# THE ROLE OF E6/E7 mRNA DETERMINATION FOR ANAL CANCER SCREENING IN HIV-POSITIVE MSM

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(Spanish HIV/AIDS ResearchNetwork, PreEC/RIS 53 )

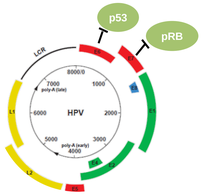
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## INTRODUCTION

- HIV-infected MSM are the highest risk population for anal cancer and the causal HPV always expresses E6 and E7 oncoproteins.
- E6/E7 Oncoproteins inhibit different tumor supressor proteins, such as p53 and pRB.



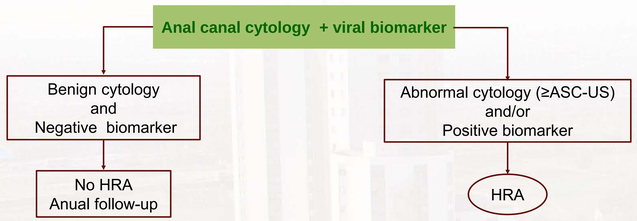

## PURPOSE

To assess anal cancer and HSIL screening strategies that include viral biomarkers (E6 and E7 mRNA and HPV DNA) to triage candidates for High-resolution anoscopy (HRA)

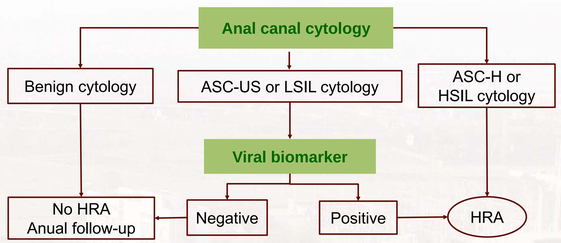
## METHODS

- Baseline visit of the 354 recruited participants of the prospective ELAVI-67 Project, that includes HIV-infected MSM visited in the HIV and STD Unit (Bellvitge University Hospital).
- All participants underwent anal smear and HRA with biopsies of areas suspicious of displasia.

### First combined triage strategy




### Second combined triage strategy




Viral biomarkers where analyzed alone and combined with anal cytology results in two different triage strategies

### Analysed viral biomarkers

- Aptima® E6/E7 mRNA test (E6/7 mRNA): detection of 14 HR-HPV types.
  - HPV-16,-18 and -45.
  - HPV-16.
- Hybrid Capture 2® (HC2) HPV DNA test: detection of 13 HR-HPV types.
- Linear Array® (LA) HPV DNA test: detection and typification of 37 HPV types.
  - 14 HPV types included in the E6/7 mRNA test
  - HPV-16,-18 and -45.
  - HPV-16.

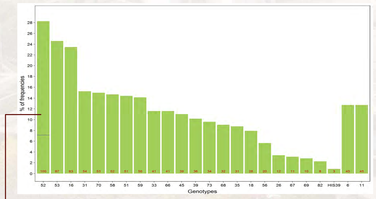
## RESULTS

### Baseline characteristics

| | |
|---|---|
| **Sociodemographic characteristics** | |
| Age (years), mean (SD) | 45.3 (11.22) |
| Current smokers, n (%) | 132 (39.05%) |
| **HIV-infection related variables** | |
| Nadir CD4 T-cell count, cells/µL mean (SD) | 335.31 (258.97) |
| Current CD4 T-cell count, cells/µL mean (SD) | 802.25 (333.14) |
| Current CD4 T-cell< 250 cells/µL, n (%) | 10 (2.82%) |
| Undetectable plasma HIV-RNA, n (%) | 309 (87.29%) |
| **Sexual behaviour** | |
| Age at first sexual intercourse, mean (SD) | 17.75 (3.87) |
| Lifetime sexual partners | |
| <30, n (%) | 119 (35.42%) |
| 30-50, n (%) | 82 (24.4%) |
| 50-100, n (%) | 48 (14.29%) |
| >100, n (%) | 87 (25.89%) |
| Receptive anal sexual intercourse, n (%) | 188 (55.46%) |
| History of Anogenital warts n(%) | 96 (29.81%) |
| History of STD (Chlamydia, gonorrhoea and/or syphilis) n (%) | 161 (45.48%) |
| ChemSex users, n (%) * | 65 (23.55%) |

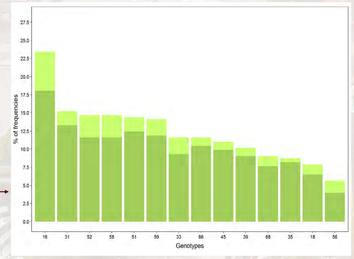
* 220 patients where asked about ChemSex.

### HPV genotypes with LA HPV DNA test




LA is unable to identify HPV-52 alone in samples containing HPV-33,-35 and/or-58, and this occurred in 76 cases. To rule out the genuine presence of HPV-52, real-time PCR was performed using specific probes to detect HPV-52. Altogether, LA and real-time PCR detected HPV-52 in 52 cases (14.7%).

### HR-HPV genotype infection




In 239 patients (67.32%), at least one of the 14 most important HR-HPV genotypes was detected with LA HPV DNA test.
Of them, 60% where infected by multiple genotypes.

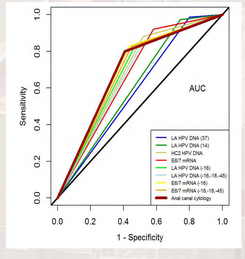
### Cytology and histology

| Cytology | HRA | | | | |
|---|---|---|---|---|---|
| | No biopsy | Benign biopsy | LSIL | HSIL | TOTAL |
| Benign | 52 | 72 | 36 | 15 | 175 (49.44%) |
| ASC-US | 18 | 18 | 9 | 13 | 58 (16.38%) |
| LSIL | 6 | 5 | 23 | 22 | 56 (15.82%) |
| ASC-H | 2 | 7 | 13 | 24 | 46 (12.99%) |
| HSIL | 2 | 4 | 3 | 1 | 10 (2.82%) |
| inadequate | 2 | 3 | 4 | 0 | 9 (2.54%) |
| TOTAL | 82 (23.16%) | 109 (30.79%) | 88 (24.86%) | 75 (21.19%) | 354 (100%) |

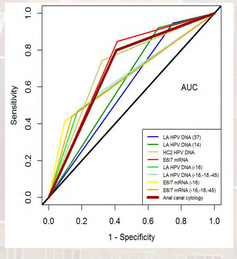
HSIL prevalence: 23,73%

### ROC Curves

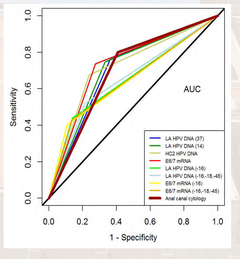
#### Biomarker alone



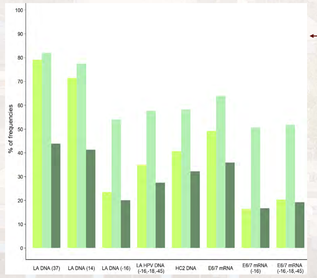

#### First combined triage strategy




#### Second combined triage strategy




### HRA performance




Percentage of participants candidates for HRA performance with each strategy.

### Biomarker performance

**Biomarkers would be performed in all participants of the «Biomarker alone strategy» and the «First combined strategy» and only in 32.2% of the «Second combined strategy»**

### Biomarker accuracy

| | Biomarker alone | | | First combined strategy (biomarker and/or aLBC) | | | Second combined strategy (biomarker if aLBC ASCUS/LSIL) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Se | Sp | AUC | Se | Sp | AUC | Se | Sp | AUC |
| LA HPV DNA test | 94.7% | 25.1% | 0.604 | 98,7% | 20,0% | 0,593 | 76% | 63.7% | 0.701 |
| LA HPV DNA test (14 HR-HPV types) | 92% | 34.1% | 0.636 | 97,3% | 25,6% | 0,614 | 74.7% | 66.7% | 0.709 |
| LA HPV DNA test (HPV-16) | 46.7% | 82.8% | 0.642 | 84,0% | 52,6% | 0,683 | 44% | 85.9% | 0.646 |
| LA HPV DNA test (HPV-16, -18 and -45) | 56% | 70.6% | 0.628 | 85,3% | 48,1% | 0,667 | 52% | 78.3% | 0.654 |
| HC2 HPV DNA test | 74.3% | 68% | 0.707 | 88,0% | 48,1% | 0,681 | 67.6% | 76.2% | 0.716 |
| E6/7-mRNA test | 84.7% | 58.6% | 0.711 | 92,0% | 41,9% | 0,669 | 73.6% | 72.4% | 0.730 |
| E6/7-mRNA test (HPV-16) | 41.3% | 90.3% | 0.664 | 82,7% | 56,7% | 0,697 | 40% | 89.3% | 0.654 |
| E6/7-mRNA test (HPV-16, -18 and -45) | 41.3% | 90.3% | 0.655 | 82,7% | 55,2% | 0,689 | 42.7% | 86.7% | 0.655 |
| p-value for AUC comparisons | <0.05 only comparing LA HPV DNA test with HC2 HPV DNA test and E6/7-mRNA test. | | | <0.05 only comparing LA HPV DNA test with and E6/7-mRNA test (HPV-16) and (HPV-16, -18 and -45). | | | >0.05 for all comparisons in this strategy | | |

## CONCLUSIONS

- **E6/7-mRNA-test could be considered for triage as an alternative to aLBC with the advantage of being a more objective and reproducible test.**
- **The second combined strategy using E6/7-mRNA-test only if aLBC ASCUS or LSIL seems to be the best strategy to triage candidates for HRA, with the highest AUC and the advantage of saving biomarker and HRA performance.**