MULTI-TEST SCREENING STRATEGIES ADD VALUE FOR PREDICTING BIOPSY-PROVEN ANAL HSIL FOR MSM

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Disclosures

I have received Speaker's Bureau fees and a contract for a vaccine trial from Merck & Co., Inc, and have edited enduring material for the ASCCP.

Dr. Young has received honorarium from Roche and Quidel for his role on their Advisory Boards and has presented an abstract with the ASCCP. Dr. Morris has edited or authored enduring material for the ASCCP.

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Analysis

- Multivariable logistic regression models estimated odds of predicting bHSIL using cytology and hrHPV tests independently and in combination
 - adjusted for age, race, HIV-infection, recent male sex partners (2 years), smoking status, and swab-collection order

• Area under receiver-operating characteristic curves (AUCs) were estimated

Results

- Most subjects were
 - White, ~55 years old, and HIV-infected.
 - Current or former use of tobacco
 - Sexually engaged with other men.
 - Showed <a>1 hrHPVs using either Dacron or NF-swab specimens.
- Abnormal cytology (<u>></u>ASCUS) detected among 34% using Dacron and 21% using NF swab.
 - Unsatisfactory swabs increased with the number of swabs tested (1-5) and Dacron swab was penalized most.
- Histological HSIL was common: 47% overall.



• At first blush, the exclusion of unsatisfactory cytology suggests that Pap test alone is predicting HSIL more accurately than when unsatisfactory cytology is included.

| | Accuracy | | | | | Accuracy | | | | |
|-------------------------------------|-----------------------------------|-----------------------------|-----------------------------------|-----------------------|--|-----------------------------------|-----------------------------|----------------------------------|-----------------------|--|
| Testing Strategy: Sequence of Tests | Fold-Improvement Adding Test 2 | HIV- uninfected (AUC) | Fold-Improvement Adding Test 2 | HIV-infected (AUC) | Testing Strategy: Sequence of Tests | Fold-Improvement Adding Test 2 | HIV- uninfected (AUC) | Fold⊣mprovement Adding Test 2 | HIV-infected (AUC) | |
| Dacron Swab Specimen Collection | | | | | Nylon Flocked Swab Specimen Collection | | | | | |
| Unsatisfactory Included | | | | | Unsatisfactory Included | | | | | |
| Dacron Cytology* | | 0.66 | | 0.71 | NF-Cytology* | | 0.72 | | 0.73 | |
| Unsatisfactory Excluded | | | | | Unsatisfactory Excluded | | | | | |
| Dacron Cytology* | | 0.72 | | 0.74 | NF-Cytology* | | 0.81 | | 0.77 | |

• When unsatisfactory cytology is included in the model: HSIL= >ASCUS (vs. NIL) + Unsatisfactory (vs. NIL)

• When unsatisfactory cytology is excluded in the model: HSIL= >ASCUS (vs. NIL) Table 1: Comparison of Test Accuracy for Eight Screening Strategies to Predict Anal Biopsy-Confirmed High-grade Squamous Intraepithelial Cancer (bHSIL) for 301 HIV-Infected and -Uninfected Gay, Bisexual and Other Men Who Have Sex With Men (MSM)

| | Accuracy | | | | | | | | | | |
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| Dacron Swab Specimen Collection | | | | | Nylon Flocked Swab Specimen Collection | | | | | | |
| Dacron Cytology* | | 0.66 | | 0.71 | NF-Cytology* | | 0.72 | | 0.73 | | |
| Cytology Alone | 1.21 [¢] | 0.67 | 1.07 ^ф | 0.72 | Cytology Alone | 4 1 15 ^φ | 0.74 | 1 10 ^ф | 0.73 | | |
| Cytology + hrHPV-DNA | 1.21 | 0.81 | 1.07 | 0.77 | Cytology + hrHPV-DNA | 1.15 | 0.81 | 1.10 | 0.78 | | |
| hrHPV-DNA Alone | 1.03 | 0.79 | 1.01 | 0.76 | hrHPV-DNA Alone | 1.09 | 0.77 | 1.06 | 0.74 | | |
| hrHPV-DNA + Cytology | | 0.81 | 1.01 | 0.77 | hrHPV-DNA + Cytology | | 0.81 | 1.00 | 0.78 | | |
| Cytology Alone | 1 21 ^{\$\$} | 0.68 | 1 17 ^Z | 0.70 | Cytology Alone | 1 18 [¢] | 1 18 [¢] | 1.18 [¢] | 0.73 | 1 16 Z | 0.73 |
| Cytology + hrHPV-E6/E7-mRNA | 1.21 | 0.82 | | 0.82 | Cytology + hrHPV-E6/E7-mRNA* | 1.10 | 0.83 | 1.10 | 0.83 | | |
| hrHPV-E6/E7-mRNA Alone | 1 01 | 0.81 | 1 01 | 0.81 | hrHPV-E6/E7-mRNA* Alone | 1.05 | 0.81 | 1 03 | 0.81 | | |
| hrHPV-E6/E7-mRNA + Cytology | 1.01 | 0.82 | | 0.82 | hrHPV-E6/E7-mRNA* + Cytology | | 0.83 | L | 0.83 | | |
| hrHPV-16 Alone** | 1.01 | 0.72 | 1.04 | 0.73 | hrHPV-16 Alone** | 1.04 | 0.72 | 1.05 | 0.74 | | |
| hrHPV-16** + Cytology | 1.01 | 0.73 | | 0.76 | hrHPV-16** + Cytology | | 0.75 | | 0.78 | | |
| Cytology | 1.06 | 0.69 | 1 07 | 0.71 | Cytology | 1 03 | 0.73 | 1 08 [¢] | 0.72 | | |
| Cytology +hrHPV-16** | | 0.73 | | 0.76 | Cytology + hrHPV-16** | | 0.75 | | 0.78 | | |
| hrHPV-18 Alone** | 1.00 | 0.71 | 1.03 | 0.69 | hrHPV-18 Alone** | 1.04 | 0.71 | 1.06 | 0.69 | | |
| hrHPV-18** + cytology | | 0.71 | | 0.71 | hrHPV-18 **+ Cytology | | 0.74 | | 0.73 | | |
| Cytology | 1.04 | 0.68 | 1.00 | 0.71 | Cytology | 1.03 | 0.72 | 1.01 | 0.72 | | |
| Cytology + hrHPV-18** | | 0.71 | | 0.71 | Cytology + hrHPV-18** | | 0.74 | | 0.73 | | |
| Molecular hrHPV Assays Only | | | | | | | | | | | |
| hrHPV-DNA Alone | 1.04 | 0.81 | 1.05 | 0.77 | hrHPV-DNA Alone | 1.01 | 0.78 | 4 00 [¢] | 0.75 | | |
| hrHPV-DNA + hrHPV-E6/E7-mRNA | | 0.84 | | 0.81 | hrHPV-DNA + hrHPV-E6/E7-mRNA* | 1.04 | 0.81 | 1.09 | 0.82 | | |
| hrHPV-E6/E7-mRNA Alone | 1.01 | 0.83 | 1.00 | 0.81 | hrHPV-E6/E7-mRNA* Alone | 4.04 | 0.80 | 1.00 | 0.82 | | |
| hrHPV-E6/E7-mRNA + hrHPV-DNA | 1.01 | 0.84 | 1.00 | 0.81 | hrHPV-E6/E7-mRNA* + hrHPV-DNA | 1.01 | 0.81 | 1.00 | 0.82 | | |

φ<0.05, Θ <0.01, Z<0.001; *hrHPV-E6/E7-mRNA tests are collected using Dacron swab; **hrHPV16/hrHPV18/hrHPVother collected using Dacron swab; False Discovery Rate = 9 observed vs. 1 expected; comparison of Dacron-cytology to NF-cytology showed no statistically significant difference, *p* =0.23

Using either swab, adding hrHPV-DNA to cytology for all comparisons improves prediction of anal HSIL 7-21%

Table 1: Comparison of Test Accuracy for Eight Screening Strategies to Predict Anal Biopsy-Confirmed High-grade Squamous Intraepithelial Cancer (bHSIL) for 301 HIV-Infected and -Uninfected Gay, Bisexual and Other Men Who Have Sex With Men (MSM)

| | | Accı | iracy | | Accuracy | | | | | |
|-------------------------------------|---------------------------------------|-----------------------------|---------------------------------------|-----------------------|--|---------------------------------------|-----------------------------|---------------------------------------|-----------------------|--|
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| Cytology Alone | 1.21 ^φ | 0.67 | 1.07 ^ф | 0.72 | Cytology Alone | 1.15 [¢] | 0.74 | 1.10 [¢] | 0.73 | |
| Cytology + hrHPV-DNA | 1.21 | 0.81 | 1.07 | 0.77 | Cytology + hrHPV-DNA | | 0.81 | | 0.78 | |
| hrHPV-DNA Alone | 1.03 | 0.79 | 1.01 | 0.76 | hrHPV-DNA Alone | 1.00 | 0.77 | 1.06 | 0.74 | |
| hrHPV-DNA + Cytology | 1.03 | 0.81 | | 0.77 | hrHPV-DNA + Cytology | 1.09 | 0.81 | | 0.78 | |
| | | | | | | | | | | |

 ϕ <0.05, $\ddot{\Theta}$ <0.01, Z<0.001; *hrHPV-E6/E7-mRNA tests are collected using Dacron swab; **hrHPV16/hrHPV18/hrHPVother collected using Dacron swab; False Discovery Rate = 9 observed vs. 1 expected; comparison of Dacron-cytology to NF-cytology showed no statistically significant difference, *p* =0.23

...but cytology does not improve performance of hrHPV-DNA testing alone

Adding hrHPV-E6/E7 to cytology for all comparisons improves prediction of anal HSIL 16-21%

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| Dacron Cytology* | | 0.66 | | 0.71 | NF-Cytology* | | 0.72 | | 0.73 |
| Cytology Alone | 1.21 ^φ | 0.68 | 1.17 ^Z | 0.70 | Cytology Alone | 1.18 [¢] | 0.73 | 1.16 ^Z | 0.73 |
| Cytology + hrHPV-E6/E7-mRNA | 1.21 | 0.82 | | 0.82 | Cytology + hrHPV-E6/E7-mRNA* | | 0.83 | | 0.83 |
| hrHPV-E6/E7-mRNA Alone | 1.01 | 0.81 | 1.01 | 0.81 | hrHPV-E6/E7-mRNA* Alone | 1.05 | 0.81 | 1.03 | 0.81 |
| hrHPV-E6/E7-mRNA + Cytology | 1.01 | 0.82 | 1.01 | 0.82 | hrHPV-E6/E7-mRNA* + Cytology | 1.00 | 0.83 | 1.03 | 0.83 |

φ < 0.05, $θ \le 0.01$, $Z \le 0.001$; *hrHPV-E6/E7-mRNA tests are collected using Dacron swab; **hrHPV16/hrHPV18/hrHPVother collected using Dacron swab; False Discovery Rate = 9 observed vs. 1 expected; comparison of Dacron-cytology to NF-cytology showed no statistically significant difference, p = 0.23

...but cytology does not improve performance of hrHPV-E6/E7 testing alone

For HIV+ men only, using NF swab, adding hrHPV16 to cytology improves prediction of anal HSIL 8% over HPV16 testing alone

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| | Accuracy | | | | | | | | | |
|--|---------------------------------------|-----------------------------|--|-----------------------|--|---------------------------------------|-----------------------------|---------------------------------------|-----------------------|--|
| Testing Strategy: Sequence of Tests | Fold- Improvement Adding Test 2 | HIV- uninfected (AUC) | Fold-Improvement Adding Test 2 | HIV-infected (AUC) | Testing Strategy: Sequence of Tests | Fold- Improvement Adding Test 2 | HIV- uninfected (AUC) | Fold- Improvement Adding Test 2 | HIV-infected (AUC) | |
| Dacron Swab Specimen Collection | | | | | Nylon Flocked Swab Specimen Collection | | | | | |
| hrHPV-16 Alone** | 1.01 | 0.72 | 1.04 | 0.73 | hrHPV-16 Alone** | | 0.72 | 4.05 | 0.74 | |
| hrHPV-16** + Cytology | | 0.73 | 0.76 | hrHPV-16** + Cytology | 1.04 | 0.75 | 1.05 | 0.78 | | |
| Cytology | 1.00 | 0.69 | 1.07 | 0.71 | Cytology | 1.02 | 0.73 | 1 09 [¢] | 0.72 | |
| Cytology +hrHPV-16** | 1.00 | 0.73 | .73 0.76 Cytology + hrHPV-16 ** | 1.03 | 0.75 | 1.00 | 0.78 | | | |
| Molecular hrHPV Assays Only | | | | | | | | | | |
| hrHPV-DNA Alone | 1.04 | 0.81 | 1.05 | 0.77 | hrHPV-DNA Alone | 1.04 | 0.78 | [¢] | 0.75 | |
| hrHPV-DNA + hrHPV-E6/E7-mRNA | | 0.84 | | 0.81 | hrHPV-DNA + hrHPV-E6/E7-mRNA* | 1.04 | 0.81 | | 0.82 | |
| hrHPV-E6/E7-mRNA Alone | 1.01 | 0.83 | 1.00 | 0.81 | hrHPV-E6/E7-mRNA* Alone | 1.01 | 0.80 | 1.00 | 0.82 | |
| hrHPV-E6/E7-mRNA + hrHPV-DNA | 1.01 | 0.84 | 1.00 | 0.81 | hrHPV-E6/E7-mRNA* + hrHPV-DNA | 1.01 | 0.81 | | 0.82 | |
| \$\$\phi < 0.05, \(\tilde{\subscript{-}} < 0.001; \(\tilde{\subscript{-}} + nrHPV-E6/E7-mRNA\) tests are collected using Dacron swab; \(\tilde{\subscript{-}} + nrHPV16/hrHPV18/hrHPV0ther\) collected using Dacron swab; False Discovery Rate = 9 observed vs. 1 expected; comparison of Dacron-cytology to NE-cytology showed no statistically significant difference n=0.23 | | | | | | | | | | |

Similarly, for HIV+ men only, using NF swab, adding hrHPV-E6/E7 to hrHPV-DNA testing improves prediction of anal HSIL 9% over hrHPV-DNA testing alone

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|-------------------------------------|---------------------------------------|-----------------------------|-----------------------------------|-----------------------|--|---------------------------------------|-----------------------------|---------------------------------------|-----------------------|
| Testing Strategy: Sequence of Tests | Fold- Improvement Adding Test 2 | HIV- uninfected (AUC) | Fold-Improvement Adding Test 2 | HIV-infected (AUC) | Testing Strategy: Sequence of Tests | Fold- Improvement Adding Test 2 | HIV- uninfected (AUC) | Fold- Improvement Adding Test 2 | HIV-infected (AUC) |
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| hrHPV-16 Alone** | 1.01 | 0.72 | 1.01 | 0.73 | hrHPV-16 Alone** | 1.04 | 0.72 | 1.05 | 0.74 |
| hrHPV-16** + Cytology | | 0.73 | 1.04 | 0.76 | hrHPV-16** + Cytology | | 0.75 | | 0.78 |
| Cytology | 1.06 | 0.69 | 1.07 | 0.71 | Cytology | 1.02 | 0.73 | 1 09 [¢] | 0.72 |
| Cytology +hrHPV-16** | 1.00 | 0.73 | 1.07 | 0.76 | Cytology + hrHPV-16** | 1.03 | 0.75 | 1.00 | 0.78 |
| Molecular hrHPV Assays Only | | | | | | | | | |
| hrHPV-DNA Alone | 1.04 | 0.81 | 1.05 | 0.77 | hrHPV-DNA Alone | 1.04 | 0.78 | 1 00 [¢] | 0.75 |
| hrHPV-DNA + hrHPV-E6/E7-mRNA | | 0.84 | | 0.81 | hrHPV-DNA + hrHPV-E6/E7-mRNA* | 1.04 | 0.81 | 1.09 | 0.82 |
| hrHPV-E6/E7-mRNA Alone | 1.01 | 0.83 | 1.00 | 0.81 | hrHPV-E6/E7-mRNA* Alone | 1.01 | 0.80 | 1.00 | 0.82 |
| hrHPV-E6/E7-mRNA + hrHPV-DNA | 1.01 | 0.84 | 1.00 | 0.81 | hrHPV-E6/E7-mRNA* + hrHPV-DNA | 1.01 | 0.81 | 1.00 | 0.82 |
| | | - | - | | | | - | | - |

\$\phi < 0.05, \vec{\u0095_20.001}, \u00e4 hrHPV-E6/E7-mRNA tests are collected using Dacron swab; \u00e4 hrHPV16/hrHPV16/hrHPV18/hrHPV0ther collected using Dacron swab; False Discovery Rate = 9 observed vs. 1 expected; comparison of Dacron-cytology to NF-cytology showed no statistically significant difference, p = 0.23

...however, among these comparisons, hrHPV-E6/E7 shows high prediction for anal HSIL consistently across swabs and HIV-infection characteristics

For high-risk MSM

- Co-testing with hrHPV assays can improve bHSIL detection over cytology alone
- One or more molecular hrHPV tests improves bHSIL detection
- Clinical Research Goals should focus on developing screening strategies that improve specificity and build consensus for acceptable sensitivity in screening.

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