Primary Human Papillomavirus (HPV) Screening in HIV+ Women

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BACKGROUND

Women living with HIV (WLWH) have high risk of cervical pre-cancer/cancer, and at each clinical visit nearly a third may have abnormal Pap tests (i.e., ASC-US+). Most of these abnormal Pap tests do not, however, reflect clinically relevant disease (i.e., a histologic diagnosis of CIN-2+).

To improve accuracy, US guidelines endorse *"Co-testing"* (i.e., concurrent Pap & oncHPV testing) for WLWH aged ≥ 30 years with repeat screening recommended in 3 years if both tests are negative. Conversely, if the Pap is normal but oncHPV[+], then screening is repeated in 12 months; whereas if genotyping detects HPV type 16 or 18, or Pap >ASC-US immediate colposcopy is warranted.

Recent US guidelines also support *Primary HPV Screening (PHS)* in women in the general population (30-65 years of age). PHS utilizes an oncHPV assay as the initial cervical cancer screening test (instead of a Pap test). For oncHPV[-] women follow-up in 3 years is indicated. Conversely, in oncHPV+ women, if HPV 16 or 18 is found then immediate colposcopy is indicated, otherwise follow-up in 1 year. Alternatively, an additional test (e.g., Pap) may be used to determine follow-up vs colposcopy in oncHPV[+] women.

However, PHS has not to our knowledge been carefully studied in WLWH. We therefore assessed PHS, as well as another promising molecular screening method, p16/ki67 dual cytologic staining, in WLWH.

METHODS

The study enrolled N=865 WLWH comprised of :

(i) N=323 newly enrolled in the *Women's Interagency HIV Study (WIHS)* cohort through three recently initiated WIHS clinical sites at *University of Alabama (UAB), Emory University, & University of North Carolina*.

(ii) N=542 WLWH enrolled through WIHS-affiliated colposcopy clinics.

New WIHS enrollees underwent routine liquid-based Pap & oncHPV tests. Those positive by oncHPV or Pap received colposcopy, as did 15% of oncHPV/Pap negatives.

WLWH enrolled at colposcopy clinics had oncHPV & Pap tests, like the WIHS enrollees, in addition to colposcopy.

Colposcopy involved ≥2 biopsies of visible acetowhite lesions, even if the diagnosis was low grade or metaplasia.

Pathology. Cytology was interpreted by two independent expert cytopathologists at UAB, with adjudication of any discrepant results by a third. Histology, similarly, was interpreted by two pathologists; a pathologist at the clinical site and one at UAB, with adjudication by an additional UAB pathologist.

OncHPV testing was conducted using the Cobas HPV Test (Roche Molecular Systems, CA), including type-specific results for HPV16/18.

p16/ki-67 dual staining (CINtec Plus®; MTM / Roche mtm laboratories AG, Manheim, Germany) was also conducted in most subjects. p16 is a cyclin-dependent kinase inhibitor that can accumulate in oncHPV[+] cells when there is high HPV E7 activity. Ki-67 is a proliferation marker. False(+) p16 has been noted in some studies, and dual staining has been shown to improve positive predictive value (PPV).

RESULTS 1

Table 1 shows selected participant characteristics at baseline. Mean age was 46 years, the majority were African American, median (IQR) CD4 count was 592 (367-846) and 95% reported antiretroviral therapy use. There were 70 CIN-2+ diagnosed, of which 32 were classified as *cervical precancer/cancer* - which based on prior papers we defined as CIN-3+ or "CIN-2 with concurrent HSIL".

Table 2 shows the sensitivity, specificity, and positive predictive value (PPV) for precancer/cancer by individual screening approach, as well as the colposcopy rate (i.e., the % of participants who would require colposcopy based on each approach). Both Pap & oncHPV had a sensitivity ≥88% for precancer with a PPV ≤11% and >32% referred to colposcopy. Results for PHS with reflex Pap testing were 84%, 16%, & 21%, respectively, & for HPV co-testing were 91%, 14%, & 26%. p16/ki-67 dual staining alone had similar sensitivity as PHS with reflex Pap testing, but moderately higher PPV & fewer colposcopies.

Table 1. Selected Participant Characteristics at Baseline

	Total	Colposcopy Patients	New WIHS Enrollees (n=313)*				
			oncHPV[+] Normal Pap	ASC-US	LSIL	HSIL**	oncHPV[-] Normal Pap
Subjects, n	865	542	46	29	18	16	204
Age, years Mean (SD)	46.3 (11.0)	47.2 (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 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)
# recent sex partners Median (IQR)	1 (0-1)	1 (0-1)	1 (0-1)	1 (0-1)	1 (1-2)	1 (0-1)	1 (0-1)

*10 new WIHS enrollees were excluded because s were missing or had inadequate Pap results and 2 had a normal Pap test but were missing HPV results
**Includes 10 with HSIL and 6 with ASC-H

Table 2. Accuracy for Diagnosis of Precancer (i.e., CIN-3+ or "CIN-2 with concurrent HSIL")

Strategy	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	Colposcopy Rate (95% CI)
Pap test only (ASC-US+)	93% (75%, 98%)	71% (67%, 74%)	11% (8%, 16%)	32% (22%, 27%)
oncHPV test only	88% (66%, 95%)	66% (75%, 81%)	9% (6%, 13%)	36% (33%, 39%)
oncHPV test with reflex Pap*	84% (68%, 93%)	82% (79%, 84%)	16% (11%, 22%)	21% (18%, 24%)
oncHPV co-testing*	91% (75%, 99%)	77% (74%, 79%)	14% (10%, 19%)	26% (23%, 29%)
Pap abnormal OR oncHPV	97% (81%, 100%)	56% (53%, 60%)	8% (6%, 11%)	46% (42%, 49%)
p16/ki67	82% (60%, 93%)	88% (85%, 90%)	21% (13%, 30%)	15% (12%, 18%)

*prospective WIHS data were used to estimate persistent oncHPV+ in WLWH with normal Pap tests

METHODS 2

Because PHS assumes that there is low 3-5 year risk of precancer/cancer following a negative oncHPV test result, we assessed this assumption in WLWH. This analysis exploited existing data from ongoing studies in the WIHS cohort that involve semi-annual follow-up to determine the effects of host immune status on oncHPV natural history and precancer/cancer risk; using results of MY09/MY11 PCR able to detect >40 individual HPV types.

RESULTS 2

Table 3 shows that there is low 3-5 year risk of precancer/cancer following a negative HPV test in WLWH, regardless of CD4 count, which is no greater than that observed in HIV[-] women.

Table 3. Cumulative incidence of precancer (i.e., CIN-3+ or "CIN-2 with concurrent HSIL") in the WIHS cohort by HIV/CD4 Group among women oncHPV[-] at Baseline

Baseline HIV Status and CD4 Count	Interval, y	No. at Start of Interval	Precancer New CIN-3+	Cumulative Incidence (95% CI)
WLWH				
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-uninfected	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)

CONCLUSIONS

The results of this study suggest that Primary HPV Screening (PHS) with a reflex Pap is a potentially useful strategy for cervical cancer screening in WLWH. It achieved a higher PPV with fewer colposcopies than PHS without reflex Pap or oncHPV co-testing. Furthermore, a single oncHPV result at baseline was shown to be associated with low risk of subsequent precancer/cancer for ≥5 years, regardless of CD4 count, and no different than in HIV[-] women. This suggests that repeat PHS with reflex Pap 3-5 years after negative test results would be safe in WLWH. The high number of colposcopies estimated using co-testing and Pap testing may partly reflect a sensitive interpretation of Paps in this study. Interestingly, p16/ki-67 dual staining provided the highest PPV & resulted in the lowest number of colposcopies. However, further study of this assay is needed to determine the long term precancer/cancer risk following a negative p16/ki-67 result in WLWH, especially as there is still limited longitudinal data in the general population regarding the frequency/periodicity of repeat screening that might be appropriate with p16/ki-67 dual staining cytology.

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WIHS Women's Interagency HIV Study