

## Which test is better for screening AIN2+ lesions: P16/ki67, anal cytology or high-risk HPV PCR in HIV+ patients?

Hidalgo Tenorio C (1), Esquivias J (1), Ramírez J (1), Gil C(1), De Jesús S (1), López MA (1), Javier R (1), Omar M(2), Juan Pasquau (1)

(1) University Hospital Virgen de las Nieves, Granada, (2) University Hospital Ciudad de Jaén, SPAIN

- BACKGROUND:** the incidence of anal squamous cell carcinoma (ASCC) is higher in HIV+ patients than in the general population. At present, ASCC is one of the most frequent non-AIDS defining malignances. Screening recommendations for premalignant lesions and ASCC in seropositive patients include annual cytology, and in cases of any grade of dysplasia, a high resolution Anoscopy (HRA). However, considering that cytology sensitivity is variable and somewhat low, the use of biological markers is being evaluated. Among these, p16/ki67, a cyclin-dependent kinase (CDK) inhibitor that decelerates the cell cycle and is overexpressed when HPV is present. Detection of p16/ki67 in anal mucosa seems to increase the diagnostic sensitivity for HSIL (1).
- OBJETIVES:** to compare the sensitivity (S), specificity (E), positive (PPV) and negative (NPV) predictive values of p16+/ki67, anal cytology and high risk(HR)-HPV PCR in screening and diagnosis of anal HSIL, as well as the grade of correlation with the histology, according to kappa index.
- PATIENTS AND METHODS:** this is a cross-sectional analysis of a prospective cohort of HIV+ patients being followed at our Anal Dysplasia outpatient clinic. In all patients, an anal cytology in liquid medium (ThinPrep® Pap Test), HPV PCR (Linear Array HPV Genotyping Test), detection of P16/ki67 in tissue sample, and HRA (Zeis 150 ©) were carried out. Data concerning sexual activity, STDs and HIV infection were also collected.

(1)-Bala R et al. Am. J. Surg. Pathol. 2013; 37: 659-68.

### RESULTS:

**Table 1. Baseline Characteristics**

|  | N=79            |
|--|-----------------|
| Age, mean                                | 40,6            |
| MSM, n(%)                                | 67 (84,8)       |
| WLHIV, n(%)                              | 12 (152)        |
| AIDS stage, n(%)                         | 32 (40.5)       |
| Nadir CD4, mean (DS)                     | 284 (212.4)     |
| Current Cd4, mean (DS)                   | 642 (276.5)     |
| History HAART, n(%)                      | 74 (93.7)       |
| Virological failure, n(%)                | 4 (5.1)         |
| Smoking, n(%)                            | 38 (48.1)       |
| ExUPD                                    | 4 (5.1)         |
| Sexual partners, 12 months, median (IQR) | 1 (1-4)         |
| Anal Warts, n(%)                         | 24 (30.4)       |
| Condom use, n(%)                         | 56 (73.3)       |
| HCV/HBV                                  | 5 (6.3)/1 (1.3) |
| Syphilis                                 | 12 (15.2)       |

**Table 2. Cytology, HR-HPV and HRA**

|                       | N=79      |
|-----------------------|-----------|
| Normal cytology, n(%) | 39 (49.3) |
| ASCUS                 | 6 (7.6)   |
| LSIL                  | 29 (36.7) |
| HSIL                  | 5 (6.3)   |
| HR-HPV (+)            | 51 (64.5) |
| P16/Ki67 (+)          | 21 (26.5) |
| HRA n(%)              |           |
| HSIL                  | 10 (12.6) |
| LSIL                  | 41 (51.9) |

**Table 3. Genotypes of HR-HPV**

| HR-HPV       | N=77      |
|--------------|-----------|
| HPV-16, n(%) | 16 (20.8) |
| HPV-18, n(%) | 7 (9.1)   |
| HPV-31, n(%) | 7 (9.1)   |
| HPV-42, n(%) | 8 (10.4)  |
| HPV-44, n(%) | 7 (9.1)   |
| HPV-51, n(%) | 10 (13)   |
| HPV-59, n(%) | 10 (13)   |
| HPV-68, n(%) | 7 (9.1)   |

**Table 4. Utility of p16/Ki67 (+), PCR of HR-HPV and cytology for screening of HSIL.**

|                   | Sensitivity | Specificity | PPV   | NPV   | OR, IC95%; p-value   | kappa |
|-------------------|-------------|-------------|-------|-------|----------------------|-------|
| P16+/Ki67         | 70%         | 79.7%       | 25%   | 94.8% | 9, (2-40); 0.003     | 0.338 |
| HR-HPV-PCR (+)    | 88.8%       | 36.8%       | 15.7% | 96.2% | 4, (0.5-39.4); 0.16  | 0.085 |
| Abnormal Cytology | 70%         | 52.7%       | 17.5% | 92.3% | 2.5, (0.6-10.7); 0.2 | 0.097 |

**Table 5. Correlation of cytology, (+) HR-HPV PCR and P16+/ki67 with histology**

|   | Normal, n (%) | LSIL, n (%) | HSIL, n (%) |
|---|---------------|-------------|-------------|
|   | N=28          | N= 41       | N=10        |
| Normal cytology (n=39)                                  | 23 (82.1)     | 13 (31.7)   | 3 (30)      |
| ASC (n=6)   | 0             | 4 (9.7)     | 2 (20)      |
| LSIL (n=29)   | 5 (17.8)      | 22(53.6)    | 2 (20)      |
| HSIL (n=5)  | 0             | 2 (4.9)     | 3 (30)      |
| HR-HPV-PCR +, (n=51)                                    | 16 (57.1)     | 27(65.3)    | 8 (88.8)    |
| P 16+, (n=21)   | 6 (21,4)      | 7 (17.1)    | 7(70)       |
| P16 + and LSIL (n=7)                                    | 2 (7.1)       | 5 (12.2)    | 0           |
| Normal cytology, positive HR-HPV and positive P16 (n=6) | 2 (7,1)       | 2 (48.7)    | 2 (20)      |
| Normal cytology and negative HPV- PCR (n=14)            | 10 (35)       | 4 (9.7)     | 0           |
| Normal cytology and positive HR-HPV PCR (n=23)          | 12 (42,9)     | 9 (21.9)    | 2 (20)      |

**MSM:** men who have sex with men; **WLHIV:** women living with HIV; **ExUDP:** ex-user to parenteral drugs **HRA:** high resolution anoscopy; **HR-HPV:** high-risk human papillomavirus; **PPV:** positive predictive value; **NPV:** negative predictive value; **OR;** odds ratio.

**CONCLUSIONS:** We recommend the combination of cytology and HR-HPV PCR for routine screening of anal HSIL in HIV patients. P16/ki67 would be more useful than PCR of HR-HPV for diagnosis of HSIL lesions due to its specificity and correlation with histology