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Comparison of PCR and Hybrid Capture Methods for Detection of Human Papillomavirus in Injection Drug-Using Women at High Risk of Human Immunodeficiency Virus Infection

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We compared Hybrid Capture, a new technique for detection of human papillomaviruses (HPV), with a PCR assay based on L1 consensus primers. By both methods, the HPV prevalence was higher in human immunodeficiency virus (HIV)-positive women than in HIV-negative women. PCR had a higher sensitivity (0.89 versus 0.48) but lower specificity (0.43 versus 0.93) for detection of Pap smear abnormalities, compared to Hybrid Capture. The higher intensity of hybridization signal by PCR was related to higher estimates of viral load by Hybrid Capture.

Injection drug use is a major source of new human immunodeficiency virus (HIV) infections in the United States. In 1988, a longitudinal follow-up of injection drug-using men and women was initiated in Baltimore (ALIVE study) (16). The study recruited and monitored nearly 3,000 intravenous drug users, of which about 20% were females. At baseline, 24% of the ALIVE participants were HIV seropositive and the HIV seroconversion rate for those at risk was 4 per 100 person-years (13). Ninety percent of the participants had antibodies to hepatitis B virus (8), and 89% had antibodies to hepatitis C virus (14).

HIV-infected women are also at high risk for infection with genital tract human papillomaviruses (HPVs) and for associated preinvasive and invasive cervical neoplasia (7, 9, 10). Cervical cancer was recognized as an AIDS-defining illness in 1992 (2). Therefore, women enrolled in the prospective component of ALIVE (all HIV seropositive and a convenience sample of HIV-seronegative women at recruitment) were invited to participate in the present study under a protocol approved by the Institutional Review Board. The study women received a biannual gynecological examination, at which time papanicolaou (Pap) smears and cervicovaginal lavage specimens for HPV diagnosis were collected. Pap smears were collected first, with a spatula, followed by a cytobrush (except in pregnancy, where a cotton swab was used). For cervicovaginal lavage, 10 ml of normal saline was sprayed on the cervical os by using a syringe with flexible tip (15) and aspirated back from the posterior vaginal fornix. The specimens were stored at −70°C until testing.

Pap smears were stained and screened by a qualified cytotechnologist by standard criteria for cytopathologic diagnosis. HPV diagnosis by PCR was accomplished by amplification of HPV genomic sequences with the use of MY09/MY11/HB01 L1 consensus primers and subsequent diagnosis of HPV types, as previously described by Hildesheim et al. (6). For positive specimens, the intensity of PCR signal was graded 1 to 4. For some analyses, the viruses were grouped into high-risk, intermediate-risk, and low-risk categories on the basis of their prevalence in cervical cancers in the international study (1). Hybrid Capture assays were performed in tubes as described previously (3), utilizing 100 μl of the lavage for testing with each probe. The specimens were tested separately with probe A (HPV types 6, 11, 42, 43, and 44) and probe B (HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56) and were classified as positive when the relative light unit (RLU) ratio (RLU of specimen/mean RLU of three positive controls) was 1 or greater. Statistical tests were performed by using Epi Info and SAS software.

We report here the results of the samples collected at recruitment. One hundred and fifty of the ALIVE women (67.6%) were HIV seropositive, and 72 (32.4%) were HIV seronegative. There was a marked contrast between HPV prevalence in these two groups, by both assays.

By PCR, the HPV prevalence in HIV-seropositive women was about 2.5-fold greater than that in HIV-seronegative women (69.3% versus 26.4%, $P < 0.001$) (Table 1). Twenty-two HPV types were identified in HIV-seropositive women, and 7 types were identified in HIV-seronegative women. The prevalence of individual HPV types in HIV-seropositive women varied from a high of 13.3% for Pap 291/MM7 to a low of 0.7% for HPV-59 and HPV-73. If we exclude the untyped HPVs (12% of isolates), the distribution of HPV types in HIV-positive women was 28, 32, and 40% for high-risk, intermediate-risk, and low-risk HPVs, respectively. The HPV prevalence in HIV-seronegative women was low, and only 12 specimens were typed. HPV types of all three risk categories were identified more frequently from HIV-positive women than from HIV-negative women, and there was no evidence that HPVs of any one risk category were more predominant than HPVs of other risk categories in HIV-infected women. Among HPV-positive women with typed HPVs, multiple HPV infections were more common in HIV-seropositive women (51.9% of 81 women) than in HIV-seronegative women (16.7% of 12 women) ($P = 0.023$). Simultaneous infection with four to seven types were detected in 12 HIV-seropositive and in none of HIV-seronegative women.

The HPV prevalences by Hybrid Capture were lower than those by PCR and showed a greater contrast between HIV-

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TABLE 1. HPV prevalence in HIV-seropositive and -seronegative ALIVE women

Test and HPV type[a]	No. (%) of subjects with HPV	
	HIV positive (n = 150)	HIV negative (n = 72)
PCR		
Any HPV/generic	104 (69.3)	19 (26.4)
High-risk types		
16	9 (6)	3 (4.2)
18	10 (6.7)	0
31	8 (5.3)	0
45	18 (12)	2 (2.8)
Subtotal (high risk)	45	5
Intermediate-risk types		
33	7 (4.7)	0
35	10 (6.7)	0
51	8 (5.3)	0
52	4 (2.7)	0
56	5 (3.3)	0
58	9 (6.0)	1 (1.4)
59	1 (0.7)	1 (1.4)
68	3 (2.0)	0
73	1 (0.7)	0
W13b/MM4	4 (2.7)	2 (2.8)
Subtotal (intermediate risk)	52	4
Low-risk types		
6	4 (2.7)	0
11	2 (1.3)	0
53	12 (8.0)	0
54	11 (7.3)	0
55	2 (1.3)	1 (1.4)
66	7 (4.7)	0
Pap 155/MM8	6 (4.0)	0
Pap 291/MM7	20 (13.3)	5 (6.9)
Subtotal (low risk)	64	6
Untyped	23	7
Hybrid Capture		
Probe A or B	25 (16.7)	1 (1.4)
Probe A	8 (3.6)	0
Probe B	22 (14.9)	1 (1.4)
Probe A and B	5 (3.3)	0

[a] HPV types 39, 26, and 40 were not detected in any specimen. Multiple isolates from a single individual are counted separately for type-specific prevalence.

positive and HIV-negative women (16.7% versus 1.4%, $P < 0.001$) (Table 1).

HPV prevalence by both PCR and Hybrid Capture increased significantly with increasing degree of immunosuppression (Table 2). HPV prevalence by PCR, which was 26.4% in HIV-seronegative women, increased almost threefold to 74.3% in women with CD4 counts of <200. The increase in HPV prevalence by Hybrid Capture was even more marked than by PCR; the prevalence rose more than 20-fold from 1.4% in HIV-seronegative women to 34.3% in women with CD4 counts of <200.

Among the 218 women for whom Pap smears were available, 28 (13.3%) had abnormal Pap smears: 9 cases of atypical squamous cells of uncertain significance, 15 cases of low-grade squamous intraepithelial lesions, and 4 cases of high-grade squamous intraepithelial lesions (HSIL). As shown in Table 3, abnormal Pap smears were highly correlated with HIV seropositivity and with degree of immunosuppression; only 1.4% of HIV-seronegative women, compared to 18% of HIV-seropositive women and 34.2% of women with CD4 counts of <200, had an abnormal Pap smear. In addition, Pap smear abnormalities were highly correlated with presence of HPVs by PCR, by Hybrid Capture, or by both tests. They were also correlated significantly with positivity by PCR alone. Pap smear abnormalities were associated with a high HPV viral load by PCR but not with HPV risk category or with multiple infections. From the data in Table 3, the sensitivities of PCR and of Hybrid Capture for detection of Pap smear abnormalities were

TABLE 2. HPV prevalence by PCR and Hybrid Capture: relationship to HIV seropositivity and CD4 cell counts

Sample (n)	PCR[a]		Hybrid Capture[b]	
	No. positive PCR	Odds ratio (95% confidence interval)	No. positive	Odds ratio (95% confidence interval)
HIV negative (72)	19	1	1	1.00
HIV positive; CD4 count:				
>500 (57)	33	3.8 (1.7–8.7)	5	6.8 (0.7–327.3)
200–499 (58)	45	9.7 (4.0–23.8)	8	11.4 (1.4–511.0)
<200 (35)	26	8.1 (2.9–22.7)	12	7.0 (4.8–1,604.8)

[a] $P = 0.058$ for HIV-positive samples; $P = 0.0001$ for all samples.
[b] $P = 0.002$ for HIV-positive samples; $P = 0.0001$ for all samples.

TABLE 3. Correlates of cytological abnormalities in ALIVE women

Sample type	No. (%) of results for women with cytological abnormalities		Odds ratio (95% confidence interval)
	No (n = 190)	Yes (n = 28)	
HIV serostatus and CD4 counts			
HIV negative	70	1 (1.4)	1.00
HIV positive; CD4 count:	120	27 (18.4)	15.8 (2.5–653.9)
>500	48	7 (12.7)	10.2 (1.2–466.5)
200–500	49	8 (14.0)	11.4 (1.4–514.2)
<200	23	12 (34.2)	36.5 (4.7–1,582.5)
HPV prevalence[a]			
PCR−	92	3 (3.2)	1.00
PCR+	98	25 (20.3)	7.8 (2.7–22.9)
HC−	176	16 (8.3)	1.00
HC+	14	12 (46.2)	9.4 (3.4–26.4)
PCR−/HC−	92	3 (3.2)	1.00
PCR+/HC−	84	13 (13.4)	4.7 (1.2–26.7)
PCR−/HC+			
PCR+/HC+	14	12 (46.2)	26.3 (5.8–155.9)
HPV risk categories			
PCR			
Low risk	19	5 (20.8)	1.00
Intermediate risk	20	9 (31.0)	1.71 (0.4–7.3)
High risk	31	9 (22.5)	1.1 (0.3–4.5)
HC			
Probe A	4	4 (50.0)	1.00
Probe B	11	12 (52.2)	1.1 (0.2–7.4)
HPV viral load			
PCR signal			
1–2	37	1	1.00
3–4	61	24	14.6 (2.1–614.5)
HC RLU			
<5	9	4	1.00
>5	5	8	3.6 (0.6–24.9)

[a] −, negative; +, positive; HC, Hybrid Capture.

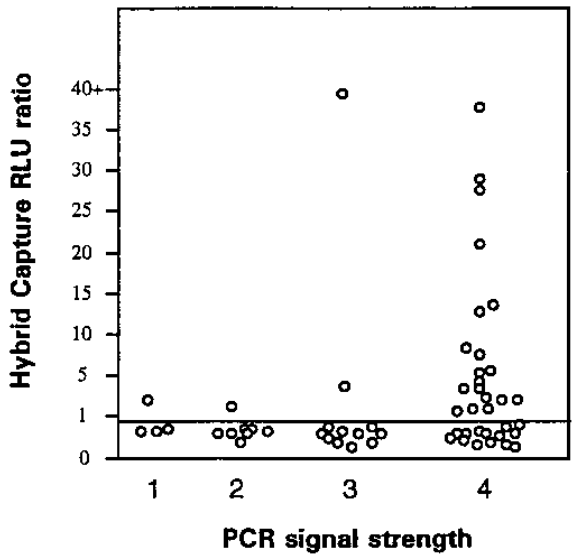

FIG. 1. Correlation between HPV quantitation by PCR and by Hybrid Capture. RLU ratios for Hybrid Capture probe B were plotted against PCR signal strength for 57 specimens which contained one or more of the nine HPV types in the probe B pool.

estimated to be 0.89 and 0.43, respectively; the corresponding specificities were 0.48 and 0.93.

PCR detects even small amounts of HPVs, whereas Hybrid Capture detects HPVs only when they are present in large amounts. Therefore, the ratio between HPV PCR prevalence and Hybrid Capture prevalence would be a crude measure of the relative amounts of HPVs in the specimens, with lower ratios indicative of greater amounts of HPV. The PCR prevalence:Hybrid Capture prevalence ratio was 19 in HIV-negative women and 4.1 in HIV-positive women (Table 1), suggesting that specimens from HIV-positive women had higher HPV viral load than specimens from HIV-negative women.

For the nine HPV types which make up the pool of Hybrid Capture probe B, we compared signal strength in PCR to RLU ratios in Hybrid Capture (Fig. 1). Fifty-seven specimens were positive in PCR for one or more of the HPV types in probe B. Of the 24 specimens with PCR signal strength of 1, 2, or 3, only 4 (16.6%) were positive by Hybrid Capture probe B, and of these, 3 had low RLU ratios. In contrast, 19 of 33 specimens (57%) with PCR signal strength of 4 were Hybrid Capture probe B positive ($P < 0.002$), and their RLU ratios covered a wide range. Overall, Hybrid Capture probe B identified HPVs in 40.4% of the specimens which were PCR positive for probe B types. All Hybrid Capture probe B positive specimens were also positive by PCR for one or more of the nine HPV types represented in probe B.

The main rationale for the use of HPV assays of genital tract specimens is that they may help identify women at high risk of concurrent or future severe cervical disease, e.g., HSIL and invasive cervical cancer. PCR-based tests for high-risk HPVs have been shown to be of value in cervical cancer screening (4, 11) and of clinical relevance (12). Similarly, the presence and amounts of HPVs, as measured by Hybrid Capture probe B, have been found to be useful in clinical management of women with low-grade or equivocal Pap smear abnormalities (3, 5, 17). The ALIVE women are being prospectively monitored for HPV infections and for cytologic and histologic evidence of cervical neoplasia. Their care is not influenced by their HPV status. When the data from this prospective study become available, we will have an opportunity to evaluate the two HPV assays for their potential usefulness in patient care.

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