

GENERAL GYNECOLOGY

The ATHENA human papillomavirus study: design, methods, and baseline results

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OBJECTIVE: The objective of the study was to describe baseline data from Addressing the Need for Advanced HPV Diagnostics, a prospective, multicenter US cervical cancer screening trial.

STUDY DESIGN: A total of 47,208 women aged 21 years or older undergoing routine screening were enrolled; liquid-based cytology and human papillomavirus (HPV) testing were performed. Women with abnormal cytology underwent colposcopy, as did high-risk HPV (hrHPV)-positive women and a random subset of women negative by both tests aged 25 years or older. Verification bias adjustment was applied; 95% confidence intervals were computed by the bootstrap method.

RESULTS: The prevalence of cytologic abnormalities was 7.1%. hrHPV, HPV 16, and HPV 18 were detected using the cobas HPV Test in 12.6%,

2.8%, and 1.0% of women, respectively. Both cytologic abnormalities and hrHPV positivity declined with increasing age. The adjusted prevalence of cervical intraepithelial neoplasia grade 2 (CIN2) or greater in women aged 25-34 years was 2.3%, decreasing to 1.5% among older women.

CONCLUSION: The Addressing the Need for Advanced HPV Diagnostics study provides important estimates of the prevalence of cytologic abnormalities, hrHPV positivity, and CIN2 or greater in a US screening population.

Key words: Addressing the Need for Advanced HPV Diagnostics study, cervical cancer screening, cervical intraepithelial neoplasia, genotype, human papillomavirus deoxyribonucleic acid testing

Cite this article as: Wright TC, Stoler MH, Behrens CM, et al. The ATHENA human papillomavirus study: design, methods, and baseline results. *Am J Obstet Gynecol* 2011;205:xx-xx.

Over the last 50 years, cytology-based cervical cancer screening has dramatically reduced the burden of invasive cervical cancer in the United States; whereas the incidence in the 1940s was estimated to be 32.6 per 100,000,¹ today it is only 8.1 per 100,000.² However, despite intensive cytologic screening, cervical cancer remains a significant cause of morbidity and mortality in the United States with more than 12,000 incident

cases of cervical cancer annually and more than 4000 deaths.³ Moreover, approximately 500,000 women in the United States are diagnosed with high-grade cervical cancer precursors (cervical intraepithelial neoplasia grades 2 and 3 [CIN2, CIN3]) annually.⁴

Cervical cancer is caused by infection with 1 of 14 high-risk types of human papillomavirus (hrHPV), with just 2 hrHPV genotypes (HPV 16 and HPV

18) causing approximately 70% of all cases.⁵ This has led to considerable interest in determining the optimal strategies for incorporating testing for hrHPV (14 pooled types) and genotyping for HPV 16 and HPV 18 into the US cervical cancer screening program to further reduce the burden of cervical disease. However, ensuring appropriate adoption of hrHPV testing into these strategies will require comprehensive assessments of the performance of cytology, hrHPV testing, and the burden of cervical disease in large US screening populations.

A recently initiated clinical trial, referred to as Addressing the Need for Advanced HPV Diagnostics (ATHENA), was designed to prospectively evaluate the performance of the cobas HPV Test, a new polymerase chain reaction-based deoxyribonucleic acid (DNA) amplification test that simultaneously identifies a pooled result for 12 hrHPV types (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and individual results for HPV 16 and HPV 18. This trial evaluated 46,887 eligible women aged 21 years and older undergoing routine screening, of

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Received April 12, 2011; revised June 14, 2011; accepted July 13, 2011.

T.C.W. has been a consultant and speaker for Merck, GlaxoSmithKline, and Roche Molecular Systems and a consultant for Gen-Probe and Becton Dickinson. M.H.S. has been a consultant in clinical trial and HPV DNA test development for QIAGEN, Roche Molecular Systems, BD, Gen-Probe, Ventana, and Merck. C.M.B., R.A., T.D., and T.L.W. are employed by Roche Molecular Systems, the sponsor of the study. Victoria Tomlinson, Health Interactions, London, England, UK, provided editorial assistance in formatting and proofing of the final draft manuscript, funded by Roche Molecular Systems.

This study was supported in part by Roche Molecular Systems, Inc, Pleasanton, CA.

Presented in part at the 26th International Papillomavirus Conference and Clinical Workshops, International Papillomavirus Society, Montreal, QC, Canada, July 3-8, 2010.

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whom 8637 women underwent colposcopy, including a randomly selected subset of women aged 25 years and older who were negative by both Papanicolaou and hrHPV testing.

This manuscript describes the ATHENA study design and methods as well as the baseline characteristics of our study population, including the distribution of cytology results, hrHPV prevalence, and cervical disease status by age and HPV status.

MATERIALS AND METHODS

Objectives

Specific objectives of the ATHENA HPV trial included determining the performance of the cobas HPV Test both as a triage test for women with abnormal cytology (atypical squamous cells of undetermined significance [ASC-US]) and as an adjunctive test to guide clinical management in women with cytology results negative for intraepithelial lesions or malignancies (NILM). A third objective was to evaluate the performance of the cobas HPV Test as a potential first-line test in the screening of women aged 25 years and older, regardless of cytology result.

Study design

The study is being conducted in 2 phases: a baseline (cross-sectional) phase and a 3 year follow-up (longitudinal) phase; data from only the baseline phase are reported here because the follow-up phase is ongoing and will be completed in December 2012. The process used to select women for colposcopy and biopsy based on age, HPV test result, and cytology result is shown in the Figure and described in detail below.

HPV tests used for subject selection were first-generation Roche HPV tests (AMPLICOR HPV test and LINEAR ARRAY HPV genotyping test; Roche Molecular Systems, Pleasanton, CA); all HPV results are based on the second-generation Roche HPV test (cobas HPV Test). The primary study endpoint for disease detection was high-grade cervical disease defined as CIN2 or greater (CIN2, CIN3, adenocarcinoma in situ, and invasive cervical cancer), as determined by a central pathology review

panel (described in the following text), and the secondary study endpoint for disease detection was CIN3 or greater. Reporting of the study endpoints was based on the highest grade lesion identified by the central pathology review panel.

Sample size was determined by the need for a sufficient number of women with CIN2 or greater in the ASC-US population to adequately evaluate the performance of the cobas HPV Test. In accordance with the sample size in similar registration trials,^{6,7} it was determined that approximately 70 women with CIN2 or greater would be needed. This estimate was used, along with published rates of ASC-US cytology⁸ and HPV positivity⁷ in the overall population, to arrive at a sample size of approximately 45,000 women.

Participants were recruited from among women presenting for routine cervical cancer screening at 61 clinical sites across 23 states between May 2008 and August 2009. Clinical centers were predominantly general obstetrics and gynecology practices that routinely perform colposcopy. The inclusion and exclusion criteria are described in detail elsewhere.⁹

The study was approved by Independent Investigational Review Board, Inc. (Plantation, FL) for the clinical sites and by Independent Investigational Review Board, Inc., the local institutional review board, or Copernicus Group Investigational Review Board (Research Triangle Park, NC) for the clinical laboratories. The study was conducted according to the International Conference on Harmonization Guideline for Good Clinical Practice.

Baseline phase (cross-sectional phase)

Participating women underwent 1 or 2 study visits at baseline, as follows.

Study visit 1 (enrollment visit [all participants]). After informed consent was obtained, a brief medical and the women's obstetrics and gynecology history were taken. A speculum examination was then performed during which 2 cervical samples (A and B) were collected using a plastic spatula and cytobrush according to

the manufacturer's instructions and placed into 2 separate vials of PreservCyt solution (Hologic, Inc., Bedford, MA) (Figure). Sample A was processed for cytologic examination and HPV testing with the aforementioned Roche tests. Sample B was used to test for HPV DNA with the Hybrid Capture 2 assay according to the manufacturer's instructions in women with ASC-US cytology (QIAGEN, Gaithersburg, MD) as well as for DNA sequencing in a subset of women selected for an HPV sequencing study (not reported here) and for long-term storage for future testing.

Study visit 2 (colposcopy visit [selected participants]). Prior to reporting screening test results back to the clinical sites, results were entered into a subject selection and randomization database that generated a subset of women selected for colposcopy. Selection/randomization was based on the results of cervical cytology and HPV testing with the first-generation AMPLICOR and LINEAR ARRAY tests (Roche).

This subset included all women aged 21 years or older with abnormal cervical cytology (ASC-US or greater), irrespective of HPV test results ($n = 3259$); women aged 25 years or older with NILM cervical cytology and a positive HPV test result by either of the first-generation HPV tests ($n = 5726$) and randomly selected women aged 25 years or older with NILM cytology who were negative for HPV by both first-generation HPV tests ($n = 1041$). Women who were not selected for colposcopy, or who decided to exit the study after the enrollment visit, were subsequently provided with the results of their enrollment cytology and HPV tests. The results of the cobas HPV Test were not used to select women for colposcopy because the test cutoff value had not been finalized at the start of enrollment into ATHENA.

Nonpregnant women selected for colposcopy underwent the procedure within 12 weeks of the enrollment visit. At the time of colposcopy, both study participants and colposcopists were blinded to cytology and HPV test results except, for safety reasons, in women with a cytologic diagnosis of cervical carci-

noma or other malignant neoplasm. A standardized colposcopy protocol was followed as described in detail elsewhere⁹ and in the Supplemental Table. Women who met the primary clinical endpoint (CIN2 or greater by consensus pathology) exited the study.

Follow-up phase (3 year longitudinal follow-up)

Women who underwent colposcopy but did not meet the primary endpoint of CIN2 or greater by consensus pathology continued to the follow-up phase of the study (3 year longitudinal follow-up). Women diagnosed by the clinical laboratory with CIN2 or greater that was downgraded to less than CIN2 by consensus pathology were included in the follow-up phase. Women requiring additional procedures (eg, loop electrosurgical excision procedure, cervical conization) were managed according to standard of care at the clinical site. If available, cervical specimens collected during such treatment procedures were submitted for consensus pathology review.

During the follow-up phase (ongoing), women are being scheduled for annual follow-up examinations at years 1, 2, and 3. At each visit a liquid-based cytology (LBC) specimen (ThinPrep Papicolaou test; Hologic, Inc, Bedford, MA) is obtained for cytology and cobas HPV testing. The residual specimen is stored for future testing. Nonpregnant women in whom cervical cytology is abnormal (ASC-US or greater) are referred for colposcopy with biopsy and/or endocervical curettage (ECC) according to the same protocol utilized during the baseline phase. Women found to have a diagnosis of CIN2 or greater will exit the study; those who do not will continue in the follow-up phase.

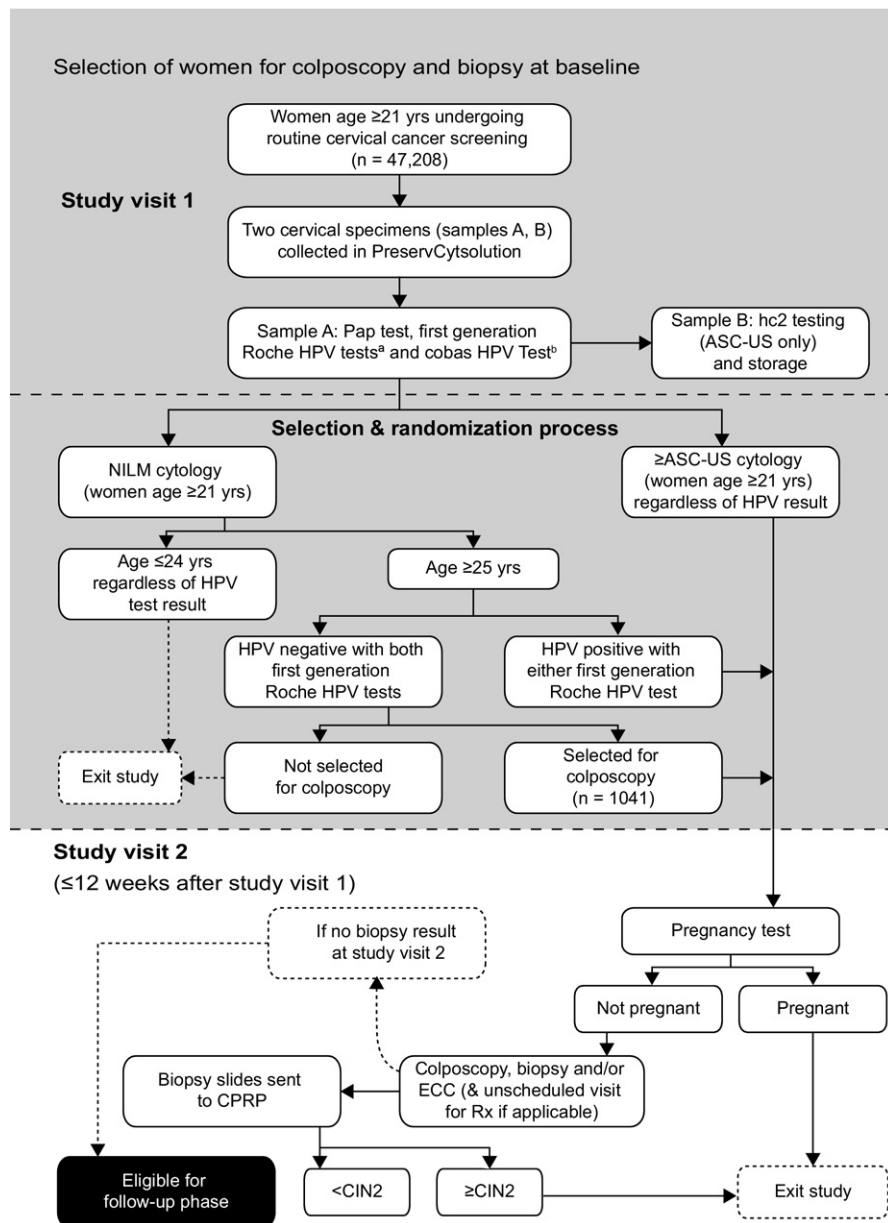
To optimize disease ascertainment at the end of the 3 year follow-up phase, an exit colposcopy and ECC will be offered to all nonpregnant women. This colposcopy will use the same protocol that was utilized at baseline with the exception that all participants will have an ECC.

Laboratory testing

Cytology and HPV testing. Cytology was conducted at four clinical laboratories

FIGURE

Selection of women for colposcopy and biopsy at baseline



A, AMPLICOR HPV test and LINEAR ARRAY HPV test (Roche Molecular Systems, Pleasanton, CA). B, cobas HPV Test not used for selection and randomization.

CPRP, central pathology review panel; hc2, Hybrid Capture 2 assay; HPV, human papillomavirus.

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and carried out as described in detail elsewhere⁹; cytologic evaluation was performed without computerized imaging. HPV testing was performed at these 4 laboratories and 1 additional laboratory. Cycle threshold cutoff values for the cobas HPV Test were established using samples from the first approximately 29,000 women enrolled;

subsequent cross-validation of the test cutoff was achieved using samples from the remaining approximately 18,000 participants.

Consensus pathology review

The consensus pathology review panel consisted of 3 study pathologists blinded to all subject and laboratory informa-

TABLE 1
Demographic data and medical history for all eligible women

Characteristics	Eligible women (n = 46,887)
Age, y	
Mean y ± SD	39.8 ± 12.3
21-29, n (%)	11,734 (25.0)
30-39, n (%)	12,528 (26.7)
40-49, n (%)	11,961 (25.5)
≥50, n (%)	10,664 (22.7)
Race, n (%)	
White	38,904 (83.0)
Black or African American	6581 (14.0)
Asian	745 (1.6)
American Indian or Alaskan Native	263 (0.6)
Native Hawaiian or Other Pacific Islander	114 (0.2)
Any combination/missing	280 (0.6)
Ethnicity, n (%)	
Hispanic or Latino	8380 (17.9)
Education, n (%)^a	
Elementary/high school (or GED)	11,929 (25.4)
Vocational/college/graduate	34,946 (74.5)
Postmenopausal, n (%)	13,442 (28.7)
Immunocompromised or immunosuppressed, n (%)	258 (0.6)
HPV vaccinated, n (%)	1224 (2.6)
History of smoking cigarettes, n (%)	
Past smoker	6612 (14.1)
Present smoker	7145 (15.2)
Nonsmoker	33,129 (70.7)
Papanicolaou test in past 5 y, n (%)	
NILM	31,876 (75.1)
ASC-US	1114 (2.6)
Greater than ASC-US	367 (0.9)
Other	482 (1.1)
Missing	8623 (20.3)
Colposcopy in past 5 y, n (%)	3646 (7.8)

ASC-US, atypical squamous cells of undetermined significance; GED, general education development; HPV, human papillomavirus; NILM, negative for intraepithelial lesions or malignancies.

^a Twelve women had missing information.

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tion. Each biopsy and ECC was initially evaluated by 2 pathologists and reported using the 3 grades of CIN (CIN1, CIN2, CIN3) as well as adenocarcinoma in situ or carcinoma. If the diagnoses were concordant, it was recorded as the central

pathology review panel diagnosis; if discordant, the biopsy/ECC was reviewed by the third study pathologist.

In cases in which all 3 diagnoses were discordant, the slides were reviewed in conference between the 3 pathologists to

arrive at a consensus pathology diagnosis. Pathology specimens obtained at an unscheduled visit (a visit after study visit 2 for a gynecologic procedure or for a study colposcopy performed outside the 12 week window) could be used to determine the histologic stage of disease at baseline, provided the specimen was obtained within 28 days of the colposcopy at study visit 2. If more than 1 pathology specimen was obtained (either as biopsy or unscheduled visit specimen), the highest grade of disease was considered the consensus pathology diagnosis. Pathology results were categorized as CIN2 or greater, less than CIN2, CIN3 or greater, and less than CIN3 for determination of study endpoints as defined in Supplemental Figure 2.

Statistical analyses

Prevalence estimates of Papanicolaou and HPV results were calculated based on all eligible women with valid Papanicolaou or HPV test results. Crude prevalence estimates of cervical disease were calculated based on women who underwent colposcopy/biopsy. The crude estimates of prevalence can result in bias because all women with positive Papanicolaou/HPV results were selected to undergo colposcopy, whereas only a small subset of women with negative test results were randomly selected to undergo colposcopy.

Verification bias adjustment was applied to account for the difference in rates of selection to colposcopy. This was accomplished by calculating the likely number of cases that would have been found if all women had undergone colposcopy and been disease verified.¹⁰

In brief, the data were divided into strata of combined age group, Papanicolaou test results, and HPV test results. Disease prevalence in each stratum was assumed to be independent of whether the women underwent biopsy. Stratum-specific probabilities were then applied to the remainder of the women who had not undergone biopsy; this permitted an estimate of the number of cases that would have been found if all women had undergone colposcopy.

Verification bias-adjusted prevalence was calculated by collapsing strata by age

TABLE 2
Distribution of cytology results

Papanicolaou test result, n (%)	Age group, y						Total (n = 46,887)
	21-24 (n = 4932)	25-29 (n = 6802)	30-39 (n = 12,528)	40-49 (n = 11,961)	50-59 (n = 7680)	60 or older (n = 2984)	
NILM	4192 (85.0)	6024 (88.6)	11,445 (91.4)	10,989 (91.9)	7158 (93.2)	2817 (94.4)	42,625 (90.9)
ASC-US	288 (5.8)	341 (5.0)	509 (4.1)	509 (4.3)	207 (2.7)	69 (2.3)	1923 (4.1)
ASC-H	13 (0.3)	17 (0.2)	25 (0.2)	8 (0.1)	2 (0.0)	1 (0.0)	66 (0.1)
LSIL	322 (6.5)	257 (3.8)	254 (2.0)	168 (1.4)	76 (1.0)	11 (0.4)	1088 (2.3)
HSIL	33 (0.7)	29 (0.4)	50 (0.4)	23 (0.2)	11 (0.1)	0 (0.0)	146 (0.3)
SCC	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.0)	0 (0.0)	0 (0.0)	2 (0.0)
AGC ^a	1 (0.0)	8 (0.1)	12 (0.1)	17 (0.1)	10 (0.1)	3 (0.1)	51 (0.1)
AGC, favor neoplastic ^b	0 (0.0)	1 (0.0)	1 (0.0)	2 (0.0)	0 (0.0)	1 (0.0)	5 (0.0)
Invalid ^c	83 (1.7)	125 (1.8)	232 (1.9)	243 (2.0)	216 (2.8)	82 (2.7)	981 (2.1)

AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot rule out HSIL; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesions or malignancies; SCC, squamous cell carcinoma.

^a AGC includes: AGC endocervical, AGC endometrial, and AGC not otherwise specified; ^b AGC, favor neoplastic includes AGC endocervical, favor neoplastic, and AGC favor neoplastic; ^c Invalid includes endometrial cells older than 40 years of age (n = 30), no result available because of inadequate cells (n = 860), and no sample tested (n = 91).

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groups. The 95% confidence intervals (CIs) were computed by bootstrap method with 1000 bootstrap samples.¹¹ The 2.5th and 97.5th percentile of the bootstrap distribution of prevalence were used as the lower and upper limits of the 95% CIs.

RESULTS

Demographics of study population

A total of 46,887 eligible women 21-93 years of age were enrolled into the study. The number of participants enrolled at any given clinical site ranged from 54 to 2824, and the median age at individual clinical sites ranged from 26 to 46 years. The flow of participants through the baseline phase of the study is shown in Supplemental Figure 1. Population demographics and medical histories of the participants at enrollment are shown in Table 1. Most were white, had more than a high school education, were premenopausal, were nonsmokers, and had had a normal cervical cytology result within the previous 5 years. Only 2.6% of the women had been vaccinated for HPV.

Prevalence of cytologic abnormalities and hrHPV at enrollment

Overall, 90.9% of the participants' enrollment LBC specimens were classified as NILM (Table 2). The overall preva-

lence of ASC-US, low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) was 4.1%, 2.3%, and 0.3%, respectively. The prevalence of cytologic abnormalities decreased with increasing age. This decrease was especially marked for LSIL and HSIL. Cytology was evaluated as LSIL in 6.5% of women in the 21-24 year age group compared with 0.4% in the 60 years and older age group. HSIL cytology was diagnosed in 0.4% of the 25-29 years age group compared with 0% in the 60 years and older age group.

The prevalence of hrHPV (14 types) detected using the cobas HPV Test also decreased with increasing age (Table 3). At enrollment hrHPV was detected in 30.5% of women 21-24 years of age, but by age 40-44 years, the prevalence of hrHPV had decreased to only 7.6%, and by 70 years and older, it had decreased to 5.0%. Similar reductions in prevalence with increasing age were also observed for both HPV 16 and HPV 18. In the vaccinated population, hrHPV was detected in 33.1% and 27.3% of women 21-24 years and 25-29 years, respectively. Immunocompromised women represented only a small subpopulation (256 women) in the study, and the prevalence of hrHPV was 16.4% (42 of 256).

Cervical disease identified during the baseline phase

Of 10,026 women selected for colposcopy, 8637 (86.1%) underwent colposcopy and valid biopsy results were available in 8383 (83.6% of those selected for colposcopy). The distribution of women undergoing colposcopy among the study populations was as follows: 2799 with abnormal cytology, 4943 aged 25 years or older with NILM cytology who were hrHPV positive with either of the first-generation HPV tests, and 895 aged 25 years or older with NILM cytology who were hrHPV negative.

Biopsy-confirmed cervical disease (consensus pathology) at baseline decreased with increasing age (Table 4). The prevalence of CIN2 or greater in women aged 21 years or older who underwent colposcopy was 5.9%. Because only a subset of the women underwent colposcopy, a verification bias adjustment was made to estimate the disease prevalence across the entire 25 years of age and older study population. This could be done for only women aged 25 years and older because only women with abnormal cytology were referred to colposcopy in the 21-24 years of age group.

The verification bias-adjusted estimate of the prevalence of CIN by con-

TABLE 3
HPV prevalence identified using the cobas HPV Test

Age group, y	Total, n	Number of women HPV positive, n (%)		
		hrHPV	HPV 16	HPV 18
21-24	4914	1498 (30.5)	428 (8.7)	118 (2.4)
Vaccinated	720	238 (33.1)	58 (8.1)	9 (1.3)
25-29	6767	1427 (21.1)	362 (5.3)	110 (1.6)
Vaccinated	451	123 (27.3)	21 (4.7)	7 (1.6)
30-34	6042	810 (13.4)	166 (2.7)	64 (1.1)
35-39	6408	634 (9.9)	120 (1.9)	56 (0.9)
40-44	6029	458 (7.6)	65 (1.1)	28 (0.5)
45-49	5860	386 (6.6)	50 (1.1)	28 (0.5)
50-54	4561	300 (6.6)	38 (0.8)	24 (0.5)
55-59	3050	181 (5.9)	22 (0.7)	13 (0.4)
60-64	1637	98 (6.0)	13 (0.8)	5 (0.3)
65-69	775	32 (4.1)	6 (0.8)	0 (0.0)
≥70	558	28 (5.0)	4 (0.7)	2 (0.4)
Overall	46,601 ^a	5852 (12.6)	1287 (2.8)	448 (1.0)

HPV, human papillomavirus; hrHPV, high-risk genotypes of human papillomavirus.

^a A total of 286 women had invalid/missing cobas HPV Test results.

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sensus pathology for women aged 25 years and older is shown in Table 5. No clear age trends are seen with either CIN1 or CIN2. However, the prevalence of CIN3 or greater decreased from 1.4% in the 25-34 years of age group to 0.5% in the 45 years of age and older group.

Cervical disease by age and cobas HPV Test result (hrHPV positive, HPV 16

positive, and HPV 18 positive) is shown in Table 6. The proportion of women positive for hrHPV (14 types) as well as for HPV 16 increased with increasing CIN grade across all age groups. The hrHPV (14 types) was identified in 65.5% of women with CIN1, 83.3% of women with CIN2, and 92.6% of women with CIN3. In addition, 87.5% of women

with a diagnosis of adenocarcinoma in situ were hrHPV positive, as were all 4 women with invasive cancers (2 additional cases with an initial diagnosis of CIN3 were subsequently diagnosed as invasive cancer by procedures performed outside the study window, and these cases were both hrHPV positive).

Among women with consensus pathology biopsy-confirmed CIN1 or CIN2, there was a significant reduction with increasing age in the proportion of the lesions that were associated with hrHPV or with HPV 16. For example, 83.8% of CIN1 cases in women 21-24 years of age were hrHPV positive compared with only 39.0% of those diagnosed in women aged 50 years and older.

Similarly, 19.8% of CIN1 lesions in women 21-24 years of age were associated with HPV 16 compared with only 3.7% of those in women 50 years old and older. The impact of increasing age on hrHPV positivity was much less pronounced for CIN3 lesions (Table 6). However, the prevalence of HPV 16 was much lower in CIN3 cases diagnosed in older as opposed to younger women.

The association of HPV 18 was relatively uncommon in almost all grades of CIN lesions compared with HPV 16 with the exception of the 16 cases of adenocarcinoma in situ, in which 6 cases (38%) were associated with HPV 16 and 8 (50%) were associated with HPV 18. Of

TABLE 4
Cervical disease status by consensus pathology in women undergoing colposcopy

CPRP diagnosis	Age group, y					Overall ^a
	21-24	25-29	30-39	40-49	≥50	
WNL, n (%)	365 (67.3)	1577 (82.0)	2191 (85.7)	1763 (90.0)	1288 (91.7)	7184 (85.7)
CIN1, n (%)	111 (20.5)	194 (10.1)	201 (7.9)	114 (5.8)	82 (5.8)	702 (8.4)
CIN2, n (%)	35 (6.5)	66 (3.4)	51 (2.0)	29 (1.5)	11 (0.8)	192 (2.3)
CIN3, n (%)	31 (5.7)	83 (4.3)	104 (4.1)	46 (2.3)	21 (1.5)	285 (3.4)
ACIS, n (%)	0 (0.0)	2 (0.1)	8 (0.3)	5 (0.3)	1 (0.1)	16 (0.2)
SCC, n (%) ^b	0 (0.0)	0 (0.0)	2 (0.1)	1 (0.1)	0 (0.0)	3 (0.0)
Adenocarcinoma, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.0)
Overall, n	542	1922	2557	1958	1404	8383

ACIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; CPRP, central pathology review panel; SCC, squamous cell carcinoma; WNL, within normal limits.

^a Includes 18 women who had invalid cobas HPV Test results, 1 with CIN1, and 17 WNL; ^b Two women with an initial diagnosis of CIN3 were found to have invasive cervical cancer after additional procedures more than 16 weeks after enrollment and are classified as CIN3 in this table.

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TABLE 5

Verification bias–adjusted estimates of the prevalence of CIN in the overall study population^a

Age group, y	Prevalence, % (95% CI)		
	CIN1	CIN2	CIN3 or greater
25–34	4.5 (3.2–6.0)	0.9 (0.7–1.1)	1.4 (1.2–1.6)
35–44	3.3 (2.0–4.9)	0.4 (0.3–0.6)	1.1 (0.7–1.9)
≥45	4.6 (2.8–6.6)	1.0 (0.3–2.1)	0.5 (0.3–1.1)
Overall	4.2 (3.3–5.2)	0.8 (0.5–1.2)	1.0 (0.7–1.3)

CI, confidence interval; CIN, cervical intraepithelial neoplasia.

^a Prevalence assessed by consensus pathology.

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note, there were no cases of adenocarcinoma in situ or invasive cancer in women below the age of 25 years and only 1 case of adenocarcinoma in situ and no invasive cancer in women less than 30 years of age.

COMMENT

This US population–based cervical cancer screening trial was designed to evaluate the medical importance of pooled hrHPV DNA in addition to HPV genotypes 16 and 18, in 3 populations of

women: those with ASC-US cytology (21 years of age or older), those with normal cytology (30 years of age or older), and those in an overall screening population that included all cytology results (25 years of age or older).

Both the demographics of the participants in the ATHENA trial and the results of the enrollment cytology indicate that the study participants are representative of women undergoing cervical cancer screening in the United States. Recent census estimates for the racial breakdown of the entire female population indicate 79% white, 13% black or African American, and 16% Hispanic or Latino ethnicity,¹² which is comparable with the distribution observed in ATHENA.

The overall rate of cytologic abnormalities in this study is almost identical to the most recent College of American

TABLE 6

Grade of cervical disease according to age and cobas HPV Test result

Consensus pathology result	Age groups, y					Overall
	21–24	25–29	30–39	40–49	≥50	
hrHPV positive, % (n/N)						
CIN1	83.8 (93/111)	76.8 (149/194)	64.5 (129/200)	49.1 (56/114)	39.0 (32/82)	65.5 (459/701)
CIN2	91.4 (32/35)	84.8 (56/66)	86.3 (44/51)	75.9 (22/29)	54.5 (6/11)	83.3 (160/192)
CIN3	96.8 (30/31)	96.4 (80/83)	92.3 (96/104)	89.1 (41/46)	81.0 (17/21)	92.6 (264/285)
ACIS	—	100.0 (2/2)	87.5 (7/8)	100.0 (5/5)	0.0 (0/1)	87.5 (14/16)
SCC/adenocarcinoma	—	—	100.0 (2/2)	100.0 (1/1)	100.0 (1/1)	100.0 (4/4)
HPV 16 positive, % (n/N)						
CIN1	19.8 (22/111)	17.0 (33/194)	12.5 (25/200)	6.1 (7/114)	3.7 (3/82)	12.8 (90/701)
CIN2	45.7 (16/35)	34.8 (23/66)	27.5 (14/51)	10.3 (3/29)	9.1 (1/11)	29.7 (57/192)
CIN3	83.9 (26/31)	54.2 (45/83)	52.9 (55/104)	30.4 (14/46)	28.6 (6/21)	51.2 (146/285)
ACIS	—	50.0 (1/2)	37.5 (3/8)	40.0 (2/5)	0.0 (0/1)	37.5 (6/16)
SCC/adenocarcinoma	—	—	50.0 (1/2)	0.0 (0/1)	0.0 (0/1)	25.0 (1/4)
HPV 18 positive, % (n/N)						
CIN1	10.8 (12/111)	5.2 (10/194)	7.5 (15/200)	2.6 (3/114)	1.2 (1/82)	5.8 (41/701)
CIN2	0.0 (0/35)	4.5 (3/66)	5.9 (3/51)	0.0 (0/29)	0.0 (0/11)	3.1 (6/192)
CIN3	6.5 (2/31)	2.4 (2/83)	6.7 (7/104)	4.3 (2/46)	9.5 (2/21)	5.3 (15/285)
ACIS	—	50.0 (1/2)	50.0 (4/8)	60.0 (3/5)	0.0 (0/1)	50.0 (8/16)
SCC/adenocarcinoma	—	—	0.0 (0/2)	100.0 (1/1)	100.0 (1/1)	50.0 (2/4)

All women 21 years old with abnormal Papanicolaou results, or women ≥25 years old with normal Papanicolaou results and positive by first-generation Roche Molecular Systems (Pleasanton, CA) HPV test results were selected to go to colposcopy/biopsy. Only a subset of women ≥25 years old with normal Papanicolaou and negative HPV test results was selected to proceed to colposcopy/biopsy.

ACIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; hrHPV, high-risk types of human papillomavirus; SCC, squamous cell carcinoma.

Wright. ATHENA HPV study. *Am J Obstet Gynecol* 2011.

Pathologists survey of cytologic abnormalities that was conducted in 2003. Based on results from 759 separate reporting laboratories, the median rate of cytologic abnormalities for ThinPrep LBC specimens (Hologic) in 2003 was 7.3% and the median rate of ASC-US was 4.0%.¹³

The rate of cytologic abnormalities detected using LBC in ATHENA is also similar to the rate reported with conventional cytology from 580,280 women undergoing routine screening in Kaiser Permanente Northern California.¹⁴ In Kaiser, the overall rates of cytologic abnormalities for women aged 30-39, 40-49, and 50-59 years were 6.1%, 5.7%, and 4.3%, respectively; in ATHENA the corresponding rates of LBC cytologic abnormalities were 6.9%, 6.2%, and 4.1%.¹⁴

The overall prevalence of hrHPV (14 genotypes), HPV 16, and HPV 18 in women aged 21 years or older enrolled in ATHENA was 12.6%, 2.8%, and 1.0%, respectively. This is similar to what was recently reported from a prevalence survey of HPV infections in 1921 women (14-59 years of age) participating in the National Health and Nutrition Examination Survey (NHANES). In NHANES the overall prevalence of hrHPV was 15.2%, with an overall prevalence of HPV 16 and HPV 18 of 1.5% and 0.8%, respectively.¹⁵

In the analysis of women undergoing routine screening at Kaiser, the overall prevalence of hrHPV detected by the Hybrid Capture 2 assay (QIAGEN) in women aged 30 years or older was 6.3%.¹⁴ In ATHENA, the prevalence of hrHPV detected by the cobas HPV Test in women aged 30 years or older was 8.4%.

The observed reduction in hrHPV prevalence with increasing age in ATHENA is consistent with that observed in other studies from the United States and countries with established cervical cancer screening programs. In NHANES, the prevalence of hrHPV decreased from approximately 28% in women 20-24 years of age to approximately 7% in women 50-59 years of age.¹⁵ In Kaiser, hrHPV prevalence was 10.8% in the 30-34 years of age group and less than 5% in the 45-79 years of age group.¹⁴ In the current trial,

hrHPV prevalence dropped from 30.5% in women 21-24 years of age to 6.6% or less in women 45-93 years of age.

HPV 16 and HPV 18 are associated with approximately 70% of all invasive cervical cancers, and there is increasing interest among clinicians and policymakers in using HPV 16 and HPV 18 status as a way of stratifying hrHPV-positive women into a low-risk group (HPV 16/18 negative) and high-risk group (HPV 16/18 positive).^{5,16,17} Therefore, it is reassuring to observe that a relatively low overall prevalence of both HPV 16 and HPV 18 was found in women 30 years of age and older and that these genotypes account for a greater proportion of hrHPV in younger women, in whom HPV DNA testing is not currently being recommended for use as an adjunct to cytology for screening.

In the current trial, the prevalence of HPV 16 in the women 30-39 years of age was only 2.3%; it decreased to 1.1% in women 40-49 years of age and to less than 1% in older women. HPV 18 was even less common and was detected in less than 1% of women 35 years of age and older.

One of the strengths of the ATHENA trial is that all women 25 years of age or older who had either an ASC-US result or greater or who were hrHPV positive with the first-generation HPV tests were referred to colposcopy, as were a subset of women who had negative results on both Papanicolaou and HPV tests.

Colposcopy was standardized across sites and included a random cervical biopsy if no lesions were visible by colposcopy. All biopsies underwent a consensus pathology review by gynecologic pathologists blinded to all clinical and laboratory information. This in-depth disease ascertainment process allows for an accurate assessment of the prevalence of CIN2 or greater in the trial population. Overall, the prevalence of CIN2 or greater in women aged 25 years and older undergoing colposcopy was 5.5%; this can be extrapolated to yield a verification bias-adjusted estimate of 1.8% for CIN2 or greater in the overall population aged 25 years and older. The verification bias-adjusted estimate for CIN3 or greater was 0.98% in this same age group.

Only 2 somewhat smaller North American studies have adjusted for verification bias by performing colposcopy in women who were both cytology and hrHPV negative and thus can produce an accurate estimate of the prevalence of high-grade cervical disease among women undergoing cervical cancer screening. One was a study of 4075 women being screened at Planned Parenthood Clinics in Washington State.¹⁸ In that study the estimated underlying prevalence of CIN3 or greater in women 30-34 years of age was approximately 5%, and in women 35-50 years of age, it was approximately 8%.

These estimates are considerably greater than the estimates of the current trial and are also considerably higher than the 1.5% prevalence of CIN3 or greater found in previously unscreened black South African women enrolled in a cervical cancer screening trial that performed colposcopy and cervical biopsy in all participants.¹⁹ A more recent Canadian study that enrolled 10,154 women 30-69 years of age estimated that the underlying prevalence of CIN2 or greater is about 1%,²⁰ which is somewhat lower than that found in the current trial.

Possible explanations for variability in estimated prevalence of underlying high-grade CIN in the screening population include differing risk factors for CIN and prior screening histories of the participants, the pathological criteria used for diagnosing high-grade CIN, and whether the pathologists were blinded to clinical information. It should also be recognized that the best approach to adjusting for verification bias when estimating disease prevalence is controversial,²¹ particularly if screening tests are reasonably sensitive and the prevalence of disease in individuals who are negative at the screening test is low.

The large number of histologically diagnosed CIN lesions (n = 1178, with valid cobas HPV Test results) observed during the baseline phase of ATHENA also allows an assessment of the distribution of hrHPV, HPV 16, and HPV 18 in CIN cases of different grades in the United States. As would be expected, hrHPV prevalence (in particular HPV 16) was found to increase with increas-

ing grade of cervical disease. HPV 16 was identified in 12.8% of CIN1 cases, 29.7% of CIN2, and 51.2% of CIN3. This is similar to what has been previously reported by metaanalyses of pooled data from around the globe, with HPV 16 identified in 18.7% of CIN1 cases and 45.3% of CIN2,3 cases.^{22,23}

HPV 18 was much less common than HPV 16 in CIN cases at baseline, and the prevalence of HPV 18 in CIN of different grades is also similar to that reported in the metaanalyses. An unexpected finding was the reduction in hrHPV positivity in consensus pathology-confirmed CIN diagnoses that occurred with increasing age. This was found for all grades of CIN and was most striking for HPV 16. It should be noted that this reduction in hrHPV positivity is based on HPV testing of the correlated cytology sample taken prior to the biopsy. Future analyses that actually genotype the tissues themselves may clarify whether this effect is due to interpretive nonspecificity of histomorphology in older vs younger women or due to age-related sampling variables.

The ATHENA trial is a large cervical cancer screening trial, enrolling 47,208 women 21 years of age or older at 61 clinical sites throughout the United States. Women were screened using both LBC and HPV DNA testing, and all women 25 years of age or older with an abnormal result on either test, as well as a subset of women who were negative on both screening tests, were referred to colposcopy.

This trial provides contemporary epidemiologic data on the prevalence of cytologic abnormalities, the prevalence of hrHPV (including HPV 16 and HPV 18), and the prevalence of biopsy-confirmed cervical disease in a US population undergoing routine cervical cancer screening. The epidemiologic data that are being obtained though ATHENA will likely

prove invaluable to US policymakers developing guidelines for both cervical cancer screening and managing women with screening test abnormalities. ■

ACKNOWLEDGMENT

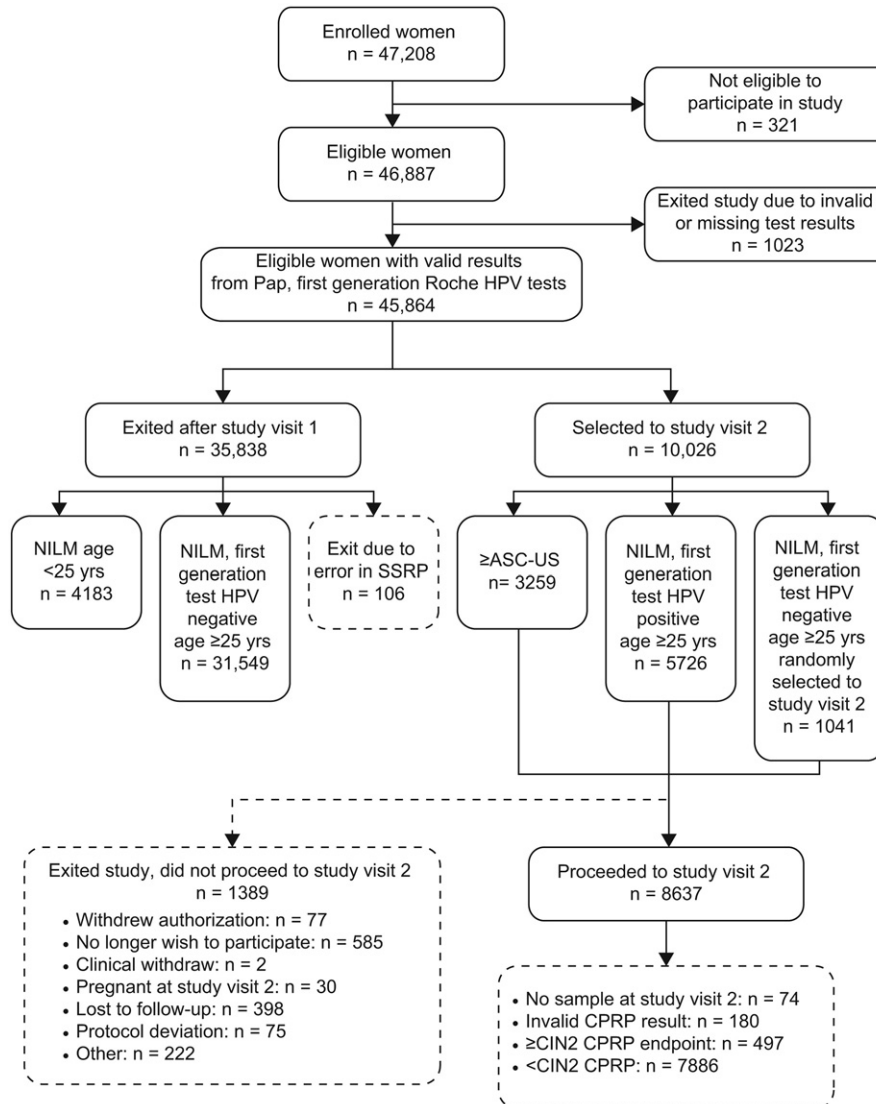
We wish to acknowledge the late Peter A. Holthe, PhD, MBA, for implementation of data management systems critical to the execution of the trial. His wit and wisdom are deeply missed.

REFERENCES

1. Kurman RJ, Ellenson LH, Ronnett BM. Blaustein's pathology of the female genital tract. New York, NY: Springer; 2002.
2. Altekruse SF, Kosary CL, Krapcho M, et al. SEER cancer statistics review, 1975-2007. Bethesda, MD: National Cancer Institute; 2009.
3. American Cancer Society. What are the key statistics about cervical cancer? Available at: <http://www.cancer.org/Cancer/CervicalCancer/DetailedGuide/index>. Accessed March 13, 2010.
4. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. *J Low Genit Tract Dis* 2007;11:223-39.
5. de Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol* 2010;11:1048-56.
6. Cervista HPV HR test instructions for use. Madison, WI: Third Wave Technologies, Inc; 2009.
7. hc2 High-Risk HPV DNA Test [package insert]. Gaithersburg, MD: Digene Corp; 2007.
8. ALTS Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 2003;188:1383-92.
9. Stoler MH, Wright TC Jr, Sharma A, Apple R, Gutekunst K, Wright TL. High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. *Am J Clin Pathol* 2011;135:468-75.
10. Zhou XH, Obuchowski NA, Obuchowski DM. Statistical methods in diagnostic medicine. New York, NY: John Wiley and Sons; 2002.
11. Chernick M. Bootstrapping methods: a practitioner's guide. New York: Wiley Inter-science, 1998:8 and 112.
12. US Census Bureau. Table 6. Resident population by sex, race, and hispanic-origin status: 2000 to 2009. Available at: <http://www.census.gov/compendia/statab/2011/tables/11s0006.pdf>. Accessed March 3, 2011.
13. Eversole GM, Moriarty AT, Schwartz MR, et al. Practices of participants in the college of American pathologists interlaboratory comparison program in cervicovaginal cytology, 2006. *Arch Pathol Lab Med* 2010;134:331-5.
14. Castle PE, Fetterman B, Poitras N, Lorey T, Shaber R, Kinney W. Five-year experience of human papillomavirus DNA and Papanicolaou test cotesting. *Obstet Gynecol* 2009;113:595-600.
15. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. *JAMA* 2007;297:813-9.
16. American Society for Colposcopy and Cervical Pathology HPV genotyping clinical update 2009. Available at: <http://www.asccp.org/consensus.shtml>. Accessed April 29, 2009.
17. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. *Am J Obstet Gynecol* 2007;197:346-55.
18. Kulasingam SL, Hughes JP, Kiviat NB, et al. Evaluation of human papillomavirus testing in primary screening for cervical abnormalities: comparison of sensitivity, specificity, and frequency of referral. *JAMA* 2002;288:1749-57.
19. Denny L, Kuhn L, De Souza M, Pollack AE, Dupree W, Wright TC Jr. Screen-and-treat approaches for cervical cancer prevention in low-resource settings: a randomized controlled trial. *JAMA* 2005;294:2173-81.
20. Mayrand MH, Duarte-Franco E, Rodrigues I, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *N Engl J Med* 2007;357:1579-88.
21. Sasieni P. Estimating prevalence when the true disease status is incompletely ascertained. *Stat Med* 2001;20:935-49.
22. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:1157-64.
23. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer* 2007;121:621-32.

SUPPLEMENTAL FIGURE 1

Accountability of women through the baseline phase of the study



First-generation Roche Molecular Systems (Pleasanton, CA) HPV tests: AMPLICOR HPV test and LINEAR ARRAY high risk HPV genotyping test.

CPRP, central pathology review panel; HPV, human papillomavirus; SSRP, subject selection and randomization process.

Wright. ATHENA HPV study. Am J Obstet Gynecol 2011.

SUPPLEMENTAL TABLE

Biopsy and ECC schedule according to visualization of the cervix

Variable	Satisfactory: visualization of cervix and SCJ		Unsatisfactory: partial visualization of SCJ		Unsatisfactory: SCJ not visualized	
	Lesion(s) visible	No lesion visible	Lesion(s) visible	No lesion visible	Lesion(s) visible	No lesions visible
Biopsy	Biopsy all lesions	Single biopsy at SCJ	Biopsy all lesions	Single biopsy at SCJ	Biopsy all lesions	No biopsy
ECC	No	No	Yes	Yes	Yes	Yes

ECC, endocervical curettage; SCJ, squamocolumnar junction.

Wright. ATHENA HPV study. Am J Obstet Gynecol 2011.

SUPPLEMENTAL FIGURE 2

Final histology diagnosis and study endpoint determination

Final histology diagnosis	≥CIN2 endpoint	≥CIN3 endpoint
No significant pathological changes	<CIN2	<CIN3
Reactive of inflammatory condition		
Atypical squamous cell or glandular changes		
Endocervical glandular atypia		
Squamous metaplasia		
CIN1		
CIN2	≥CIN2	≥CIN3
CIN3		
Adenocarcinoma in situ		
Squamous cell carcinoma		
Adenocarcinoma/adenosquamous carcinoma		

CIN, cervical intraepithelial neoplasia.

Wright. ATHENA HPV study. *Am J Obstet Gynecol* 2011.