Screening Perspectives Panel

HPV Testing and P16: What is the Role in Anal Disease

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Disclosures

- I have no significant financial relationships to disclose that would pose a potential conflict of interest.

Non-FDA-Approved

- There are NO FDA-approved HPV tests for anal HSIL screening.

Introduction

- Rationale and limitations
- Review data on various testing methods
- Which test HPV DNA (HC or PCR), HPV genotyping, HPV mRNA, or p16ki67 dual staining?
- Prospective data in women??
- Future directions and conclusions

Stages of Cervical or Anal SCCA Development

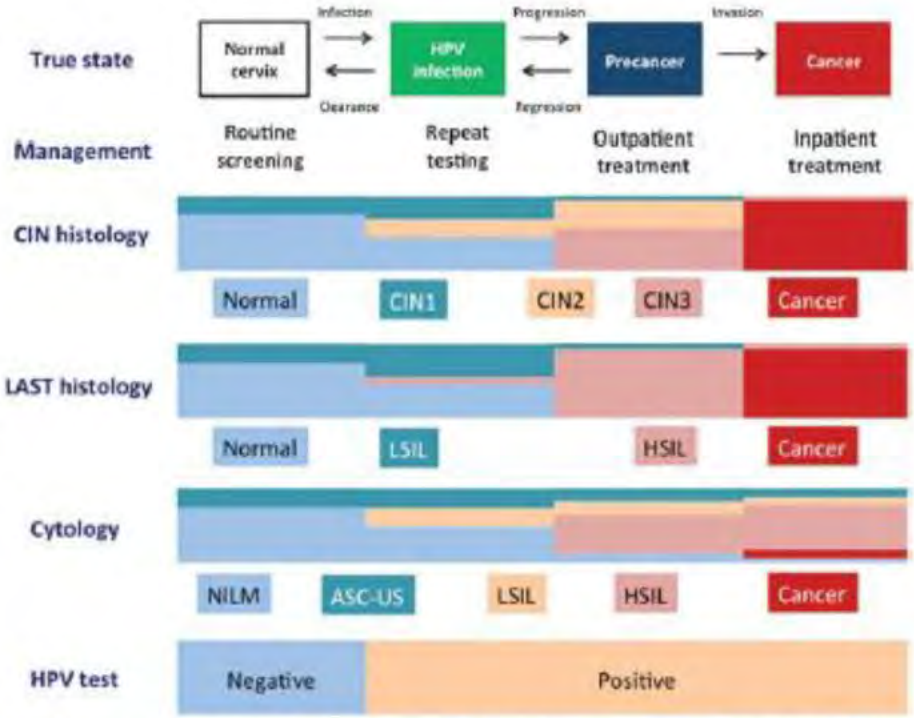
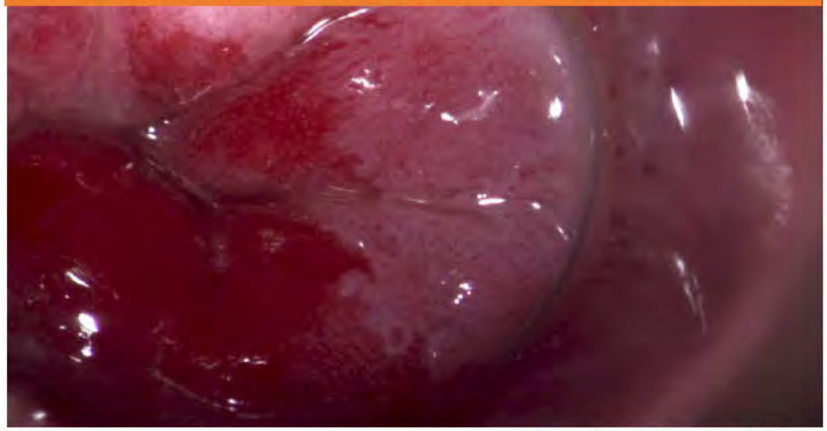


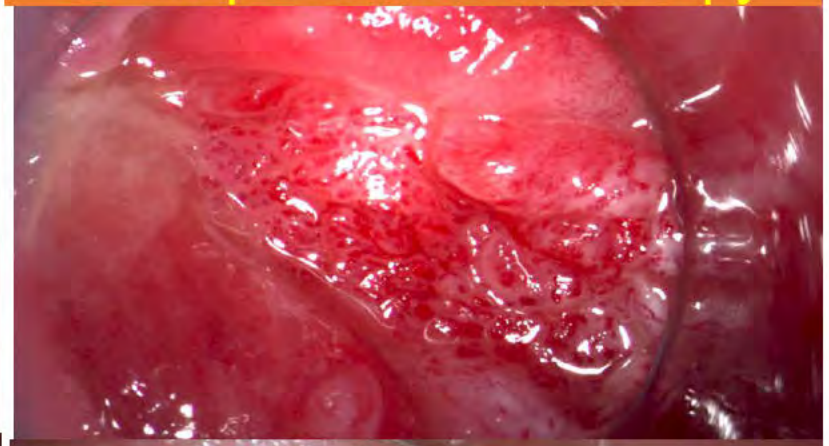
FIGURE 1. Comparison of common test results and categories in cervical screening programs to the stages of cervical cancer development. Cervical screening programs generate a large number of terms and categories that only imperfectly correspond to the now established stages in cervical carcinogenesis (top row). A focus on diagnosis of each stage, with simplification of terms, could usefully organize available test methods and maximize concordant clinical action (rescreening at an extended interval when screening indicates a normal cervix, accelerated retesting for high-risk HPV infection, colposcopic biopsy to diagnose precancer treatable by outpatient procedures, and curative or palliative treatment of invasive cancer). The figure indicates that no screening or diagnostic test is perfect. For example, histology, our current diagnostic reference standard, tends to overcall precancer because we cannot yet determine which severe intraepithelial microscopic abnormalities indicate that the lesion would invade if untreated (rather than regress or persist). The impact of replacing the CIN scale with LAST criteria incorporating p16 testing to clarify precancer is not yet known. In any case, histology cannot accurately distinguish HPV infection from the normal cervix. Similarly, cytologic categories are prone to misclassification of HPV status and whether infection has progressed to precancer/cancer. Human papillomavirus testing is most effective at establishing normalcy (i.e., lack of infection implying extremely low risk of precancer/cancer), but it cannot distinguish between benign infection and precancer/cancer; secondary triage tests and extended testing intervals are needed to prevent substantial overtreatment. Note that this crude conceptual figure is not drawn to any scale, and the color distributions are not representative of actual proportions, which may vary between populations.

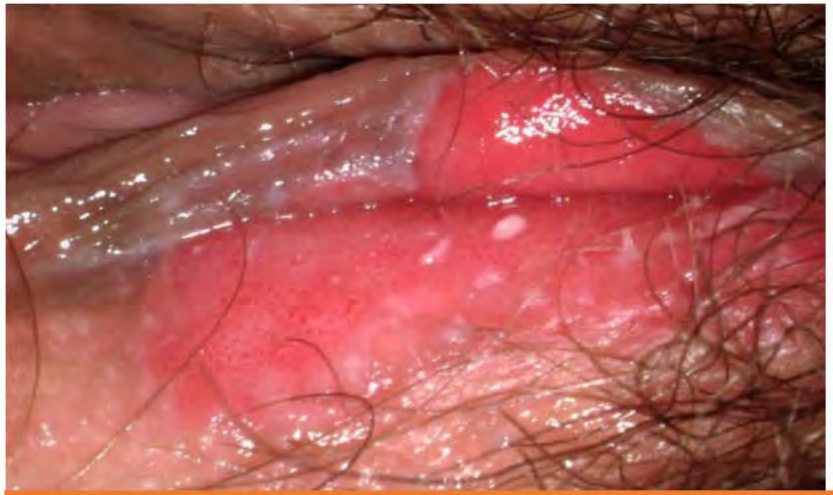
Anal Cancer has Many Faces

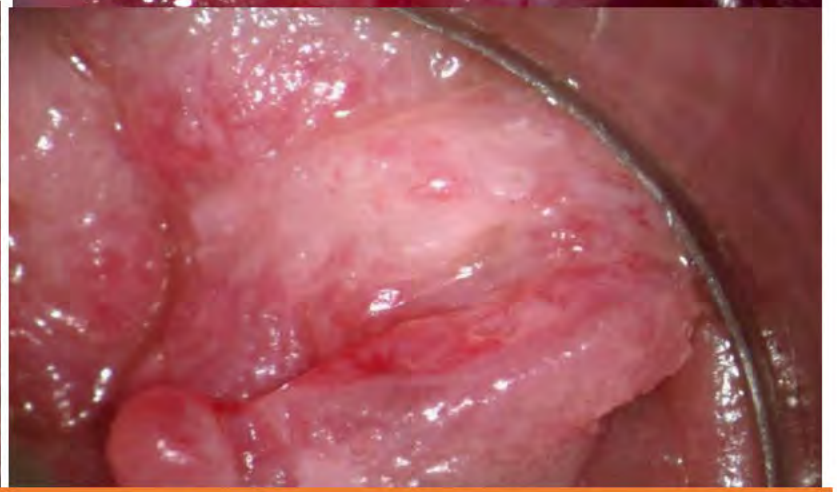
Woman/Normal Cx Paps
Submucosal Nodule



Woman/Normal Cx Paps
SI suspicious/Colonoscopy







Simultaneous Vulvar and Anal Cancer

Limitations of HPV Testing

- Very few studies performed with high-quality HRA, particularly in women
- Heterogeneity of HPV testing methods and subjects, mostly cross-sectional or cohort
- Limited or no longitudinal data
- Lack of data confirming that detection and treatment of HSIL effectively prevents cancer

HRHPV, Anal HSIL and Risk Group

	HIV-positive Women	LGT-HPV	HIV-negative Women	
Anal Ca incidence per 100,000	3.9-30	0.8-6*	0.55-2.4	Higher in women with LGT-HPV
Anal HPV infection	16-85%	23-36%	4-36%	Higher in anus than cervix
HSIL cytology	0-5%	0-29%	0-0.3%	
HSIL histology	3-26%	0-3%	0-9%	
HSIL histology UCSF	54%	52%	37%	Only women without HSIL on referral

Adapted from Stier et. al. AJOG 2015 and Berry-Lawhorn et. al. IANS Meeting 2015

HC2 and Anal HSIL

Goldstone et. al. IJC: 131, 1641-1648, 2012

- ~300 participants, 97% male and 45% HIV+
- 111 or 37% with first screening
- All had anal cytology, HC2 HPV DNA testing, and HRA/biopsy

Table 1. Study data for anal specimens

		A) Histology and cytology test results for study patients								
		Anal histology								
		Benign	%	AIN1	%	AIN2	%	AIN3	%	Total
Anal cytology	Benign	74	61.2	23	19.0	17	14.0	7	5.8	121
	ND	8	38.1	4	19.0	5	23.8	4	19.0	21
	ASCUS	41	46.1	17	19.1	23	25.8	8	9.0	89
	LSIL	1	1.9	18	34.0	21	39.6	13	24.5	53
	ASC-H	2	40.0	0	0.0	1	20.0	2	40.0	5
	HSIL	0	0.0	0	0.0	1	33.3	2	66.7	3
	Total	126		62		68		36		292

- Overall, HSIL prevalence=35.6% and positive HC2 =71%
  - 51.5% in HIV positive and positive HC2=83%
  - 22.5% in HIV negative and positive HC2=61%

[1]Cytolog
($\alpha = 0.0$

Conclusions

- Significantly increased sensitivity of HC2 with slight loss of specificity compared with cytology
- Significantly increased sensitivity of cytology plus HC2 with slight loss of specificity compared with cytology
- Only 9 of 104 participants with HSIL had negative HC2
- Significance was largely contributed by HIV positive participants and was not significant in HIV negative
- “HC2 might be useful for primary screening or as an adjunct to cytology, especially patients with benign or ASCUS cytology.”

UCSF Study Evaluating Performance Characteristics

- 85 HIV -, 35 HIV +, and 5 unknown=125 MSM
- Anal cytology, HPV testing by PCR, HRA/biopsy
- There were significant differences in performance characteristics between HIV+ and HIV- MSM
- HPV testing was more useful in HIV – MSM and increased sensitivity from 55% to 90% when HRHPV+ and HPV 16
- Also in HIV- MSM the NPV was 93% when participants had negative cytology and no HRHPV

HPVE6E7 mRNA Testing

- HC2 and PCR are HPV DNA tests indicating the presence or absence of HRHPV
- E6 and E7 are transforming proteins produced by HRHPV
- Overexpression of E6 and E7 are key steps in carcinogenesis and may increase specificity of finding HSIL
- Commercial tests are available to test for mRNA in the Cervix such as: PreTect HPV Proofer, NucliSENS Easy Q, and Aptima
- Aptima is a target amplification assay using trancription-mediated amplification

UCSF Aptima Study for Anal HSIL

- Option for pooled HRHPV or genotype specific for HPV 16 or HPV 18 and 45
- 656 HIV-positive and 287 HIV-negative (834 men, 104 women, 5 transgender)
- Anal swab for Thinprep anal cytology, a second anal swab into Thinprep media for Aptima HPV RNA testing, and high resolution anoscopy with biopsy of visible disease.
- Aptima-positive specimens were further tested for HPV16 and HPV18/45 RNA.
- 309 (33%) patients were diagnosed with HSIL.
- 425 (45%) patients were Aptima-positive, including 131 (14%) for HPV16 and 73 (8%) for HPV18/45.

Relative risk of anal HSIL for selected HPV result comparisons, by cytology

Cytology	HPV result comparison	Absolute risks (%)	Relative risk (95% CI)
Normal	Aptima+ vs. Aptima-	16.7 vs. 1.6	10.4 (2.9 to 37.0)
ASC-US	Aptima+ vs. Aptima-	26.2 vs. 8.0	3.3 (1.8 to 6.0)
LSIL	Aptima+ vs. Aptima-	30.4 vs. 11.6	2.6 (1.4 to 4.9)
Normal	Aptima 16+ vs. Aptima 16-	40.0 vs. 4.2	9.4 (2.8 to 32.4)
ASC-US	Aptima 16+ vs. Aptima 16-	35.3 vs. 12.2	2.9 (1.4 to 6.0)
LSIL	Aptima 16+ vs. Aptima 16-	44.7 vs. 17.6	2.5 (1.6 to 4.1)
Normal	Aptima 18/45+ vs. Aptima 18/45-	20.0 vs. 4.7	4.3 (0.7 to 27.2)
ASC-US	Aptima 18/45+ vs. Aptima 18/45-	0.0 vs. 14.5	NC
LSIL	Aptima 18/45+ vs. Aptima 18/45-	31.8 vs. 21.2	1.5 (0.8 to 2.9)
Normal	HPV DNA PCR+ vs. HPV DNA PCR-	7.6 vs. 2.6	2.9 (0.8 to 10.5)
ASC-US	HPV DNA PCR+ vs. HPV DNA PCR-	17.6 vs. 5.3	3.3 (1.3 to 8.3)
LSIL	HPV DNA PCR+ vs. HPV DNA PCR-	23.2 vs. 10.0	2.3 (0.6 to 8.9)
Normal	HPV PCR 16+ vs. HPV PCR 16-	25.0 vs. 4.8	5.2 (0.9 to 31.3)
ASC-US	HPV PCR 16+ vs. HPV PCR 16-	36.4 vs. 12.0	3.0 (1.3 to 7.1)
LSIL	HPV PCR 16+ vs. HPV PCR 16-	43.6 vs. 17.1	2.5 (1.6 to 4.1)

Palefsky J. Manuscript in preparation

P16/Ki-67 Cytology Testing

- Another approach to improve detection and reduce inter-rater variability is adding p16/Ki-67 staining to cytology specimens

- Data from Kaiser Study: Human papillomavirus genotyping, mRNA expression and p16/Ki-67 cytology to detect anal cancer precursors in HIV-infected MSM (Wentzensen et. al. AIDS. 2012 November 12; 26(17): 2185-92)

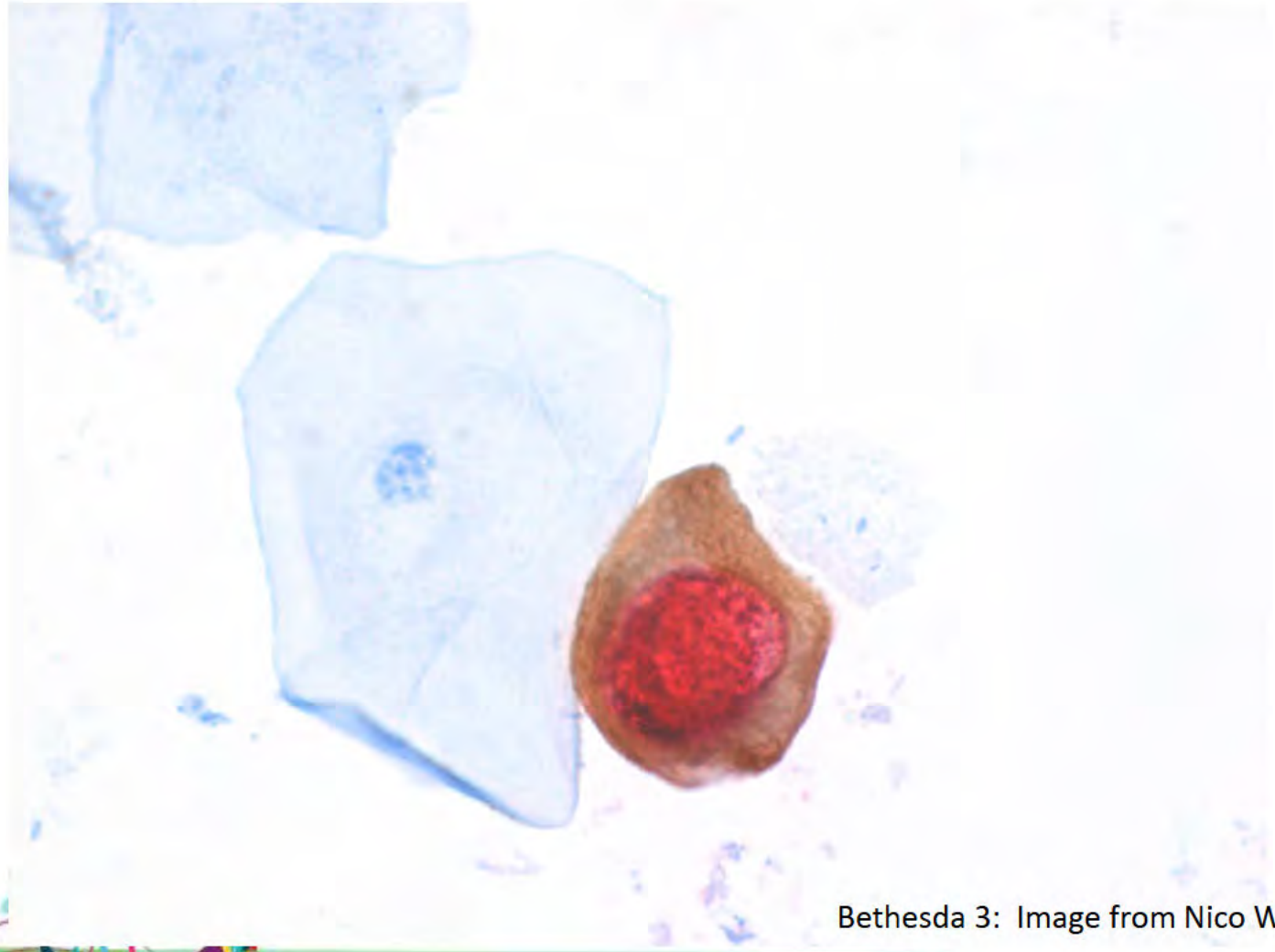
- 363 HIV+ MSM:
  - HSIL cytology 16.5%
  - HSIL histology 22.0%
  - Negative 30.8%
  - Composite HSIL 30.0%

Bethesda 3
Chapter 8 Figures
P16/ki67 dual staining

Ki-67 = nuclear red stain

p16 = cytoplasmic brown stain

Anal Cytology: p16/ki-67 stain



Bethesda 3: Image from Nico Wetzensen

Kaiser Results

Table 2

Biomarker positivity in disease categories.

Test	No dysplasia	AIN1	AIN2	AIN3	Total	*P* trend	
Cobas HR-HPV	88/147 (59.9%)	87/103 (84.5%)	50/50 (100%)	59/59 (100%)	284/359 (79.1%)	<0.001	Highest sensitivity
Cobas HPV16/18	27/147 (13.4%)	41/103 (39.8%)	31/50 (62.0%)	39/59 (66.1%)	138/359 (38.4%)	<0.001	
mRNA	38/141 (27.0%)	47/98 (48.0%)	38/48 (79.2%)	47/58 (81.0%)	170/345 (49.3%)	<0.001	
p16/Ki-67	56/133 (42.1%)	65/92 (70.7%)	45/49 (91.8%)	54/58 (93.1%)	220/332 (66.3%)	<0.001	Highest sensitivity

Combined disease endpoints: no dysplasia, including men with a nondysplastic biopsy or without a biopsy and with <HSIL cytology; AIN1, including men with AIN1 histology and <HSIL cytology; AIN2, including men with AIN2 histology or with lower grade, normal, or no histology and with HSIL-AIN2; AIN3, including men with AIN3 histology or with lower grade, normal, or no histology and with HSIL-AIN3. *P* trend is based on the χ^2-test. AIN1, anal intraepithelial neoplasia grade 1; AIN2, anal intraepithelial neoplasia grade 2; AIN3, anal intraepithelial neoplasia grade 3; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion.

- For all biomarkers, significant trend of greater proportion of positive results in those with most severe disease

- When threshold for p16/K1-67 positivity was increased from at least 1 to 5+ cells there was significantly higher specificity with unchanged sensitivity for detecting AIN 3

Future Research

- Currently AMC 084 is evaluating anal cytology, Aptima, HC2, and OncoHealth HPVE6/E7 oncoprotein in 300 HIV+ women

- Women who have never been screened have above tests and simultaneous HRA with biopsy of visible lesions or random biopsy of at least 2 areas

Conclusions

- Most HPV testing methods appear to function in a similar manner in the anus as in the cervix
- No specific recommendations can be made for any form of HPV testing or p16/Ki-67 staining for primary screening
- HPV DNA or HPV mRNA testing with or without genotyping may be useful in populations with a low prevalence of HPV as primary testing, but requires validation

Conclusions

- Co-testing or reflex testing may be useful in triaging patients for HRA, who have abnormal cytology <HSIL or ASC-H, but requires validation
- Currently best use of HPV testing may be for its negative predictive value and determining need and interval for follow-up