ONLINE RESOURCES

Cancer Causes and Control

Risk of abnormal cervical cytology in HIV-infected women testing positive for both human papillomavirus and Epstein-Barr virus in genital tract specimens

Jennifer E. Cameron[#1,4], D'Antoni C. Dennis[1,a], Nicholas R. Herrel[1,b], Andrew G. Chapple[3] and Michael E. Hagensee[2,4]

Departments of [1]Microbiology, Immunology & Parasitology and [2]Medicine, School of Medicine; [3]Biostatistics Program, School of Public Health; and [4]The Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA.

[#]CORRESPONDING AUTHOR

Jennifer E. Cameron, Ph.D. Louisiana State University Health Sciences Center, Department of Microbiology, Immunology & Parasitology, 1901 Perdido St., New Orleans, LA 70112, USA. Phone: 504-568-2196. Fax: 504-568-2918. Email address: jcame2@lsuhsc.edu
ORCID: https://orcid.org/0000-0002-5785-2410

Online resource 1. HPV genotype prevalence and association with EBV detection.

HPV genotype	Overall cohort *n* = 115	EBV negative *n* = 100	EBV positive *n* = 15	*p*-value
Low-Risk	No. (%)	No. (%)	No. (%)	
HPV-6	10 (8.7)	6 (6.0)	4 (26.7)	**0.008**
HPV-11	1 (0.9)	1 (1.0)	0 (0)	0.697
HPV-40	3 (2.6)	2 (2.0)	1 (6.7)	0.290
HPV-42	6 (5.2)	4 (4.0)	2 (13.3)	0.130
HPV-53	11 (9.6)	8 (8.0)	3 (20.0)	0.141
HPV-54	16 (13.9)	15 (15.0)	1 (6.7)	0.385
HPV-66	14 (12.2)	12 (12.0)	2 (13.3)	0.883
HPV-84 (MM8)	14 (12.2)	12 (12.0)	2 (13.3)	0.883
High-Risk				
HPV-16	4 (3.5)	3 (3.0)	1 (6.7)	0.470
HPV-18	10 (8.7)	7 (7.0)	3 (20.0)	0.096
HPV-26*	4 (3.5)	3 (3.0)	1 (6.7)	0.470
HPV-31	5 (4.3)	4 (4.0)	1 (6.7)	0.637
HPV-33	2 (1.7)	2 (2.0)	0 (0)	0.581
HPV-35	8 (7.0)	5 (5.0)	3 (20.0)	**0.033**
HPV-39	5 (4.3)	5 (5.0)	0 (0)	0.376
HPV-45	9 (7.8)	9 (9.0)	0 (0)	0.226
HPV-51	10 (8.7)	10 (10.0)	0 (0)	0.200
HPV-52	20 (17.4)	18 (18.0)	2 (13.3)	0.657
HPV-55*	14 (12.2)	13 (13.0)	1 (6.7)	0.484
HPV-56	4 (3.5)	3 (3.0)	1 (6.7)	0.470
HPV-58	14 (12.2)	13 (13.0)	1 (6.7)	0.484
HPV-59	17 (14.8)	15 (15.0)	2 (13.3)	0.865
HPV-68	8 (7.0)	7 (7.0)	1 (6.7)	0.962
HPV-82 (MM4)*	2 (1.7)	2 (2.0)	0 (0)	0.581
HPV-83 (MM7)*	26 (22.6)	24 (24.0)	2 (13.3)	0.357
HPV-73 (MM9)*	9 (7.8)	6 (6.0)	3 (20.0)	0.060

Abbreviations: HPV, human papillomavirus. EBV, Epstein-Barr virus.

Probability (*p*) was calculated using Chi-square analysis or Fisher Exact test.

*These intermediate-risk HPV types have been re-classified as low-risk viruses but were treated as high-risk viruses for the primary analysis presented in this report.

Online resource 2. Risk factors[a] for EBV detection in cervicovaginal lavage of HIV-positive women who are also hrHPV-positive.

	hrHPV = IARC-12 + HPV-68 *n* =85			hrHPV = IARC-12 + HPV-26, 55, 68, 73, 82, 83 *n* = 102		
	EBV Negative (*n* = 72) n (%)	EBV Positive (*n* = 13) n (%)	*p*-value[b]	EBV Negative (*n* = 88) n (%)	EBV Positive (*n* = 14) n (%)	*p*-value[b]
Race/Ethnicity			0.056[c]			**0.039[c]**
Caucasian	9 (90.0)	1 (10.0)		10 (11.4)	1 (7.1)	
African-American	63 (85.1)	11 (14.9)		78 (88.6)	12 (85.7)	
Hispanic	0 (0)	1 (100.0)		0 (0)	1 (7.1)	
Age			0.264			0.298
20-30	27 (93.1)	2 (6.9)		31 (35.2)	2 (14.3)	
31-40	28 (82.4)	6 (17.6)		33 (37.5)	7 (50.0)	
>40	17 (77.3)	5 (22.7)		24 (27.3)	5 (35.7)	
Mean (SD)	34.4 (8.1)	37.9 (7.4)	0.156	34.8	37.8	0.195
Marital status			1.000			1.000
Married/co-habitant partner	6 (84.6)	1 (15.4)		7 (8.0)	1 (7.1)	
Single/divorced/widowed	66 (85.7)	12 (14.3)		81 (92.0)	13 (92.9)	
Education level			0.315			0.313
6-11 years	7 (70.0)	3 (30.0)		8 (9.5)	3 (21.4)	
High School graduate	58 (87.9)	8 (12.1)		68 (80.9)	8 (57.1)	
College graduate or above	4 (80.0)	1 (20.0)		8 (9.5)	3 (21.4)	
Smoking history			1.000			0.937
Never	37 (88.1)	7 (15.9)		45 (51.1)	7 (50.0)	
Ever	35 (85.4)	6 (14.6)		43 (48.9)	7 (50.0)	
Parity			0.582			0.588
Nulliparous	6 (100.0)	0 (0)		6 (7.3)	0 (0)	
$\geq$ 1	60 (82.2)	13 (17.8)		76 (92.7)	14 (100)	
History of abnormal Pap smear			0.742			0.535
Yes	41 (80.4)	10 (19.6)		49 (65.3)	11 (78.6)	
No	19 (86.4)	3 (13.6)		26 (34.7)	3 (21.4)	
Male sex partners, past year			0.398			0.644
None	7 (77.8)	2 (22.2)		9 (13.0)	2 (22.2)	
1	36 (92.3)	3 (7.7)		41 (59.4)	4 (44.4)	

2 or more	16 (84.2)	3 (15.8)		19 (27.5)	3 (33.4)	
Male sex partners, past month			0.688			0.713
None	14 (82.4)	3 (17.6)		16 (27.6)	3 (37.5)	
1	32 (88.9)	4 (11.1)		39 (67.2)	5 (62.5)	
2 or more	2 (100.0)	0 (0.0)		3 (5.2)	0 (0)	
CD4+ T cell count			0.972			0.981
<200	20 (83.3)	4 (16.7)		24 (27.9)	4 (28.6)	
200-500	27 (84.4)	5 (15.6)		33 (38.3)	5 (35.7)	
>500	24 (85.7)	4 (14.3)		29 (33.7)	5 (35.7)	
Mean (SD)	396 (268)	359 (216)	0.640	398 (273)	401 (258)	0.977
Peripheral HIV viral load			0.682			0.557
<400	19 (90.5)	2 (9.5)		24 (27.9)	2 (14.3)	
400-10,000	23 (82.1)	5 (17.9)		25 (29.0)	5 (35.7)	
>10,000	29 (82.9)	6 (17.1)		37 (43.0)	7 (50.0)	
Mean, x 10^3 (SD)	84.6 (193.7)	56.0 (97.5)	0.605	95.4 (202.7)	53.4 (94.2)	0.450

[a]All data is self-reported on demographic survey, except CD4+ T cell counts and peripheral HIV viral load which were extracted from the clinical chart. Sum of individual data may not equal total column *n* due to missing data (participant did not respond, marked 'unknown', or information was otherwise unavailable).

[b]Probability (*p*) was derived from Chi square analysis, or Fisher Exact test when expected counts in one or more category was <5.

[c]Fisher Exact test *p*-value for Caucasians vs. African-Americans = 1.000.

Abbreviations: IARC, International Agency for Research on Cancer. HIV, human immunodeficiency virus. EBV, Epstein-Barr virus. hrHPV, high-risk human papillomavirus. SD, standard deviation.

Online resource 3. Multinomial regression model predicting abnormal cytology for women who had high-risk HPV infection.

A) Model for SIL vs Normal Cytology
hrHPV: IARC-12 + HPV-26, -55, -68, -73, -82, -83

Variable	Coefficient	Test Statistic	*p*-value
Intercept	2.089	1.844	0.065
Age	-0.580	-2.309	**0.021**
African American Race	-2.113	-1.817	0.069
CD4+ T Cell Count	-0.671	-2.532	**0.011**
EBV	1.866	2.121	**0.034**

B) Model for SIL vs Normal Cytology
hrHPV: IARC-12 + HPV-68

Variable	Coefficient	Test Statistic	*p*-value
Intercept	2.132	1.834	0.067
Age	-0.572	-2.026	**0.043**
African American Race	-1.822	-1.529	0.126
CD4+ T Cell Count	-0.803	-2.684	**0.007**
EBV	1.373	1.530	0.127

Age and CD4 counts were standardized.

Abbreviations: IARC, International Agency for Research on Cancer. HPV, human papillomavirus. EBV, Epstein-Barr virus. SIL, squamous intraepithelial lesion (includes low-grade and high-grade SIL).

Online resource 4. Impact of genital high-risk HPV (IARC-12 +HPV-68) and human herpesvirus detection on cervical squamous intraepithelial lesions (SIL) in HIV-infected women.

Genital hrHPV	Genital HHV	SIL-Negative No. (%)	SIL-Positive No. (%)	Odds Ratio (95% CI)	*p*-value[b]
	Any HHV				
-	-	107 (84.9)	19 (15.1)	Reference	
+	-	31 (47.7)	34 (52.3)	6.18 (3.10-12.31)	**<0.0001**
-	+	18 (72.0)	7 (28.0)	2.19 (0.81-5.96)	0.12
+	+	7 (35.0)	13 (65.0)	10.46 (3.69-29.61)	**<0.0001**
+	-	31 (47.7)	34 (52.3)	Reference	
+	+	7 (35.0)	13 (65.0)	1.69 (0.60-4.79)[a]	0.32
	EBV				
-	-	112 (83.6)	22 (16.4)	Reference	
+	-	34 (47.2)	38 (52.8)	5.69 (2.97-10.90)	**<0.0001**
-	+	13 (76.5)	4 (23.5)	1.57 (0.47-5.26)	0.50
+	+	4 (30.8)	9 (69.2)	11.45 (3.24-40.53)	**0.0001**
+	-	34 (47.2)	38 (52.8)	Reference	
+	+	4 (30.8)	9 (69.2)	2.01 (0.57-7.14)[a]	0.37

[a]Odds ratio of SIL in women with both hrHPV and HHV (top of table) or EBV (bottom of table) compared to women with hrHPV but no HHV or EBV, respectively.

[b]Fisher's Exact Test p-value is presented.

Abbreviations: IARC, International Agency for Research on Cancer. hrHPV, high-risk human papillomavirus (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 68). HHV, human herpesvirus. SIL, squamous intraepithelial lesion. CI, confidence interval. EBV, Epstein-Barr virus.

Online resource 5. Association of cervical high-risk HPV (IARC-12 +HPV-68) and EBV detection with cervical squamous intraepithelial lesions (SIL) in HIV-infected women.

Cervical hrHPV[a]	Cervical EBV	SIL-Negative No. (%)	SIL-Positive No. (%)	Odds Ratio (95% CI)	*p*-value[b]
-		164 (82.0)	36 (18.0)	Reference	
+		51 (53.7)	44 (46.3)	3.93 (2.29-6.75)	**<0.001**
	-	129 (79.6)	33 (20.4)	Reference	
	+	86 (64.7)	47 (35.3)	2.14 (1.27-3.60)	**0.004**
-	-	96 (82.8)	20 (17.2)	Reference	
+	-	33 (71.7)	13 (28.3)	1.89 (0.85-4.22)	0.116
-	+	68 (81.0)	16 (19.0)	1.13 (0.55-2.34)	0.743
+	+	18 (36.7)	31 (63.3)	8.27 (3.89-17.58)	**<0.001**
+	-	33 (71.7)	13 (28.3)	Reference	
+	+	18 (36.7)	31 (63.3)	4.37 (1.84-10.39)[c]	**<0.001**

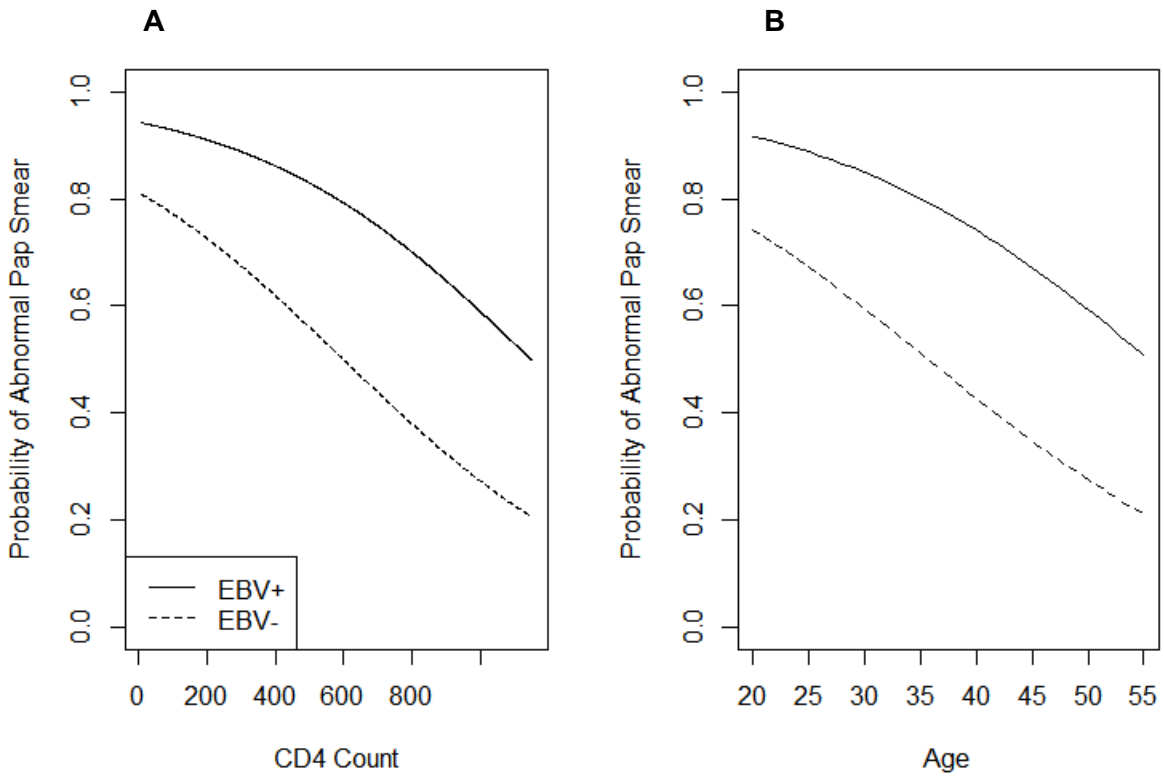
[a]Epstein-Barr virus and high-risk HPV were detected in cervical swabs collected from HIV-positive women enrolled in cohort 2.

[b]Fisher's Exact test p-value is presented.

[c]Odds ratio of SIL in women with both hrHPV and EBV compared to women with hrHPV but no EBV.

Abbreviations: IARC, International Agency for Research on Cancer. HIV, human immunodeficiency virus. hrHPV, high-risk human papillomavirus (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 68). EBV, Epstein-Barr virus. SIL, squamous intraepithelial lesion. CI, confidence interval.

Online resource 6. Predicted probability of an abnormal cervical cytology A) as a function of CD4 count at the mean age of 35.0 years old and B) as a function of age at the mean CD4 count of 390.7 for African Americans in the study using the model described in online resource 3, panel B (using the IARC-12 + HPV-68 classification of hrHPV).



Online resource 7. Estimated Odds ratios from multivariable logistic regression model (online resource 3, panel B) for abnormal cervical cytology and 95% confidence intervals. Confidence intervals that do not overlap with the horizontal line indicate a significantly different odds ratio from 1.0. This analysis uses the IARC-12 + HPV-68 classification of hrHPV.