPV), and negative (NPV) predictive values for precancer of the several cervical cancer screening strategies studied, as well as their estimated colposcopy referral rates (Colpo). "*Concurrent HPV and Pap testing*" (Co-Testing) had 91% Se for precancer, 12% PPV, and 40% Colpo, whereas *Primary HPV Screening* (*PHS*) had 87% Se for precancer, 9% PPV, and 35% Colpo. "*PHS with reflex HPV16/18-genotyping and Pap testing*" (*PHS*-genotype/pap) had 84% Se, 16% PPV, 30% Colpo. However, "*PHS with reflex HPV16/18-genotyping*" (*PHS*-genotype) without reflex Pap testing had 24% Colpo. "*p16/Ki67 immunocytochemistry*" had the highest PPV (20%), but also had 13% specimen inadequacy. The NPV was ≥99% for all screening strategies tested. The differences in Se between screening strategies (as a group) were not statistically significantly, but Sp and PPV did differ significantly ($P < .0001$ and $P = .049$, respectively) between cervical cancer screening strategies.

Table 1. Participant Characteristics at Baseline, by Enrollment Site and Additional Clinical Data

Variable	Total	Colposcopy Patients	New WIHS Enrollees (n = 313)[a]				
			OncHPV(+)/Normal Pap	ASC-US	LSIL	HSIL[b]	Controls[c]
Subjects, n	865[a]	542	46	29	18	16	204
Age, y (Mean; SD)	46.3 (11.0)	472 (12.1)	47.1 (8.9)	42.8 (9.7)	44.9 (9.8)	43.8 (9.3)	44.9 (8.6)
Race/Ethnicity, n (%)							
White	67 (8%)	35 (7%)	8 (17%)	2 (7%)	0 (0%)	2 (13%)	19 (9%)
Hispanic	171 (20%)	155 (30%)	3 (7%)	1 (3%)	0 (0%)	1 (6%)	11 (5%)
Black	561 (67%)	292 (56%)	34 (74%)	25 (86%)	17 (94%)	13 (81%)	171 (84%)
Other	43 (5%)	37 (7%)	1 (2%)	1 (3%)	1 (6%)	0 (0%)	3 (1%)
Smoking, n (%)	339 (54%)	192 (62%)	20 (43%)	14 (48%)	10 (56%)	9 (56%)	89 (44%)
CD4 count (cells/μL), Median (IQR)	592 (367–846)	560 (342–843)	631 (362–849)	565 (374–800)	392 (258–566)	396 (209–577)	695 (444–905)
HIV RNA, copies/mL, Median (IQR)	20 (20–77)	35 (20–91)	20 (20–52)	20 (20–99)	20 (20–2640)	20 (20–459)	20 (20-20)
HAART use, n (%)	784 (95%)	491 (97%)	38 (83%)	27 (93%)	18 (100%)	16 (100%)	185 (91%)
lifetime # of sex partners, Median (IQR)	9 (4–20)	6 (4–15)	11 (6–40)	10 (6–50)	10 (8–50)	12 (5–60)	15 (7–40)
# of sex partners in last 6 mo, Median (IQR)	1 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)	1 (1–2)	1 (0–1)	1 (0–1)

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high grade SIL; IQR, interquartile range; LSIL, low grade squamous intraepithelial lesions; oncHPV, oncogenic HPV; SD, standard deviation; WIHS, Women's Interagency HIV Study.

[a] 10 WIHS enrollees were excluded because 8 were missing or had inadequate Pap results and 2 had a normal Pap test but were missing HPV results. Thus, total number of new WIHS enrollees is 313 (per Figure 1).

[b] Includes 10 with HSIL and 6 with ASC-H.

[c] Controls = WIHS enrollees (undergoing routine screening) who are oncHPV[−] with a Pap result within normal limits (normal).

For *PHS* to be safe and effective in WLWH, it assumes that a single negative oncHPV result by itself, with no additional screening or testing, indicates very low subsequent risk of cervical precancer, with no need for follow-up screening for at least 3–5 years. To assess this assumption, we conducted life-table analysis of precancer risk in all WIHS WLWH and HIV[−] women studied in the WIHS enrollment cohorts with long-term follow-up (enrolled 1994–1995 and 2001–2002). The dataset included n = 545 WLWH and n = 163 HIV[−] women. A total of 10 precancer cases were detected during 5 years of subsequent follow-up (Table 5). This included 5 cases among the WLWH and 5 among the HIV[−] women. No cancers occurred.

DISCUSSION

The results of this study suggest that *PHS* with reflex HPV16/18-genotyping (*PHS*-genotype) may be a useful cervical cancer screening strategy for WLWH. Specifically, both *PHS* and *PHS*-genotype had lower colposcopy rates (35% and 24%, respectively) than Co-Testing (40%), albeit with a modest nonsignificant reduction in Se. Importantly, in separate analysis, we also observed that WIHS women who tested oncHPV[−] had a very low risk of cervical precancer over more than 5 years of follow-up, no greater than in HIV[−] women. This is noteworthy because most prior studies only assessed the NPV of oncHPV testing in WLWH with normal Pap test results, whereas in *PHS* and *PHS*-genotype if the oncHPV test is negative then cytology is not conducted and it is several years until cervical cancer screening is repeated. Taken as a whole, the current data suggest that *PHS*-genotype may result in substantially fewer colposcopy tests than Co-Testing, while still allowing a 3–5 year screening interval, given the strong 5-year NPV of *PHS*.

It is noteworthy that *PHS*-genotype resulted in fewer colposcopies than *PHS*-genotype/Pap. While it may seem counterintuitive that a screening approach with one reflex assay (*PHS*-genotype) would result in fewer colposcopies than an approach with two reflex assays (*PHS*-genotype/Pap), the reasons for this are shown in Table 4. Briefly, *PHS*-genotype refers only HPV16/18[+] WLWH to immediate colposcopy, and those who are positive for other oncHPV types are retested in 1-year. In contrast, *PHS*-genotype/Pap refers both WLWH with HPV16/18[+], as well as those with oncHPV[+]/ASC-US+, to immediate colposcopy. These differences in referral strategy had substantial impact in the current study, as more than 40% of all baseline results that warranted repeat testing in 1-year resolved prior to rescreening.

Dual staining by p16/Ki-67 had the highest PPV (20%), the highest Sp (88%), a low immediate colposcopy rate (15%), and very high NPV similar to that of the other screening methods we tested. While the Se of p16/Ki-67 was nonsignificantly less than *PHS*, the major concern with p16/Ki-67 was the high rate

Table 2. Diagnosis and Results Among all Participants who Underwent Colposcopy and (if Indicated) Biopsy

Variable	Normal Colposcopy	CIN-1	CIN-2	CIN-3
Subjects, n	n = 407	n = 198	n = 43	***n = 27***[a]
CD4 count (cells/μL)	633	456	506	399
Median (IQR)	(414–891)	(281–690)	(209–657)	(243–675)
OncHPV (any), n (%)	138 (35%)	98 (51%)	31 (72%)	24 (89%)
HPV16	22 (6%)	13 (7%)	7 (17%)	7 (26%)
HPV18	13 (3%)	18 (9%)	4 (10%)	1 (4%)
Pap, n (%)				
WNL	277 (73%)	91 (49%)	15 (36%)	2 (7%)
ASC-US	54 (14%)	40 (22%)	7 (17%)	4 (15%)
LSIL	36 (10%)	37 (20%)	12 (29%)	6 (22%)
HSIL (including ASC-H)	11 (3%)	16 (9%)	8 (19%)	15 (56%)
Subgroup, n (%)				
(a) *Colposcopy*[c]	*Subgroup = 335 (82%)*	*155 (78%)*	*33 (77%)*	***18 (67%)***
OncHPV- (normal Pap)[b]	179 (44%)	48 (24%)	9 (21%)	***1 (4%)***[a]
OncHPV+ (normal Pap)	44 (11%)	20 (10%)	3 (7%)	***0 (0%)***[a]
ASC-US	42 (10%)	31 (16%)	6 (14%)	***2 (7%)***[a]
LSIL	31 (8%)	29 (15%)	12 (28%)	***5 (19%)***[a]
HSIL (including ASC-H)	9 (2%)	13 (7%)	2 (5%)	***10 (37%)***[a]
(b) *New WIHS enrollees*[c]	*Subgroup = 72 (18%)*	*43 (22%)*	*10 (23%)*	***9 (33%)***
OncHPV- (normal Pap)[b]	27 (7%)	15 (8%)	0 (0%)	***0 (0%)***[a]
OncHPV+ (normal Pap)	26 (6%)	8 (4%)	3 (7%)	***1 (4%)***[a]
ASC-US	12 (3%)	9 (5%)	1 (2%)	***2 (7%)***[a]
LSIL	5 (1%)	8 (4%)	0 (0%)	***1 (4%)***[a]
HSIL (including ASC-H)	2 (0.5%)	3 (2%)	***6 (14%)***[a]	***5 (19%)***[a]

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells cannot exclude HSIL; CIN, cervical intraepithelial neoplasia; HSIL, high grade SIL; IQR, interquartile range; LSIL, low grade squamous intraepithelial lesions; oncHPV, oncogenic HPV; WIHS, Women's Interagency HIV Study; WNL; Pap within normal limits.

[a]***Precancers*** shown in ***bold and italics*** = CIN-3+ and CIN-2 confirmed by concurrent HSIL.

[b]21% random subset of all oncHPV[–] WIHS women with a Pap within normal limits underwent colposcopy.

[c]10 (3)% of 323 New WIHS enrollees had an inadequate Pap test and 17 (16%) were nonadherent with colposcopy referral. 17 (3%) of 541 WIHS-Affiliated colposcopy patients had an inadequate Pap test and in 28 (5%) patients the colposcopist neglected to obtain a Pap specimen.

of specimen inadequacy observed with this assay (13%): a difficulty similarly noted in an earlier study of p16 testing in WLWH [27]. Nonetheless, further assessment of p16/Ki-67 cytology is warranted because of the interval between specimen collection and testing. Specifically, specimens needed to be shipped in batches to the manufacturer (MTM/Roche Laboratories AG) in

Table 3. Cross-sectional Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of Several Cervical Cancer Screening Approaches for Precancer (ie, CIN-3+ or CIN-2 with Concurrent HSIL), Including Primary HPV Screening (*PHS*)

Screening Strategy	Cross-sectional Indication for Colposcopy	Sensitivity[a] (95% CI)	Specificity[a] (95% CI)	PPV[a,c] (95% CI)	NPV[c] (95% CI)
OncHPV Co-Testing	HPV16/18+ or other oncHPV+ AND ASC-US or LSIL+	91% (75%, 97%)	73% (70%, 76%)	12% (8%, 16%)	99% (98%, >99%)
Primary HPV Screening[b]	Any oncHPV+	87% (71%, 95%)	66% (63%, 69%)	9% (6%, 13%)	99% (98%, >99%)
PHS-genotype/Pap	HPV16/18+ or other oncHPV+ AND reflex ASC-US+	84% (68%, 93%)	78% (75%, 80%)	13% (9%, 18%)	99% (98%, >99%)
p16/Ki-67	p16/Ki-67+	82% (60%, 93%)	88% (85%, 90%)	20% (13%, 30%)	99% (98%, >99%)

OncHPV Co-Testing involves concurrent oncogenic human papillomavirus (oncHPV), HPV16/18 genotype, and Pap testing. *Primary HPV Screening (PHS)* involves use of an oncHPV assay as the initial cervical cancer screening test without reflex HPV16/18 genotyping or Pap tests; *PHS-genotype/Pap* includes reflex HPV16/18 genotype and Pap testing. *p16/Ki- 67* involves dual immunocytological staining for Ki67 (a proliferation marker) and p16 (a cyclin-dependent kinase inhibitor that can accumulate in HPV-positive cells when there is overexpression of the viral oncoprotein E7).

While differences in disease and exposure prevalence can effect PPV, it does not influence sensitivity and specificity, which are characteristics of the tests themselves. While the high prevalence of disease in the patients studied raises the possibility that PPV might be overestimated, any impact would be expected to equally effect each of the several screening strategies we compared.

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; CI, 95% confidence interval; HPV, human papillomavirus; HSILs, high grade squamous intraepithelial lesions; oncHPV, oncogenic HPV; WIHS, Women's Interagency HIV Study.

[a]**Statistical Significance:** The overall test statistic for differences between the cervical cancer screening approaches as a whole for ***Sensitivity*** was $P = .26$ (nonsignificant);***Specificity*** $P < .0001$; for ***PPV*** $P = .049$.

[b]*PHS* with genotyping was not separately analyzed to determine sensitivity/specificity/PPV/NPV because cross-sectionally PHS with reflex genotyping does not differ from PHS any Onc although they differ in overall Colposcopy rate (see Table 4) be estimated based on WIHS data [see Table 4]).

[c]**Calculation of Positive and Negative Predictive Value** (see also Statistical Methods) - we simultaneously estimated both PPV and NPV for a given assay or algorithm in a single statistical model by using "agreement" (ie, whether the assay was positive in the presence of disease and negative in the absence of disease) as the binary outcome variable. The Model is therefore: $\log P(agreement) = \alpha_0 + \alpha_1 test\_positive + \alpha_2 test\_type + \alpha_3 test\_positive * test\_type + \alpha_4 z$. It was used to estimate and compare PPVs of Test 1 and 2, $e^{\alpha_0+\alpha_1+\alpha_4 z}$and $e^{\alpha_0+\alpha_1+\alpha_2+\alpha_3+\alpha_4 z}$as well as the NPVs of Test 1 and 2, $e^{\alpha_0+\alpha_4 z}$and $e^{\alpha_0+\alpha_2+\alpha_4 z}$. The relative PPV is $e^{\alpha_2+\alpha_3}$ and the relative NPV is e^{α_2}.

Table 4. Estimated % of HIV[+] Women Who Would: (i) Undergo "immediate colposcopy" (colpo), (ii) Require "retesting" at 1-Year (iii) Have ASC-US+ or oncHPV at 1-Year Retesting (iv) the "overall colpo rate"– see Details in Footnote

Approach	*% Immediate Colpo* indication	*% Retesting in 1-Year* indication	*% 1-Year Persistent ASC-US+/ oncHPV*[a]	*Overall Colpo Rate*[a] 95% CI
oncHPV Co-Testing	**29%** HPV16/18, LSIL+, or oncHPV[+] ASC-US	**17%** oncHPV[+] ASC-US[–] oncHPV[–] ASC-US[+]	**56%** Persistent oncHPV or ASC-US[+]	**40%** 35%, 42%
Primary HPV Screening (PHS)	**35%** Any oncHPV+	NA	NA	**35%** 33%, 39%
PHS with reflex genotyping[b]	**9%** HPV16/18	**26%** Non16/18 oncHPV	**57%** Persistent oncHPV	**24%** 21%, 27%
PHS-genotype/Pap	**25%** HPV16/18 or oncHPV[+] ASC-US[+]	**10%** oncHPV[+] ASC-US[–]	**45%** Persistent oncHPV	**30%** 26%, 33%
P16/Ki-67	**15%** cross-sectional only	NA	NA	NA

"Colpo" = colposcopy; "Indication" = reason for colposcopy; "Retesting in 1-Year" = referral to repeat the screening test(s)approximately 1-year after the initial cervical cancer screening results were obtained; "1-Year Persistent ASCUS±/oncHPV" = repeated detection of ASCUS+ and/or oncHPV when retested approximately 1-year after the initial cervical cancer screening results were obtained; "Overall Colpo Rate" = % immediate colpo referral + (% referral after 1-year x% persistent ASC-US and/or oncHPV for 1 year).

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; CCSS, Cervical Cancer Screening Study; CI, 95% confidence interval; HAART, highly reactive antiretroviral therapy; HIV, humn immunodeficiency virus; HPV, human papillomavirus; oncHPV, oncogenic HPV; NA, not applicable; PHS, Primary HPV Screening; WIHS, Women's Interagency HIV Study; WLWH, women living with human immunodeficiency virus.

[a]Calculation of the Overall Colpo Rate was based on both CCSS and longitudinal data in the WIHS. That is, CCSS data were used to determine the % requiring immediate colposcopy referral and follow-up in 1 year. However, the probability of oncHPV and/or ASC-US persistence was estimated using semi-annual follow-up data in the WIHS cohort as a whole; ie, analyzed utilizing multivariate Cox models that incorporated the Wei-Lin-Weissfeld method to address repeated observations (such as contemporaneous infection with multiple different HPV types), stratified by HIV-status, and adjusted for age, race, smoking, and, amongst WLWH, also adjusted for HAART use and baseline CD4 count.

[b]Notes: "*PHS with reflex genotyping*" is shown in this Table but does not appear in Table 3, because cross-sectionally *PHS* with reflex genotyping does not differ from PHS (ie, any oncHPV), whereas they do differ in terms of overall colpo rates (ie, accounting for immediate versus 1 year re-testing). The "*Overall Colposcopy Rate*" could not be calculated for p16/Ki-67 testing, because national guidelines for the frequency of re-testing following a negative p16/Ki-67 finding have not yet been established.

Table 5. 5-Year Cumulative Incidence of Precancer (ie, CIN-3+ or CIN-2 Concurrent with Cytologic HSIL) Following a Negative oncHPV Test at Baseline, Stratified by HIV-Status and CD4 Count[a]

Baseline HIV Status and CD4 Count	Interval, y	No. at Start of Interval	No. of New Precancer	Cumulative Incidence (95% CI)
HIV-positive				
CD4 < 350/μL	0	71	1	1 (0–4)
	0–1	70	0	1 (0–4)
	1–2	60	1	3 (0–7)
	2–3	56	0	3 (0–7)
	3–4	48	0	3 (0–7)
	4–5	45	0	3 (0–7)
CD4 350–499/μL	0	56	0	0 (0)
	0–1	56	0	0 (0)
	1–2	46	1	2 (0–7)
	2–3	40	0	2 (0–7)
	3–4	32	0	2 (0–7)
	4–5	29	0	2 (0–7)
CD4 ≥ 500/μL	0	147	1	1 (0–2)
	0–1	146	0	1 (0–2)
	1–2	139	1	1 (0–3)
	2–3	127	0	1 (0–3)
	3–4	117	0	1 (0–3)
	4–5	106	0	1 (0–3)
HIV-negative	0	163	0	0 (0)
	0–1	163	0	0 (0)
	1–2	156	2	1 (0–3)
	2–3	143	0	1 (0–3)
	3–4	131	2	3 (0–6)
	4–5	116	1	4 (0–7)

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; CCSS, Cervical Cancer Screening Study; CI, 95% confidence interval; HAART, highly reactive antiretroviral therapy; HIV, humn immunodeficiency virus; HPV, human papillomavirus; oncHPV, oncogenic HPV; NA, not applicable; PHS, Primary HPV Screening; WIHS, Women's Interagency HIV Study; WLWH, women living with human immunodeficiency virus.

[a]For *PHS* to be safe and effective in WLWH, it assumes that a single negative oncHPV result by itself, with no additional testing, indicates very low subsequent risk of cervical precancer, and no need for follow-up screening for at least 3–5 years. Therefore, we determined the risk of precancer in WLWH and HIV[–] women who tested oncHPV[–] at baseline, using life-table analysis of precancer risk in all WIHS WLWH and HIV[–] women with long-term semi-annual follow-up (ie, those enrolled in 1994–95 and 2001–02). Lifetable methods were utilized to estimate the cumulative incidence of precancer, and 95% CIs were calculated based on the life-table estimator under a normal approximation assumption (stratified by HIV status and CD4 count) [1, 2].

Germany on dry ice, 2–12 months after collection (depending on collection date and laboratory schedule).

There are several additional limitations to the current investigation that warrant consideration when interpreting the study data. First, our results are most relevant to WLWH similar to those studied, namely, WLWH engaged in long-term clinical follow-up, who are using an effective HAART regimen, and have moderate to good immune status. Second, the oncHPV prevalence in precancer cases (87%) among the WLWH studied was moderately lower than has been reported in precancer cases in the general population [22], albeit similar to the results in other studies of cervical precancer among urban WLWH [23]. One possibility is that a small amount of blood (too little to be detected on Pap tests) was present in a subset of the liquid Pap specimens from the high risk women we studied, and impacted HPV PCR amplification. If correct, this would likely also impact screening in real world settings.

Overall, this study presents evidence that *PHS* with and without reflex HPV16/18-genotyping may be useful as a cervical cancer screening strategy for WLWH, resulting in less unnecessary colposcopies than standard Co-Testing. If confirmed in larger, more comprehensive studies, the high NPV of PHS would suggest that cervical cancer screening using *PHS* or *PHS*-genotype every 3–5 years would be safe and appropriate (and possibly superior to Co-Testing). P16/Ki-67 immunocytochemistry for cervical cancer screening also warrants further investigation in WLWH, as do other promising new technologies that may improve Se, Sp, PPV, and reduce unnecessary colposcopies. Selected examples include the use of type-specific oncHPV viral load, epigenetic changes in oncHPV, and cervical epithelial DNA [28, 29], as well as expression levels of E6/E7 protein in exfoliated cervical cells [30].

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References

1. Engels EA, Biggar RJ, Hall HI, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. Int J Cancer **2008**; 123:187–94.
2. Abraham AG, D'Souza G, Jing Y, et al; North American AIDS Cohort Collaboration on Research and Design of IeDEA. Invasive cervical cancer risk among HIV-infected women: a North American multicohort collaboration prospective study. JAIDS **2013**; 62:405–13.
3. Hernández-Ramírez RU, Shiels MS, Dubrow R, Engels EA. Cancer risk in HIV-infected people in the USA from 1996 to 2012: a population-based, registry-linkage study. Lancet HIV **2017**; 4:e495–504.
4. Robbins HA, Shiels MS, Pfeiffer RM, Engels EA. Epidemiologic contributions to recent cancer trends among HIV-infected people in the United States. AIDS **2014**; 28:881–90.
5. Massad LS, Xie X, Minkoff H, et al. Longitudinal assessment of abnormal Papanicolaou test rates among women with human immunodeficiency virus. Int AIDS **2020**; 34:73–80.
6. Keller MJ, Burk RD, Xie X, et al. Risk of cervical precancer and cancer among HIV-infected women with normal cervical cytology and no evidence of oncogenic HPV infection. JAMA **2012**; 308:362–9.
7. USPHS C, NIH. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents. Published 2018. https://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed 7 February 2020.

8. Keller MJ, Burk RD, Massad LS, et al. Cervical precancer risk in HIV-infected women who test positive for oncogenic human papillomavirus despite a normal Pap Test. Clin Infect Dis **2015**; 61:1573–81.
9. D'Souza G, Burk RD, Palefsky JM, Massad LS, Strickler HD; WIHS HPV Working Group. Cervical human papillomavirus testing to triage borderline abnormal Pap tests in HIV-coinfected women. AIDS **2014**; 28:1696–8.
10. Kirby TO, Allen ME, Alvarez RD, Hoesley CJ, Huh WK. High-risk human papillomavirus and cervical intraepithelial neoplasia at time of atypical squamous cells of undetermined significance cytologic results in a population with human immunodeficiency virus. J Low Genit Tract Dis **2004**; 8:298–303.
11. Curry SJ, Krist AH, Owens DK, et al; US Preventive Services Task Force. Screening for cervical cancer: US Preventive Services Task Force Recommendation Statement. JAMA **2018**; 320:674–86.
12. Fontham ETH, Wolf AMD, Church TR, et al. CA Cancer J Clin **2020**. doi:10.3322/caac.21628
13. Huh WK, Ault KA, Chelmow D, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecol Oncol **2015**; 136:178–82.
14. Learman LA, Garcia FAR. Screening for cervical cancer: new tools and new opportunities. JAMA **2018**; 320:647–9.
15. Gustinucci D, Giorgi Rossi P, Cesarini E, et al. Use of cytology, E6/E7 mRNA, and p16INK4a-Ki-67 to define the management of human papillomavirus (HPV)-positive women in cervical cancer screening. Am J Clin Pathol **2016**; 145:35–45.
16. Savone D, Carrone A, Riganelli L, Merlino L, Mancino P, Benedetti Panici P. Management of HPV-related cervical disease: role of p16INK4a immunochemistry. Review of the literature. Tumori **2016**; 102:450–8.
17. Barkan SE, Melnick SL, Preston-Martin S, et al. The women's interagency HIV study. WIHS Collaborative Study Group. Epidemiology **1998**; 9:117–25.
18. Adimora AA, Ramirez C, Benning L, et al. Cohort profile: the Women's Interagency HIV Study (WIHS). Int J Epidemiol **2018**; 47:393–394i.
19. Massad LS, Jeronimo J, Katki HA, Schiffman M; National Institutes of Health/American Society for Colposcopy and Cervical Pathology Research Group. The accuracy of colposcopic grading for detection of high-grade cervical intraepithelial neoplasia. J Low Genit Tract Dis **2009**; 13:137–44.
20. Gage JC, Hanson VW, Abbey K, et al; ASCUS LSIL Triage Study (ALTS) Group. Number of cervical biopsies and sensitivity of colposcopy. Obstet Gynecol **2006**; 108:264–72.
21. Cuzick J, Ahmad AS, Austin J, et al. A comparison of different human papillomavirus tests in PreservCyt versus SurePath in a referral population-PREDICTORS 4. J Clin Virol **2016**; 82:145–51.
22. Castle PE, Stoler MH, Wright TC Jr, Sharma A, Wright TL, Behrens CM. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol **2011**; 12:880–90.
23. Massad LS, Xie X, Burk RD, et al. Association of cervical precancer with human papillomavirus types other than 16 among HIV co-infected women. Am J Obstet Gynecol **2016**; 214:354.e1–6.
24. Keller MJ, Burk RD, Massad LS, et al. Racial differences in human papilloma virus types amongst United States women with HIV and cervical precancer. AIDS **2018**; 32:2821–6.
25. Schiffman M, Herrero R, Desalle R, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology **2005**; 337:76–84.
26. Castle PE, Adcock R, Cuzick J, et al; New Mexico HPV Pap Registry Steering Committee; p16 IHC Study Panel. Relationships of p16 immunohistochemistry and other biomarkers with diagnoses of cervical abnormalities: implications for LAST terminology. Arch Pathol Lab Med **2020**; 144:725–34.
27. McGrath CJ, Garcia R, Trinh TT, et al. Role of p16 testing in cervical cancer screening among HIV-infected women. PLoS One **2017**; 12:e0185597.
28. Gradissimo A, Lam J, Attonito JD, et al. Methylation of high-risk human papillomavirus genomes are associated with cervical precancer in HIV-positive women. Cancer Epidemiol Biomarkers Prev **2018**; 27:1407–15.
29. Mirabello L, Frimer M, Harari A, et al. HPV16 methyl-haplotypes determined by a novel next-generation sequencing method are associated with cervical precancer. Int J Cancer **2015**; 136:E146–53.
30. Valdez M, Jeronimo J, Bansil P, et al. Effectiveness of novel, lower cost molecular human papillomavirus-based tests for cervical cancer screening in rural china. Int J Cancer **2016**; 138:1453–61.