Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study

J. Thomas Cox, Phillip E. Castle, Catherine M. Behrens, Abha Sharma, Thomas C. Wright Jr, Jack Cuzick, Athena HPV Study Group
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J. Thomas Cox, MD; Phillip E. Castle, PhD, MPH; Catherine M. Behrens, MD, PhD; Abha Sharma, PhD; Thomas C. Wright Jr, MD; Jack Cuzick, PhD; and the Athena HPV Study Group

OBJECTIVE: The objective of the study was to compare 9 cervical cancer screening strategies to the current screening standard (cytology with human papillomavirus [HPV] triage of atypical squamous cells of undetermined significance) for the detection of high-grade cervical disease.

STUDY DESIGN: Women (n = 34,254) aged 30 years or older from the Addressing the Need for Advanced HPV Diagnostics (ATHENA) study underwent screening with cytology and HPV testing with simultaneous HPV16/18 genotyping; those with atypical squamous cells of undetermined significance cytology or greater or HPV-positive status were referred for colposcopy.

RESULTS: In general, screening strategies that offered greater sensitivity also required more referral to colposcopy. HPV testing was more sensitive than cytology for detection of cervical intraepithelial neoplasia grade 2 or greater, but strategies that depended on cytology for triage of HPV-positive women decreased this sensitivity. Various strategies of cotesting with cytology increased sensitivity but did so by increasing testing. Strategies that included integrated HPV16/18 testing provided more efficient referral to colposcopy.

CONCLUSION: Strategies that maximize detection of women at greatest risk of cervical intraepithelial neoplasia grade 3 or greater by immediate referral to colposcopy, with follow-up testing of women at intermediate risk, maximize the benefits of cervical cancer screening while decreasing the potential harm. Incorporating screening with HPV and triage of HPV-positive women by a combination of genotyping for HPV16/18 and cytology provided a good balance between maximizing sensitivity (benefit) and specificity by limiting the number of colposcopies (potential harm).

Key words: Addressing the Need for Advanced HPV Diagnostics, atypical squamous cells of undetermined significance, cotesting, human papillomavirus, human papillomavirus 16/18, low-grade squamous intraepithelial lesion


In March 2012, new primary cervical screening guidelines were jointly issued by the US Preventative Services Task Force (USPSTF) and a consortium of the American Cancer Society (ACS), the American Society for Colposcopy and Cervical Pathology (ASCCP), and the American Society of Clinical Pathologists (ASCP). Based on the evidence that human papillomavirus (HPV) testing is more sensitive and therefore provides better negative predictive values (NPV) than cytology, these new guidelines recommend that women aged 30 years and older be screened every 3 years.

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using cervical cytology alone or every 5 years using a combination of cervical cytology and high-risk HPV testing (referred to as cotesting).

The better NPV of HPV testing permits a safe extension of the screening inter-val, thereby reducing harms caused by screening. The ACS/ASCCP/ASCP guidelines endorsed the cotesting option as the preferred approach for women aged 30 years and older,

whereas the USPSTF endorsed it as acceptable, and the American College of Obstetricians and Gynecologists (ACOG) expressed support of these recommendations. The ACS/ASCCP/ASCP guidelines recommend that cytology-negative/HPV-positive women undergo follow-up in 12 months with repeat cytology and HPV testing or, alternatively, cytology-negative/HPV-positive women can be genotyped for HPV 16 and HPV 18. With the latter option, women who are found to have either HPV 16 or HPV 18 are referred for colposcopy, whereas those without these highest-risk HPV types are cotested again in 12 months.

The Addressing the Need for Advanced HPV Diagnostics (ATHENA) HPV study is a prospective 3 year cervical cancer screening trial designed to compare the performance of the newly introduced cobas HPV Test (Roche Molecular Diagnostics, Pleasanton, CA) both alone and in combination with cervical cytology among women aged 21 years and older in the United States. Based on the cross-sectional data from the ATHENA trial, the US Food and Drug Administration recently approved for use in the United States the cobas HPV Test, which detects 11 pooled high-risk HPV genotypes (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 1 possible high-risk type (HPV 66) and concurrently provides separate results for HPV 16 and HPV 18.

The current manuscript further analyzes the enrollment results of the ATHENA trial to investigate alternative screening strategies to those endorsed by the most recent US cervical cancer screening guidelines. Ten different cervical cancer screening strategies, including several that use HPV testing alone as the initial screening method, were investigated. The performance of each strategy for detection of cervical intraepithelial neoplasia grade 2 (CIN2) or more severe or CIN3 or more severe was explored, as was the potential harm estimated by the number of tests and the number of colposcopies (a metric used by the recent guideline process) needed to detect each high-grade lesion at baseline. These strategies included results of testing with cytology and/or various combinations of HPV testing, including HPV genotyping for HPV 16 and HPV 18.

**Materials and Methods**

**Study protocol**

As previously described, the ATHENA HPV study enrolled more than 47,000 women aged 21 years and older who presented for cervical cancer screening; all eligible participants had both Papanicolaou testing (by liquid-based cytology, ThinPrep; Hologic, Bedford, MA) and HPV testing (by Amplicor HPV test, Linear Array high-risk HPV genotyping test, and the cobas HPV Test, all from Roche Molecular Systems). The protocol was approved by the institutional review boards at all study sites, and all women provided written informed consent before undergoing any study procedures. The current analysis focuses only on the subset of women aged 30 years old and older to compare alternative management strategies with those endorsed in the current guidelines.

All women in this subgroup who either abnormal cytology (atypical squamous cells of undetermined significance [ASC-US] or greater) or who tested positive for HPV (by Amplicor or Linear Array test) were scheduled for colposcopy. In addition, to adjust for ascertainment bias, a randomly selected subset of women who tested negative for both cytology and HPV had colposcopy. Colposcopic biopsies were performed according to a standardized protocol, and a random biopsy was required in all women with adequate colposcopy in whom no lesion was seen; patients and colposcopists were blinded to the cytology and HPV results.

An expert central pathology review (CPR) panel of 3 pathologists read all bi-opsies masked to any clinical data. Women achieving the study endpoint of CIN2 or more severe by CPR exited the study; those who did not reach this endpoint proceeded to the 3 year follow-up phase of the study, scheduled to conclude December 2012. The current analysis is restricted to disease detected at enrollment; disease detected over the subsequent 3 years of follow-up will be analyzed separately.

**Screening strategies**

The 10 screening strategies were evaluated based on review of the published cervical cancer screening literature and appear to be the strategies most likely to be considered potentially attractive by the clinical and public health communities. Strategies 1 and 2 are cytology screening strategies (Figure 1). Strategy 1 consists of screening with cytology with reflex HPV testing (pooled high-risk HPV test for 14 genotypes) of ASC-US and referral of all women with HPV-positive ASC-US or low-grade squamous intraepithelial lesion (LSIL) or greater to colposcopy. Because this is the strategy most widely used in the United States, it serves as the comparator for the other 9 strategies.

Strategy 2 consists of screening with cytology alone, with referral of all women with ASC-US or greater to colposcopy. Strategies 3, 4, and 5 (Figure 2) incorporate cotesting with both cytology and HPV testing. They vary as to whether genotyping for HPV 16 and HPV 18 is used and by the cytological threshold for referral to colposcopy. Strategies 6 through 10 (Figure 3) use HPV testing alone (pooled high-risk HPV test with, or without, genotyping for HPV 16/18) as the initial screening test and differ by which triage tests are used to evaluate HPV-positive women. In the strategies described, women who did not meet the criteria for either immediate colposcopy or return to routine screening would be deferred to a 1 year follow-up per the current guidelines as indicated in Figures 2 and 3.

**Statistical analysis**

For each screening strategy, the number of tests (cytology and/or HPV test with
or without integrated HPV 16/18 geno-type detection) required at baseline was calculated, as was the number of colposcopies required to detect 1 case of CIN2 or more severe or CIN3 or more severe. From the total catchment of CIN2 or more severe and CIN3 or more severe detected, the number of cases not identified at baseline, and an estimate of the number that could potentially be identified by each strategy at 12 month follow-up were calculated. The crude sensitivity and specificity for detection of CIN2 or more severe or CIN3 or more severe and its sensitivity and specificity relative to strategy 1 were also determined (Tables 2 and 3).

The cobas HPV Test results were categorized as follows: HPV positive (positive for any of 14 high-risk HPV types); HPV negative (negative for all 14 high-risk HPV types); HPV 16/18 positive (positive for HPV 16 and/or HPV 18, regardless of the presence or absence of 12 other HPV types); positive for 12 other HPV types (positive for 1 or more of the 12 other HPV types and negative for HPV 16 and HPV 18).

For calculations of sensitivity and specificity, only those cases in which colposcopy was performed and a valid biopsy result was obtained were considered. Crude estimates are given because the intent was to report on the utility of the strategies as would be observed in a clinical situation. Verification bias adjustment would not change the relative sensitivities or specificities of the various strategies or represent what happens in clinical practice.

Results
A total of 34,254 women aged 30 years or older were eligible for this analysis; the mean age was 44.7 years, and the demographics are shown in Table 1. Among the eligible women, 2872 (8.4%) tested positive with the cobas HPV Test, and 1966 (5.7%) had abnormal cytology; 280 women were diagnosed with CIN2 or more severe and 189 with CIN3 or more severe. The most sensitive screening strategy was screening with HPV alone (pooled 14 high-risk types) with referral of all HPV-positive women to colposcopy (strategy 6), detecting 242 of CIN2 or more severe lesions (86.4%) and 170 (89.9%) of the CIN3 or more severe lesions (Tables 2 and 3). However, this strategy also had the highest false-positive rate for CIN3 or more severe (38.0%). In terms of the utilization of colposcopy resources, it was almost as inefficient as the strategy of screening with cytology alone and referring all woman with ASC-US or more severe to colposcopy (strategy 2) since it required 9.7 colposcopic evaluations to find a single case of CIN2 or more severe and 13.8 to find a single case of CIN3 or more severe.

All strategies depending on cytology alone or on cytology as the sole reflex test had the lowest sensitivities for detection of CIN2 or more severe and CIN3 or more severe. These included cytology with HPV triage of ASC-US (strategy 1; 51.4% and 56.1%, respectively) and cytology alone (strategy 2; 53.2% and 57.7%, respectively). Strategy 3 was also cytology based because it screened with cytology and HPV testing (cotesting) but used only the results of cytology and reflex HPV testing of ASC-US to determine immediate referral to colposcopy. Therefore, this strategy had an identical performance to strategy 1 during the first round of screening, except that cotesting also identified cytology-negative/HPV-positive women in need of additional follow-up in 12 months from which 72 additional cases of CIN3 or more severe could potentially be detected (subsequently described as "cases identified for 12 month round follow-up" [Tables 2 and 3]). HPV alone with cytology triage of HPV-positive women (strategy 7) had the lowest cross-sectional sensitivity because the triage test negates the increased sensitivity of HPV testing. In contrast to the lower sensitivity of each of these strategies, all but strategy 2 were among the most specific, with false-positive rates for detection of CIN3 or more severe be-

![Figure 1: Cytology primary screening options](image-url)
Cotesting primary screening options

**Strategy 3:** Cotesting with reflex for ASC-US

- HPV and Pap Test (Cotesting)
  - Both Negative or ASC-US/HPV-
    - ASC-US/HPV+ & > ASC-US → Routine screening
    - NILM/HPV+ → Colposcopy
    - Repeat cotest in 12 mo.

**Strategy 4:** Cotesting with genotyping and cytology triage: HPV 16/18 & ASC-US HPV+ threshold

- HPV with 16/18 genotyping and Pap Test (Cotesting)
  - Both Negative or ASC-US/HPV-
    - ASC-US/HPV+ or > ASC-US or NILM HPV16/18+ → Colposcopy
    - NILM/HPV+ but not 16/18 → Repeat cotest in 12 mo.

**Strategy 5:** Cotesting with genotyping and cytology triage: HPV 16/18 & LSIL threshold

- HPV with 16/18 genotyping and Pap Test (Cotesting)
  - Both Negative or HPV-/ASC-US
    - ≥ LSIL or NILM or ASC-US HPV16/18+ → Colposcopy
    - ASC-US or NILM/HPV- but not 16/18 → Repeat cotest in 12 mo.

Strategies 3-5 screened initially with both cytology and testing for high-risk HPV. Strategies 4 and 5 also utilized the information provided when the HPV test also included separate results for HPV 16 and HPV 18 genotyping or, if not, when reflex genotyping could be done. Strategy 3, 1 of 2 recommended cotesting options for women aged 30 years or older, referred to colposcopy women with ASC-US HPV positivity and LSIL or more severe, irrespective of HPV result, whereas women with cytology-negativity/HPV positivity had repeat cotesting in 12 months and women who were cytology negative/HPV negative and ASC-US/HPV negative continued routine screening. Strategy 4, also a recommended cotesting strategy, referred to colposcopy women who were ASC-US/HPV positive and LSIL or more severe irrespective of HPV result, although all women who were cytology negative/HPV negative/ASC-US/HPV negative continued routine screening. Strategy 5, also a cotesting strategy, referred to colposcopy women who were ASC-US/HPV positive and LSIL or more severe irrespective of HPV result, whereas women who were cytology negative/HPV positive but not positive for HPV 16 or HPV 18 had repeat cotesting in 12 months, and women who were cytology negative/HPV negative and ASC-US/HPV negative continued with routine screening. Strategy 5 was similar to strategy 4 except that the threshold for referral to colposcopy was LSIL or more severe or cytology negative/HPV 16/18 positive or ASC-US/HPV 16/18 positive, whereas ASC-US or negative cytology/HPV positive but not HPV 16/HPV 18 positive had 12 month follow-up.

ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion.

Women with any abnormal cytology ASC-US or more severe were also referred to colposcopy, whereas women who were cytology negative/HPV positive for non-HPV 16/HPV 18 had 12 month follow-up. Strategy 10 screened initially with a panel of HPV plus genotyping for HPV 16 and HPV 18, referring all women who were HPV 16/HPV 18 positive to colposcopy and reflex testing by cytology those HPV positive but not positive for HPV 16/HPV 18.

Colposcopy screening algorithms

**Strategy 6:** HPV alone

- HPV Test
  - Neg: Routine screening
  - Pos: Colposcopy

**Strategy 7:** HPV with reflex to cytology

- HPV Test
  - Neg: Routine screening
  - Pos: Pap Test
    - NILM: Follow-up in 12 mo.
    - ASC-US: Pap Test
      - @ASC-US: Colposcopy

**Strategy 8:** HPV with genotyping

- HPV Test and 16/18 Genotyping
  - Neg: Routine screening
  - HPV16/18+: Colposcopy
  - Follow-up in 12 mo.
  - 12 Other HR+: Colposcopy

**Strategy 9:** HPV with genotyping and reflex cytology: ASC-US threshold

- HPV Test and 16/18 Genotyping
  - Neg: Routine screening
  - HPV16/18+: Colposcopy
  - Follow-up in 12 mo.
  - 12 Other HR+: Colposcopy

**Strategy 10:** HPV with genotyping and reflex cytology: LSI threshold

- HPV Test and 16/18 Genotyping
  - Neg: Routine screening
  - HPV16/18+: Colposcopy
  - Follow-up in 12 mo.
  - 12 Other HR+: Colposcopy

Strategies 6-10 screened initially with HPV testing. Strategy 6 referred all HPV-positive women to colposcopy and all HPV-negative women to routine screening. Strategy 7 reflex tested all HPV-positive women with cytology, referring to colposcopy only those with ASC-US or more severe, whereas those with negative cytology had follow-up in 12 months. Strategy 8 screened initially with a panel of HPV plus genotyping for HPV 16 and HPV 18, referring to colposcopy all women testing positive for HPV 16 and/or HPV 18 and to 12 month follow-up women positive for other HPV genotypes but not positive for HPV 16/HPV 18. Strategy 9 screened initially with a panel of HPV plus genotyping for HPV 16 and HPV 18, referring all women who were HPV 16/HPV 18 positive to colposcopy and reflex testing by cytology those HPV positive but not positive for HPV 16/HPV 18.

Using genotyping to triage HPV-positive women to colposcopy (strategy 8) slightly decreased sensitivity for CIN2 or more severe compared with using cytology at an ASC-US threshold (strategy 7; 43.6% vs 47.5%) but increased sensitivity for CIN3 or more severe (53.4% vs 51.9%), indicating a trend toward increased predictive value of HPV 16/HPV 18 genotyping as a triage test compared with cytology.

Of all the screening strategies, HPV with cytology triage (strategy 7) and HPV with genotyping triage (strategy 8) required both the least number of colposcopies (596 and 580, respectively) and the least number of colposcopies to detect 1 CIN2 or more severe (approximately 5). However, both strategies had relatively low baseline sensitivity.

Strategies 8, 9, and 10 used HPV alone as the initial screen and incorporated genotyping triage for HPV-positive women. Strategies 9 and 10 added cytology triage for HPV-positive women who were HPV 16/HPV 18 negative to increase baseline detection of CIN2 or more severe caused by the other 12 HPV types, thereby reducing the number of cases deferred for identification to 12 month follow-up. This produced a gain in the detection of CIN3 or more severe compared with using cytology alone (strategy 7; 53.4% vs 51.9% specificity).

Women with any abnormal cytology ASC-US or more severe were also referred to colposcopy, whereas women who were cytology negative/HPV positive for non-HPV 16/HPV 18 had 12 month follow-up. Strategy 10 screened initially with a panel of HPV plus genotyping for HPV 16 and HPV 18, referring all women who were HPV 16/HPV 18 positive to colposcopy and reflex testing by cytology those HPV positive but not positive for HPV 16/HPV 18. Women with LSI or more severe were also referred to colposcopy, whereas women who were cytology negative/HPV positive and ASC-US/HPV positive for non-HPV 16/HPV 18 had 12 month follow-up.

ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus.

in sensitivity of approximately 14-20% when compared with that achieved by strategy 8. Of these 2 strategies, strategy 9 was more sensitive than strategy 10 for CIN2 or more severe (63.6% vs 57.9%) and CIN3 or more severe (72.0% vs 66.7%) but slightly less specific (85.2% vs 88.0% and 85.7% vs 88.5%, respectively, for CIN2 or more severe and CIN3 or more severe) because of the lower threshold for referral to colposcopy of ASC-US, as opposed to LSIL.

Figure 4 demonstrates in graphical form the trade-offs between sensitivity for CIN3 or more severe and the number of colposcopies that each strategy would produce. Presenting the data in this manner delineates 3 groups. One includes a single strategy, HPV alone, which was significantly more sensitive than all the other strategies but is also the least efficient, as measured by the number of colposcopies. In contrast, the 5 strategies grouped with the lowest sensitivity if poor specificity can lead to potential harm.

The recent ACS/ASCCP/ASCP cervical cancer screening guidelines specifically acknowledge that the benefits of screening should be balanced against the potential harms, with the total number of colposcopies serving as the primary measure of harm. Colposcopy was chosen as the measure of harm because it has been shown to be associated with considerable psychological distress, physical discomfort during the procedure, and the potential to lead to more invasive treatments with short- and long-term risks. The baseline data from the ATHENA study provide us with an exceptional opportunity to evaluate both the benefit and potential harms that would be produced during a single round of screening using cervical cancer of colposcopies (810-1202). Within this group, cotesting with genotyping and cytology triage at the ASC-US HPV-positive threshold (strategy 4) was the most sensitive but also required the most colposcopies. HPV with genotyping and cytology triage at the LSIL threshold (strategy 10) was the least sensitive and required the fewest colposcopies. Cotesting with genotyping and cytology triage with LSIL (strategy 5) and HPV with genotyping and reflex cytology triage with ASC-US (strategy 9) were very similar in both sensitivity and number of colposcopies, but strategy 5 required nearly twice the number of initial screening tests (Tables 2 and 3).

**Comment**

The ideal cervical cancer screening strategy would provide maximum sensitivity to minimize missing disease as well as maximum specificity to minimize false positive results and overreferral. Unfortunately, cervical cancer screening strategies that maximize both sensitivity and specificity have proven elusive because strategies that maximize sensitivity have typically produced relatively poor specificity. The development of invasive cervical cancer is a slow process that takes decades rather than years to occur. This fact reduces the relative benefits of achieving maximum sensitivity if poor specificity can lead to potential harm.

**Table 1**

Demographic and clinical characteristics of ATHENA population aged 30 years or older at baseline

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Evaluable subjects (n = 34,264)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
</tr>
<tr>
<td>Mean ± (SD)</td>
<td>44.7 ± (10.1)</td>
</tr>
<tr>
<td>30-39, n (%)</td>
<td>12,248 (35.6)</td>
</tr>
<tr>
<td>≥40, n (%)</td>
<td>22,066 (64.2)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>28,821 (84.1)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>4903 (13.1)</td>
</tr>
<tr>
<td>Asian</td>
<td>503 (1.5)</td>
</tr>
<tr>
<td>American Indian or Alaskan native</td>
<td>184 (0.5)</td>
</tr>
<tr>
<td>Native Hawaiian or other Pacific Islander</td>
<td>78 (0.2)</td>
</tr>
<tr>
<td>Any combination/missing*a</td>
<td>165 (0.5)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>6144 (17.9)</td>
</tr>
<tr>
<td>Postmenopausal, n (%)</td>
<td>12,743 (37.2)</td>
</tr>
<tr>
<td>HPV vaccine, n (%)</td>
<td>50 (0.1)</td>
</tr>
<tr>
<td>Immunosuppressed or immunocompromised, n (%)</td>
<td>224 (0.7)</td>
</tr>
<tr>
<td>Family history of cervical disease related to cervical cancer, n (%)</td>
<td>1920 (5.6)</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>31,988 (93.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>346 (1.0)</td>
</tr>
<tr>
<td>Pap cytology test in past 5 y, n (%)</td>
<td>31,089 (90.8)</td>
</tr>
</tbody>
</table>

ATHENA, Addressing the Need for Advanced HPV Diagnostic; Pap, Papanicolaou.

*a Any combination/missing* refers to participants who selected more than 1 race or for whom the information was missing.

screening strategies based on various combinations of cytology, pooled testing for 12 high-risk HPV types, and genotyping for HPV 16 and HPV 18.

To evaluate trade-offs between sensitivity and potential harms of the different screening strategies, we used a scatterplot of each strategy’s sensitivity for CIN3 or more severe vs the number of colposcopies. However, the low sensitivity of these strategies for detection of CIN3 or more severe at baseline increases the burden of cases that are missed at baseline and that burden would depend on potential identification for those strategies that have 12 month follow-up.

The most attractive strategies from the perspective of a benefit-vs-harm analysis appear to be the 4 strategies occupying the middle portion of the scatterplot. These strategies all combine what we consider to be a reasonable sensitivity for CIN3 or more severe with only a modest (less than 2-fold) increase in the required number of colposcopies compared with the less sensitive strategies. All 4 of the strategies that occupy the middle portion of the scatterplot use HPV testing with genotyping for HPV 16/HPV 18, and 2 are cotesting strategies. Of these, cotesting with genotyping triage (strategy 4) is the most sensitive strategy but requires the most colposcopies and twice the initial tests as the non-cotesting options in this group, strategies 9 and 10. HPV with genotyping and cytology triage with LSIL (strategy 10) requires the fewest colposcopies and therefore would be most applicable in settings in which potential harm from colposcopy is of greater concern than benefit.

2 strategies within this tier that seem to optimize the balance between sensitivity and specificity are cotesting with genotyping and cytology triage with LSIL (strategy 5) and HPV with genotyping and cytology triage with ASC-US (strategy 9). The latter strategy results in a 50% reduction in the number of required screening tests and is also slightly
more sensitive and requires slightly fewer colposcopies to detect 1 CIN3 or more severe case.

An obvious limitation of this analysis is that it uses only the baseline data from the ATHENA trial and does not include the results of those women identified as needing 12 month follow-up. Because the design of the ATHENA study referred all women with abnormal screening test results at enrollment for colposcopy, many women with CIN2 or more severe lesions missed by a given screening test were identified by the other screening test, and their CIN2 or more severe lesions were treated. Therefore, it is impossible to know what percentage of the CIN2 or more severe lesions missed at enrollment by a given screening test would be detected on subsequent screening or follow-up.

In a setting in which it would be considered unethical to follow up women with known CIN3 or more severe lesions, a randomized trial in which women are assigned to each of the different screening strategies would be required to determine exactly how subsequent rounds of follow-up or screening would perform. We therefore consider it reassuring that our approach of comparing benefits and harms of different screening strategies arrived at many of the same conclusions as have analyses incorporating multiple rounds of screening and mathematical modeling studies. For example, in this analysis, a strategy of cytology with HPV triage of ASC-US (strategy 1) is clearly superior to cytology alone (strategy 2) because both have similar sensitivities but with cytology alone almost doubling the number of required colposcopies.

Multiple cost-effectiveness analyses and metaanalyses have previously documented the attractiveness of cytology with triage of ASC-US by HPV over a strategy of cytology with referral of all women with abnormal cytology results to colposcopy. Likewise, the group of screening strategies that appear to be the most attractive after comparing benefits and harms includes the strategy recently recommended by the ACS as the preferred strategy when screening women aged 30 years or older (cotesting).2

However, of the 2 cotesting strategies recommended by the ACS/ASCCP/ASCP, cotesting with genotyping triage (strategy 4) detects 26.4% more CIN3 or more severe than cotesting with 12 month follow-up of all cytology-negative/HPV-positive women (strategy 3) but requires nearly 50% more colposcopies at the ini-

<table>
<thead>
<tr>
<th>Strategy number and name</th>
<th>Tests performed, n</th>
<th>Colposcopies performed, n</th>
<th>Colposcopies to detect 1 CIN3 or more severe, n</th>
<th>CIN3 or more severe cases identified, n</th>
<th>Cases identified for 12 month follow-up (eliminated), n</th>
<th>Sensitivity, %</th>
<th>Specificity relative to ASC-US triage</th>
<th>False-positive rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Cytology with reflex HPV (ASC-US triage)</td>
<td>35,546</td>
<td>816</td>
<td>7.7</td>
<td>106</td>
<td>0</td>
<td>56.1</td>
<td>1.00</td>
<td>12.4</td>
</tr>
<tr>
<td>2 Cytology alone</td>
<td>34,524</td>
<td>1644</td>
<td>15.1</td>
<td>109</td>
<td>0</td>
<td>57.7</td>
<td>1.03</td>
<td>26.8</td>
</tr>
<tr>
<td>3 Cytology with reflex for ASC-US</td>
<td>66,508</td>
<td>1202</td>
<td>8.3</td>
<td>108</td>
<td>72</td>
<td>56.1</td>
<td>1.00</td>
<td>12.4</td>
</tr>
<tr>
<td>4 Cytology with genotyping and cytology triage: HPV 16/18 and ASC-US HPV: positive threshold</td>
<td>66,508</td>
<td>1202</td>
<td>8.3</td>
<td>108</td>
<td>72</td>
<td>56.1</td>
<td>1.00</td>
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</tr>
<tr>
<td>5 Cytology with genotyping and cytology triage: HPV 16/18 and LSIL threshold</td>
<td>66,508</td>
<td>1030</td>
<td>7.7</td>
<td>134</td>
<td>44</td>
<td>70.9</td>
<td>1.20</td>
<td>15.7</td>
</tr>
<tr>
<td>6 HPV alone</td>
<td>34,254</td>
<td>2344</td>
<td>13.8</td>
<td>170</td>
<td>0</td>
<td>89.0</td>
<td>1.60</td>
<td>38.0</td>
</tr>
<tr>
<td>7 HPV with reflex to cytology</td>
<td>37,226</td>
<td>596</td>
<td>8.1</td>
<td>98</td>
<td>72</td>
<td>51.9</td>
<td>0.92</td>
<td>8.7</td>
</tr>
<tr>
<td>8 HPV with genotyping</td>
<td>34,254</td>
<td>596</td>
<td>8.1</td>
<td>98</td>
<td>72</td>
<td>51.9</td>
<td>0.92</td>
<td>8.7</td>
</tr>
<tr>
<td>9 HPV with genotyping and reflex cytology: ASC-US threshold</td>
<td>36,423</td>
<td>982</td>
<td>7.2</td>
<td>106</td>
<td>34</td>
<td>72.0</td>
<td>1.28</td>
<td>14.8</td>
</tr>
<tr>
<td>10 HPV with genotyping and reflex cytology: LSIL threshold</td>
<td>36,423</td>
<td>982</td>
<td>6.4</td>
<td>126</td>
<td>44</td>
<td>68.7</td>
<td>1.19</td>
<td>12.0</td>
</tr>
</tbody>
</table>

ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion.

Scatterplot of sensitivity for CIN3 or more severe and number of colposcopies for each screening strategy. Clear outliers as measured by numbers of colposcopies are in red. Strategies with the lowest sensitivity but also the lowest number of initial colposcopies are in black and those with intermediate sensitivity and number of colposcopies are in blue. Strategies that are presently recommended in the American Society for Colposcopy and Cervical Pathology guidelines are in bold.

ASC-US, atypical squamous cells of undetermined significance; CN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LSL, low-grade squamous intraepithelial lesion.

It is difficult to compare these analyses with ours because they are based on 1-5 year follow-up data, and each of these studies had quite different study designs compared with ATHENA. Moreover, neither the Finnish nor The Netherlands trials evaluated strategies that included HPV 16/HPV 18 genotyping. It should be stressed, however, that the ATHENA baseline data do not support a conclusion that HPV with cytology triage is competitive with any of the HPV 16/HPV 18 genotyping strategies that include cytology, unless an absolute reduction in baseline colposcopies is the primary goal. In addition, another recent report from a large screening population in The Netherlands (Population-Based Screening Study Amsterdam [POBASCAM]) has documented that early detection of CIN3 or more severe was associated with HPV 16 was a major component of the benefit of testing for HPV. Other more complex HPV genotype-based approaches may further improve upon these strategies. In particular, several studies, including ATHENA, have shown that HPV 16 has a much higher baseline positive predictive value (PPV) than the other high-risk types and that, at least cross-sectionally, HPV 18 has a similar PPV to the pool of 12 other high-risk types. This raises the question as to whether HPV 18-positive women would be more efficiently managed by short-term (6-12 months) repeat testing for HPV 18 to establish persistence before referral to colposcopy, similar to the management of women positive for one of the other 12 high-risk HPV types. However, HPV 18 is associated more with cancer than these other types and also is associated with endocervical lesions that are difficult to detect.

In ATHENA, 3 of the 6 cancers detected at baseline were HPV 18 positive, as were 8 of the 16 adenocarcinoma in situ cases and 1 cancer detected in follow-up. So although the PPV for CIN3 or more severe of a positive HPV 18 test at baseline is lower than typically recommended for immediate colposcopy, the greater severity of the disease burden substantiates this approach. Other questions related to the behavior of different HPV types also need to be addressed, such as whether HPV 45, which shows a similar predilection for adenocarcinoma as HPV 18, should be managed in the same way as HPV 18.

The strategies with 12 month follow-up for women at intermediate risk will increase both the ultimate sensitivity of each of these strategies as well as the total number of colposcopies and must also take into account loss to follow-up. Hence, any strategy selected should consider the trade-offs of sensitivity and specificity in the context of real-world clinical practice. As illustrated by Kitchener et al., poor follow-up of HPV-positive women not sent immediately to colposcopy could negate some of the benefits of HPV testing to identify women at risk for CIN2 or more severe while leaving unaffected the added safety of a negative HPV test. Within the context of poor follow-up of screen-positive patients, more sensitive methods of managing HPV-positive women may be preferred over methods with increased specificity and PPV, so that the opportunity for immediate detection and treatment of precancerous lesions will not be missed.

In conclusion, this analysis demonstrates that multiple cervical cancer screening strategies are more effective than the present standard of cytology screening with ASC-US triage. Strategies that maximize early detection of CIN3 or more severe without excessive increases in initial screening tests and colposcopies, yet also identify women at intermediate risk in need of 12 month follow-up, would appear to provide optimal balance between benefit and harms. Of these options, strategies that incorporate initial screening with HPV and triage of HPV-positive women by a combination of genotyping for HPV 16/HPV 18 and cytology may best fulfill these requirements for more balanced screening, although other options may also be compelling in different settings. When the 3 year follow-up data from ATHENA become available, formal cost-effectiveness modeling can be performed to better determine the benefits of cervical cancer screening strategies that incorporate HPV with genotyping for HPV 16/HPV 18 and cytology.

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REFERENCES

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