Video Article

cobas Screening and Improved Cervical Cancer Screening and Prevention

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Abstract

Cervical cancer is of significant global and national concern. Scientific data accumulating steadily over the past 10 years support a shift from traditional cytology-based screening to the use of "high-risk" HPV (hrHPV) testing as the most effective screening approach to prevent cervical cancer. However when hrHPV testing is used together with cytology for screening, some women will be found to be hrHPV positive but cytology negative. The management of this clinically important subset of women can be greatly facilitated by HPV genotyping. Genotyping for HPV 16 and 18 which are the two most commonly found hrHPV genotypes in women with invasive cervical cancer allows women at greatest risk for having an occult high-grade cervical cancer precursor or invasive cancer to undergo appropriate workup, while allowing women who are relatively low risk for significant cervical disease to be followed. Understanding the research that has led to improved clinical screening guidelines1 will help clinicians implement these improved technologies in cervical cancer screening and management, thereby decreasing the incidence of the disease.

Video Link

The video component of this article can be found at http://www.jove.com/video/3488/

Introduction

Worldwide, cervical cancer is the second most common cancer among women. It is estimated that annually there are 529,828 cases and 275,128 deaths globally[2]. Cervical cancer occurs in relatively young women, accounting for an average of 17 potential years of life lost for every death from invasive cervical cancer occurring before the age of 70[3]. There are marked global disparities in both the incidence of, and mortality from, cervical cancer[4]. More than 85% of the cases of cervical cancer occur in developing countries[5]. In large part the global disparity reflects differences in cervical cancer screening rates.

Cytology-based cervical cancer screening (*i.e.*, Papanicolaou or Pap test) was first introduced by Dr. Papanicolaou 50 years ago and is widely considered to be the most successful form of cancer screening. Over the last four decades as cytology-based screening was introduced, a dramatic reduction in the incidence of invasive cervical cancer occurred in North America and Western Europe. In the US, the incidence of cervical cancer has decreased 75% and mortality has decreased 74% since 1949[6]. The impact of nationwide cervical cancer screening programs has also been clearly demonstrated by the Scandinavian experience[7-9]. In Finland, a national cytology-based screening program to prevent cervical cancer, begun in the 1950's, dramatically lowered rates of cervical cancer to 5.5 cases per 100,000 women and the experience in Sweden was similar. In contrast, in Norway, which more recently developed a nationwide screening program, there has been a much smaller reduction in cervical cancer rates and the rate of cervical cancer continues to be three times higher (15.6 per 100,000) than in Finland.

Limitations of Current Cancer Screening Methods

Despite the success of cytology-based cervical cancer screening, cytology has a number of limitations. One of the most important is the low sensitivity of a single cervical cytology for detecting high-grade cervical cancer precursors (referred to as high-grade cervical intraepithelial neoplasia grades 2 and 3 or CIN 2,3) and invasive cervical cancers. Meta-analyses have estimated that the sensitivity of a single cervical cytology test is only approximately 50%[10,11]. Since it takes on average 10-15 years for a CIN 2,3 lesion to progress to an invasive cervical cancer, screening programs have compensated for the low sensitivity of cytology by recommending repeat testing at periodic intervals. However, this increases both the cost of the screening program and the likelihood that women will not comply with screening recommendations. It is also important to stress that the low sensitivity of cytology-based testing means some women who have been screened within the recommended screening interval will develop invasive cervical cancer. Reviews of the screening histories of women diagnosed with invasive cervical cancer in the Kaiser Northern California healthcare system, as well as in Sweden, have found that 24%-32% have had a recent negative cervical cytology[12,13]. another limitation of cervical cytology is that its specificity has dropped considerably over the last decade. Surveys of U.S. laboratories carried out by the College of American Pathologists show increasing rates of cytological abnormalities since 1996. In 1996 the median rate of cytological abnormalities was 6%[14]. By 2003 it had increased to 7.5%, and by 2006 it was 10.4%[15,16]. This means that today 1 out

of every 10 women undergoing routine cytology-based screening in the U.S. will have some form of reported abnormality requiring further testing or diagnostic workup.

HPV Genotyping in Cervical Cancer Screening

It is now accepted that infection with one of 14 oncogenic HPV genotypes (referred to as "high-risk" genotypes) is required for the development of CIN 2,3 and invasive cervical cancer[17]. This underlying causal association has spurred considerable interest in utilizing high-risk HPV (hrHPV) testing to improve cervical cancer screening and has resulted in commercially available sensitive molecular tests to detect hrHPV genotypes in clinical specimens. A number of large clinical trials and cost-effectiveness analyses have firmly established the clinical utility of using hrHPV testing to determine which women with an equivocal cytologic abnormality referred to as atypical squamous cells of undetermined significance (ASC-US) require colposcopy[18-20]. Since 2001, national Pap management guidelines have recommend the use of hrHPV testing as the "preferred" approach for evaluating ASC-US. Today, hrHPV testing is almost universally utilized in the North America to triage women with ASC-US[21,22] into two groups: one group is hrHPV positive and requires colposcopy; the other group is hrHPV negative and can simply be followed with repeat testing.

hrHPV testing is also increasingly being used to improve the sensitivity of cervical screening in women ≥30 years undergoing routine screening. Multiple studies have convincingly demonstrated that hrHPV testing is both more sensitive and more reproducible than cervical cytology[23-25]. Combining cytology and hrHPV testing for screening greatly improves sensitivity and this improved sensitivity allows women negative on both tests to wait for at least 5 years before undergoing rescreening[21,26].

There also is increasing interest in using HPV genotyping assays that identify the specific HPV 16 or 18 genotype with which a woman is infected. Of the 14 different known hrHPV genotypes, these two account for approximately 70% of all cervical carcinomas globally[27]. Prospective follow-up studies have shown that women infected with HPV 16 and/or HPV 18 have a much greater risk for subsequently developing CIN 2,3 than do women infected with other hrHPV genotypes[28-30]. Recognition of the increased risk of CIN 2,3 associated with infection by HPV 16 and/or 18 has led professional societies to recommend that women ≥30 years infected with either HPV 16 and/or 18 undergo colposcopy, even if they have a negative cervical cytology result[21,31].

Characteristics of Clinically useful hrHPV Tests

It is important for clinicians to understand that molecular testing for hrHPV is inherently different than molecular testing for other important cervical pathogens such as *Chlamydia trachomatis*, *N. gonorrhea*, or even herpes simplex virus (HSV). In laboratories that offer molecular testing, most tests are *analytically* validated, but may not be *clinically* validated. This is an important distinction. The Clinical Laboratory Improvement Amendments (CLIA) of 1988 that regulate laboratory testing in the U.S. require that a laboratory establish analytic validation of tests that they offer. For most infectious diseases, clinical utility translates into maximum analytic sensitivity. This allows a test to detect a small number of organisms that may be causing clinically significant disease. However, HPV is different. The majority of sexually-active women become infected with hrHPV at some point during their lives, but only a small number of these infected women go on to develop significant disease, CIN 2,3 or invasive cervical cancer. Therefore, a very high analytic sensitivity of an hrHPV test is undesirable, as it would result in the detection of an unnecessarily large number of women with clinically unimportant hrHPV infections[25,32,33]. Thus, the FDA requires that hrHPV tests be clinically validated using the clinical disease endpoints such as of CIN2,3 or cervical cancer (*e.g.*, CIN2+). This also explains why large-scale prospective clinical studies are required to allow an hrHPV test to be clinically optimized in terms of the tradeoff between sensitivity and specificity.

Addressing the Need for Advanced HPV Diagnostics: the ATHENA Study and the cobas HPV Test

Herein we describe an ongoing prospective clinical trial referred to as ATHENA. This trial was designed to clinically validate the performance of the new Roche cobas HPV Test. This test is performed on the cobas 4800 system which is comprised of the *cobas x 480* instrument for sample extraction and purification of nucleic acids, and the *cobas z 480* analyzer dedicated to PCR amplification and detection. This system can process up to 94 specimens (+ 2 controls) in a single run, and has been configured to meet the demands of typical laboratory workload. The test is currently used for cervical cell specimens collected in cobas PCR cervical collection media and PreservCyt liquid cytology media. Furthermore, the cobas HPV test is the only FDA approved cervical cancer screening test capable of distinctly identifying hrHPV genotypes 16 and 18 with concurrent detection of the other 12 hrHPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in a single assay. Data obtained from the ATHENA trial provide clinical validation of this assay. Additionally, the trial allows comparison of the cobas HPV test's performance characteristics to hybrid capture 2 (hc2), which has been a gold standard in the United States for hrHPV detection, in women with ASC-US. The data demonstrate equivalent sensitivities of the two tests for CIN2+ and CIN3+ in women with ASC-US. The cobas HPV test has sensitivities of 90% and 94% for CIN2+ and CIN3+,respectively, while hc2 has sensitivities of 87% and 91%. Positive and negative predictive values were also shown to be equivalent[34]. Despite these equivalencies, the cobas HPV Test offers several distinct advantages: the assay requires only 1 ml of pre-aliquoted PreservCyt liquid cytology specimen, compared to the 4 ml of residual specimen following Pap slide processing necessary for hc2 analysis (in an instance of low cellularity, there may be insufficient specimen to obtain the 4 ml necessary for hc2 analysis, and if repeat analyses are necessary to further evaluate an indeterminate finding, a reportable result may not be able to be obtained); the cobas HPV Test utilizes a pre-aliquot of the ThinPrep fluid which avoids the risks of contamination that may occur during Pap slide preparation with the ThinPrep processor (the removal of a pre-aliquot is performed in accordance with the FDA approved pre-aliquoting procedure for PreservCyt vials[35].); sample preparation takes place within an automated processing sequence during which PCR inhibitors common to gynecologic specimens are removed concurrently with nucleic acid purification; and the assay includes robust PCR amplification and the inclusion of a human Beta-globin internal control, providing straightforward interpretation without the need to repeat indeterminate results.

The concurrent identification of HPV genotypes 16 and 18 is a particularly attractive feature of the cobas HPV Test, as these high-risk genotypes are associated with approximately 70% of invasive cervical cancers worldwide[27]. The ATHENA data provide additional support for HPV genotype characterization, as genotyping was found to influence both the associated risks and relative risks of CIN2 or worse and CIN3 or worse in women with NILM and ASC-US cervical cytologies[34]. These findings indicate increased risks for CIN2 and CIN3 for HPV genotype 16+ patients compared to those who were positive for the other 12 non-16/18 genotypes[34]. With its technical advancements and ability to further characterize

the HPV genotype status of hrHPV positive women with abnormal cervical cytologies, the cobas HPV Test is a compelling key element for a comprehensive cervical cancer-screening program.

Protocol

1. Performing a Full Workflow

1. On the day of the procedure, use powder free gloves and pipetting devices with an aerosol barrier to transfer 1 ml of each specimen to individual 13 ml round-based Sarstedt tubes.
2. After loading samples, reagents are loaded via a simple "scan, scan, pour process," followed by consumables onto the x 480 instrument for sample preparation and click "Start Run."
3. After the eluted samples have cooled, transfer them from the deep well plate to their corresponding wells in the microwell plate. Then, once the sample processing is complete, seal the microwell plate and transfer it to the z 480 analyzer for amplification and detection within 90 min to maintain validity of the run.
4. The detection of amplified DNA is performed during thermal cycling and is facilitated through the oligonucleotide probes labeled with different fluorescent dyes detected in each of 4 different channels as shown here in **Table 2**.
5. The cobas HPV Test produces a qualitative result that is calculated using a kinetic algorithm. The software also can be configured to report results for the high risk HPV panel only (pooled 14 high risk HPV genotypes) or for both the pool of 12 high-risk HPV genotypes plus independent result reporting for HPV 16 and HPV 18. Here **Table 3** summarizes the clinical significance of results obtained from cobas HPV Test analyses for patients with cytological abnormalities based on clinical trials data (**Table 3**).

Description of ATHENA (Addressing the Need for Advanced HPV Diagnostics)[34]

ATHENA enrolled 46,887 women 21 years and older who were undergoing routine cervical cancer screening at 61 different clinical sites around the U.S. It is the largest U.S. cervical cancer screening trial to date. At enrollment, all women had cervical samples taken for liquid-based cervical cytology and hrHPV testing. The trial was designed to clinically validate the cobas HPV Test for three different indications:

1. As a triage test to determine which women ≥21 years with an ASC-US cytology result should be referred for colposcopy.
2. As an adjunct test to guide clinical management in women ≥30 years with cervical cytology results of negative for intraepithelial lesions or malignancy (NILM) (*e.g.*, cotesting with both cytology and hrHPV test).
3. As a first line cervical cancer screening test for women ≥25 years (*e.g.*, screening with hrHPV testing *without* cytology).

At enrollment, all women had cervical samples taken for liquid-based cervical cytology and hrHPV testing. Once the cervical cytology and hrHPV test results were available, women were entered into a subject selection database that generated a subset of women for colposcopy. Women referred to colposcopy included: a) all those with a ≥ASC-US cytology result; b) all those ≥25 years with NILM cytology who were hrHPV positive; and 3) a random subset of women ≥25 years with NILM cytology who were hrHPV negative, **Figure 1**. At the time of colposcopy both the participants and colposcopists were blinded to cytology and hrHPV test results. The primary study endpoint for disease detection was biopsy-confirmed CIN2+ (cervical intraepithelial neoplasia grades 2 and 3, invasive cervical cancer, and adenocarcinoma in situ) as determined by a central pathology review panel. The study is being conducted in two phases: a Baseline, cross-sectional, Phase, and a 3-year Follow-up, longitudinal, Phase; data from the Baseline Phase only are discussed here, as the Follow-up Phase is ongoing.

Demographics

A total of 46,887 eligible women ages 21 - 93 years were enrolled. **Table 4** shows the population demographics and medical histories of the participants at enrollment. Most were white, pre-menopausal, nonsmokers, and had had a normal cervical cytology result within the previous 5 years.

Overall, 92.9% of the participants' enrollment liquid based cytology, or LBC, specimens were classified as NILM. The overall prevalence of ASC-US, low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) were 4.2%, 2.4%, and 0.3%, respectively. The prevalence of cytologic abnormalities decreased with increasing age. This decrease was especially marked for LSIL and HSIL. LSIL was diagnosed in 6.6% of women in the 21-24 year group compared to 0.4% in the ≥60 year old group. HSIL was diagnosed in 0.4% of the 25-29 year old group compared to 0% in the ≥60 year old group.

The prevalence of the 14 types of high-risk HPV detected using the cobas HPV Test also decreased with increasing age, as demonstrated here in **Table 4**. At enrollment high-risk HPV was detected in 30% of women 21-24 years of age, but by age 40-44 years, the prevalence of high-risk HPV had dropped to only 8%, and by ≥70 years to 5%. Similar reductions in prevalence with increasing age were also observed for both HPV 16 and HPV 18, **Figure 2**.

Representative Results

Clinical Validation of cobas HPV Test in Women with ASC-US

A total of 1,578 women with ASC-US had valid HPV test results and underwent colposcopy. The mean age of women with ASC-US was 37 years and 23% were post-menopausal. The prevalence of hrHPV (14 genotypes) and HPV 16 and HPV 18 detected using the cobas HPV Test in women with ASC-US was 32%, 8% and 3% respectively. For comparison, the prevalence of hrHPV positivity (13 genotypes) detected in this same population using hc2 was 31%. As was observed for the entire population, among women with ASC-US the prevalence of hrHPV (14 genotypes) as well as HPV 16 and 18, declined with increasing age.

Among women with ASC-US who underwent colposcopy, biopsy-confirmed CIN1, CIN2, and CIN3 were detected in 10%, 2%, and 3%, respectively. There were no cases of invasive cervical cancer or adenocarcinoma in-situ. The sensitivity of cobas HPV Test for CIN2+ or CIN3+ was 90% and 94%, respectively, which is equivalent to that of hc2, at 87% and 91%, respectively, as presented in **Table 5**. Both the positive and negative predictive values of cobas HPV Test and hc2 were also equivalent.

Data from ATHENA clinically validates the cobas HPV Test (14 genotypes) by demonstrating that its performance characteristics are essentially identical to that of hc2, considered to be an established standard in the United States. Moreover the sensitivity of the cobas HPV test in women with ASC-US is similar to that found in a 2005 meta-analysis of published studies of hrHPV testing which reported a pooled hrHPV sensitivity of 94% for detection of CIN2+[36].

It is important to note that in ATHENA, the specific hrHPV genotype with which a woman with ASC-US is infected, had a dramatic impact on the absolute risk of CIN2+. The absolute risk of CIN2+ in those women who had HPV 16 and/or HPV 18 was 24%, whereas for women with a non16/18 hrHPV genotype the absolute risk of CIN2+ was only 9%. Although it is likely that clinical guidelines will continue to recommend colposcopy for all hrHPV positive women with ASC-US, irrespective of their specific HPV genotype, the risk of CIN2+ associated with being infected with HPV 16 and/or 18 is so great that it is likely that these women will require more intensive follow-up post-colposcopy.

Clinical Validation of cobas HPV Test as Adjunct Test to Guide Clinical Management in Women ≥ 30 Years with Cytology NILM Results

A total of 32,260 women enrolled into ATHENA were ≥30 years, had NILM cytology, and had a valid HPV test result. Of these, 4,219 were hrHPV positive and referred for colposcopy. An additional 886 women with NILM cytology who were hrHPV negative were randomized to undergo colposcopy in order to adjust for verification bias. Of these, 4,258 underwent colposcopy and had valid biopsy results. The mean age of the women in the NILM population who underwent colposcopy was 45 years.

In women ≥30 years with NILM cytology, the overall prevalence of hrHPV (14 types) detected using cobas HPV Test was 6.7% and the overall prevalence of HPV-16, HPV-18, and 12 other HR-HPV genotypes was 1.0%, 0.5%, and 5.2%, respectively. As was observed for both the entire population and in women with ASC-US, in the NILM population the overall prevalence of hrHPV (14 types), as well as HPV-16 and HPV-18 individually, decreased with increasing age, as observed here in **Figure 3**.

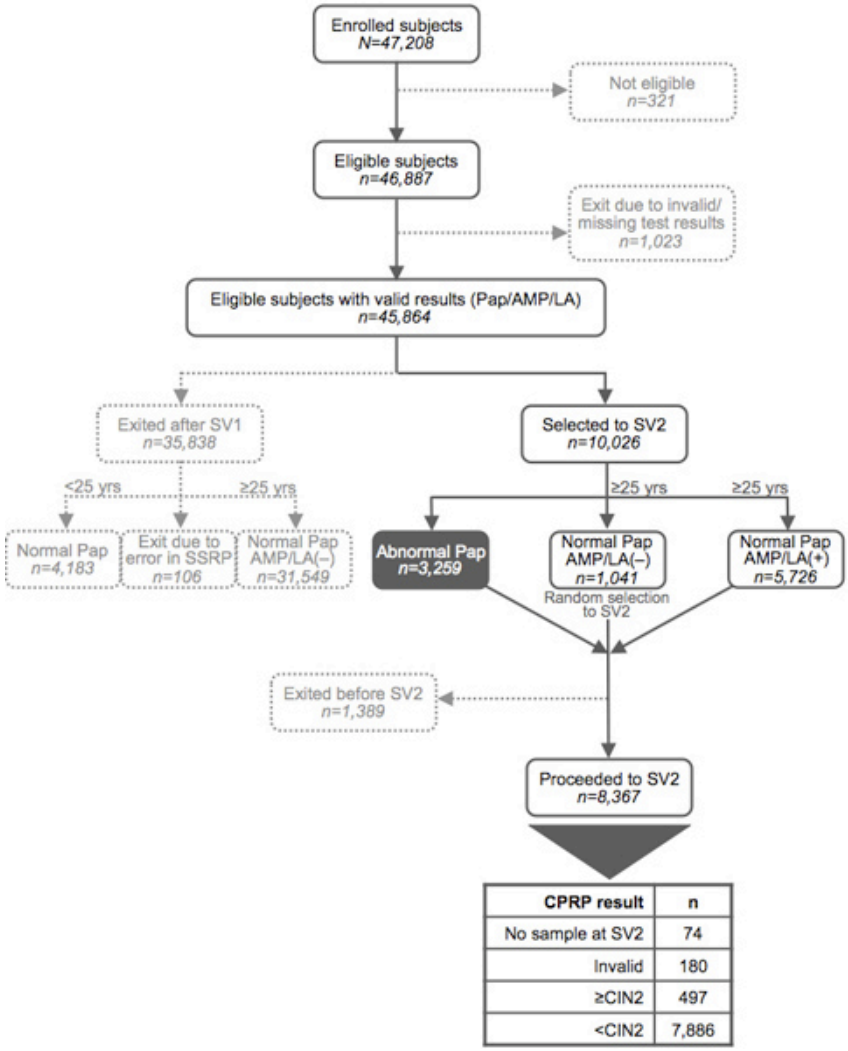
Verification bias-adjusted estimates of the overall absolute risk among women ≥30 years or older with NILM cytology were 1.2% (95% CI, 0.6-1.8%) for CIN2+ and 0.5% (95% CI, 0.3-0.9%) for CIN3+, **Table 6**. The estimated absolute risk was dependent on hrHPV status with HPV 16 positive women having the highest absolute risks. The estimated absolute risk for CIN3+ ranged from 0.3% (95% CI, 0.02-0.7%) in women who were negative for hrHPV to 11.7% (95% CI, 7.9-15.8%) in women who were positive for HPV 16.

These results clearly demonstrate that the absolute risk of CIN2+ and CIN3+ in women ≥30 years with NILM cytology is impacted not only by whether or not they are hrHPV (14 genotypes) positive, but even more so by whether or not they are infected with HPV 16 and/or 18. Moreover, the absolute risk for CIN3+ in HPV 16/HPV 18 positive women ≥30 years with NILM in ATHENA (9.8%) is approximately the same as for the women ≥21 years with ASC-US who are hrHPV positive (14 genotypes). Since colposcopy is widely accepted as appropriate for hrHPV positive women with ASC-US, these findings support the 2006 American Society of Colposcopy and Cervical Pathology (ASCCP) Consensus Conference approved a strategy of incorporating HPV 16/18 genotyping when managing women ≥30 years with NILM cytology who are hrHPV positive. With this strategy, HPV 16/HPV 18 positive women with NILM are referred for immediate colposcopy, whereas those who are positive for the 12 other hrHPV genotypes undergo repeat testing with both cytology and hrHPV testing at 12 months[8].

Clinical Validation of cobas HPV Test as a First Line Cervical Cancer Screening Test for Women ≥25 Years

Using enrollment data from ATHENA, Castle *et al.* modeled how hrHPV testing and HPV 16/18 genotyping would perform as a primary screening test among women ≥25 years[37]. Enrolment screening test results alone and in combination are provided in **Table 7**.

In a post-hoc analysis, Castle *et al.* went on to assess different combinations of liquid-based cytology and genotyping for HPV 16 and/or 18 as potential triage tests for determining which hrHPV positive women should be referred to colposcopy, as seen in **Table 8**. This data clearly supports the use of genotyping for HPV 16/18 as a triage test for hr HPV positive women found when hrHPV testing is used as the primary cervical cancer-screening test. Using a combination of genotyping for HPV 16/18 together with cervical cytology to triage hrHPV positive women improves sensitivity over using either cytology or HPV genotyping alone and results in only a modest increase in the number of women referred to colposcopy and a modest reduction in the positive predictive value.



CPRP result	n
No sample at SV2	74
Invalid	180
≥CIN2	497
<CIN2	7,886

Figure 1. Flow of patients through the ATHENA trial. 47,208 women ≥21 years of age were enrolled into ATHENA. At Study Visit 1 (SV1) women had a speculum exam with samples obtained for liquid-based cytology and hrHPV testing using the first generation Roche HPV tests (Amplicor and Linear Array). Women with abnormal cervical cytology, those ≥25 years who were hrHPV positive, as well as a random subset of women ≥25 years who were both cytology and hrHPV negative were referred to Study Visit 2 (SV2). A total of 8,367 women were referred to SV2 at which time they underwent colposcopy. Click here to view larger figure.

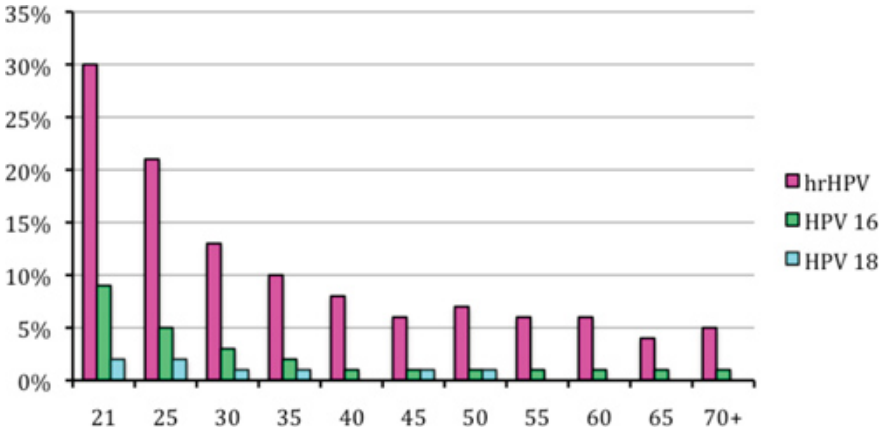


Figure 2 Prevalence of hrHPV in Women Undergoing Routine Cervical Cancer Screening in the U.S. In women ≥21 years undergoing routine screening in the U.S., the overall prevalence of the 14 types of hrHPV detected using cobas HPV Test was 13% and the overall prevalence of HPV-16, HPV-18 was 3.0% and 1%, respectively. The overall prevalence of hrHPV (14 types), as well as of HPV-16 and HPV-18 individually, decreased with increasing age.

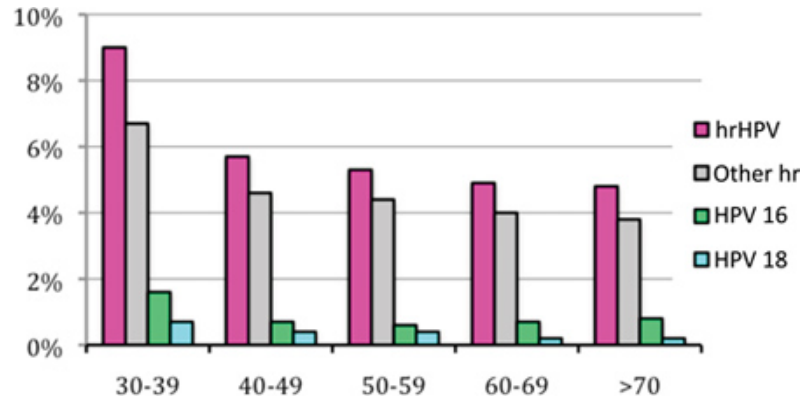


Figure 3. Prevalence of hrHPV in Women Aged 30 Years or Older with NILM Cytology. In women ≥30 years with NILM cytology, the overall prevalence of the 14 types of hrHPV detected using cobas HPV Test was 6.7% and the overall prevalence of HPV-16, HPV-18, and 12 other HR-HPV genotypes was 1.0%, 0.5%, and 5.2%, respectively. As was observed for both the entire population and in women with ASC-US, in the NILM population the overall prevalence of hrHPV (14 types), as well as HPV-16 and HPV-18 individually, decreased with increasing age.

Channel	Plasmid
Channel 1	*HPV 39 plasmid*
Channel 2	HPV 16 plasmid
Channel 3	HPV 18 plasmid
Channel 4	Beta-globin plasmid

Table 1. HPV positive control plasmids and their corresponding detection channels.

Channel	Target
Channel 1	*Other high risk HPV**
Channel 2	HPV type 16
Channel 3	HPV type 18
Channel 4	Human Beta-globin
*Other high risk HPV genotypes:	31,33,35,39,45,51,52,56,58, 59,66,68

Table 2. HPV targets and detection channels for the cobas HPV Test

Results	Interpretation
Other high risk HPV* NEG HPV 16 NEG HPV 18 NEG	Very low likelihood of underlying ≥ CIN2
Other high risk HPV* POS HPV 16 NEG HPV 18 NEG	Increased likelihood that underlying ≥ CIN2 will be detected at colposcopy
HPV 16 POS and/or HPV 18 POS	Highest likelihood that underlying ≥ CIN2 will be detected at colposcopy
*Other high risk HPV genotypes:	31,33,35,39,45,51,56,58,59,66,68

Table 3. Clinical significance of cobas HPV Test results

Characteristics	Eligible Subjects

	n = 46,887[b]
Age (Years)	
Mean ±(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	6,581 (14.0)
Asian	745 (1.6)
Other/Missing	263 (1.4)
Ethnicity, n (%)	
Hispanic or Latino	8,380 (17.9)
Post-Menopausal, n (%)	13,442 (28.7)
Immunocompromised or Immunosuppressed n (%)	258 (0.6)
History of Smoking Cigarettes, n (%)	
Present Smoker	7,145 (15.2)
Pap Test in Past 5 Years, n	42,462

Table 4. Demographic Data and Medical History for All Eligible Subjects*

	Final Biopsy Diagnosis			
	≥CIN2			
	cobas HPV test		**hc2**	
	%	95% CI	%	95% CI
Sensitivity	90%	82-95	87 (68/78a)	78-93
Specificity	71 (1056/1498)	68-73	71 (1056/1485b)	69-73
PPV	14 (72/514)	13-16	14 (68/497)	12-15
NPV	99 (1056/1064)	99-100	99 (1056/1066)	99-100
	≥CIN3			
Sensitivity	94 (43/46)	83-98	91% (42/46)	80-97
Specificity	69 (1061/1532)	67-72	70% 91062/1517)	68-73
PPV	8.4% (43/514)	8-9	9% (42/497	8-9
NPV	100% (1061/1064)	99-100	100% (1062/1066)	99-100

Modified from reference[34]

Table 5. Comparative performance of cobas HPV test and hc2 test in ASC-US. The sensitivity of cobas HPV Test was determined to be equivalent to that of Hybrid Capture 2 (hc2) in women with ASC-US. (PPV: positive predictive value; NPV: negative predictive value)

Estimated Absolute Risk, % (95% CI)		
cobas HPV Test Result	**CIN 2+**	**CIN 3+**
HR-HPV+	6.1 (4.9-7.2)	4.1 (3.1-5.0)
HPV-16/HPV-18+	11.4 (8.3-14.7)	9.8 (6.9-12.6)
HPV-16+	13.6 (9.5-18.0)	11.7 (7.9-15.8)
HPV-18+	7.0	5.7

	(2.9-11.9)	(1.7-9.9)
12 other HR-HPV+	4.6 (3.5-5.7)	2.4 (1.6-3.3)
HR-HPV-	0.8 (0.3-1.5)	0.3 (0.02-0.7)
Overall	1.2 (0.6-1.8)	0.5 (0.3-0.9)

Table 6. Impact of hrHPV Status on Estimated Absolute Risk of CIN in Women with NILM. Verification bias-adjusted estimates of the overall absolute risk among women ≥30 years or older with NILM cytology was dependent on hrHPV status, with HPV 16 positive women having the highest absolute risks.

Test Combination	% of Total (n=40,901)	% of CIN3 cases (n=254)	% of ACIS/Cancer (n=20)
LBC (+)	6%	52%	70%
hrHPV (+)	10%	92%	90%
LBC and hrHPV (-)	86%	4%	0
LBC (-) / hrHPV (+)	8%	44%	30%
LBC (+) / hrHPV (-)	4%	4%	10%
LBC (+) / hrHPV (+)	3%	48%	60%

Modified with permission from reference[37]

Table 7. Enrollment screening test results among women ≥25 years. Data from an enrollment screening test for hrHPV and HPV16/18 genotyping with or without concomitant LBC analysis in women ≥25 years. The results demonstrated that the sensitivity of cervical cytology for CIN3+ lesions is much lower than that of hrHPV testing and that co-testing appears to provide little benefit over hrHPV testing alone for screening, even in women as young as 25 years of age. (LBC: liquid-based cytology)

Test Combination (n=40,901)	Number to Colposcopy	Sensitivity CIN3+	Positive Predictive Value (PPV) for CIN3+
All hrHPV (+) women	3502	100%	7%
Cytology			
≥ASC-US	940	53%	14%
≥LSIL	564	40%	18%
Genotyping			
HPV 16	693	50%	18%
HPV 16 or 18	966	60%	16%
Combinations			
HPV 16 or ≥ASC-US	1380	75%	14%
HPV 16 or ≥LSIL	1089	68%	16%
HPV 16 or 18 or ≥ASC-US	1569	78%	13%
HPV 16 or 18 or ≥LSIL	1314	72%	14%

Modified with permission from reference[37]

Table 8. Performance of different triage tests for hrHPV positive women ≥25 years These data represent the different combinations of LBC and genotyping for HPV 16 and/or 18 that were assessed as potential triage tests for determining which hrHPV positive women ≥25 years should be referred to colposcopy. It was determined that using a combination of genotyping for HPV 16/18 together with cervical cytology to triage hrHPV positive women improves sensitivity over using either cytology or HPV genotyping alone. (LBC: liquid-based cytology; LSIL: low grade squamous epithelial lesion)

Discussion

ATHENA is the largest US cervical cancer screening trial that has ever been conducted. The trial was specifically designed to assess the performance of the new Roche cobas HPV test in women undergoing routine cervical cancer screening in the U.S. The Roche cobas HPV test uses PCR-based DNA amplification to identify 14 hrHPV genotypes and also provides integrated genotyping for HPV 16 and HPV 18. The results from the baseline cross-sectional phase of ATHENA clinically validate the use of the cobas HPV Test both as a triage test for women with ASC-US cytology results and as an adjunctive test to guide clinical management of women ≥30 years with NILM cytology results. In women with ASC-US, the cobas HPV Test performed similarly to hc2 in terms of both it's sensitivity for CIN2+ and CIN3+ as well as the proportion of

women with ASC-US found to be hrHPV positive and who would be referred to colposcopy if the test were utilized in routine clinical practice. Genotyping for HPV 16 and 18 in women with ASC-US was found to identify a subset of women at particularly high risk for having CIN 2+. One in four women with ASC-US who were HPV 16/18 positive were found to have biopsy-confirmed CIN 2+ compared to only 1 in 10 who had other (non-16/18) hrHPV genotypes.

In women ≥30 years with NILM cervical cytology, the cobas HPV test also was demonstrated to provide clinically useful information on risk of an occult CIN 2,3 lesion, adenocarcinoma in-situ, or even an invasive cervical cancer. In ATHENA, one in ten women with a NILM cytology result who were positive for HPV 16/18 with the cobas HPV test had an occult CIN 3+ lesion. Based on the extremely high absolute risks for CIN3+ observed in HPV 16 and/or 18 positive women with NILM cytology results, the ATHENA results provide support for the 2006 ASCCP management algorithm, which recommends colposcopy for HPV 16/18 positive women with NILM.

It is now widely acknowledged that cervical cancer screening that uses only hrHPV testing would be considerably more sensitive than cytology-based screening and would provide lead-time detection of CIN2,3 lesions[38]. Moreover, hrHPV testing allows the detection of glandular precursor lesions and adenocarcinomas of the cervix that are quite difficult to detect with cervical cytology[39]. Therefore it is of considerable interest that the findings from enrolment examinations of ATHENA demonstrate that hrHPV testing has a higher sensitivity than cervical cytology. The enrolment findings also suggest that hrHPV testing when used alone as the primary screening test will be a highly sensitive and efficient strategy for screening women ≥25 years. As has been demonstrated in other large screening studies, these results indicate that the sensitivity of cervical cytology for CIN3+ lesions is much lower than that of hrHPV testing[23,40-43]. In fact, the sensitivity of cervical cytology for CIN3+ found in ATHENA is almost identical to the performance of cervical cytology reported by older meta-analyses (approximately 50%), despite the fact that in ATHENA all cytology was liquid-based and processed in highly regulated U.S. cytology laboratories[10,11]. As has been found in several other large screening studies, the data from ATHENA show that cervical cytology adds little to the sensitivity of hrHPV testing for the detection of CIN3+[40,41,44]. Thus co-testing using both hrHPV testing and cervical cytology appears to provide little benefit over hrHPV testing alone for screening, even in women as young as 25 years of age.

Despite the potential benefits in terms of cost that would be achieved by using only hrHPV testing to screen, there remain considerable concerns as to how to best manage hrHPV positive women. The analysis of Castle *et al.* based on the enrolment examinations in ATHENA suggest that the most efficient way to triage hrHPV positive women would be to use a combination of HPV 16/18 genotyping and cervical cytology. As results from the 3-year follow-up phase of ATHENA become available, we will be able to better evaluate the merits of using the cobas HPV test alone as the primary screening test and of triaging hrHPV positive women using a combination of HPV 16/18 genotyping and cervical cytology.

From the laboratory perspective, the cobas HPV test has a number of advantages over other hrHPV assays that are currently available within the U.S. Using the cobas 4800 automated sample preparation, amplification and detection system it is possible to generate up to 94 results in approximately 4-5 hr. The automated nature of the system and its operating software facilitate instrument interfacing with a variety of laboratory information systems, providing additional opportunities for gains of efficiencies within laboratory workflow models. The nature of the cobas 4800 system and the cobas HPV Test lends itself to ease of uses and streamlined QC monitoring: the pre-aliquoting step significantly reduces opportunities for sample contamination during Pap slide preparation, while automated sample processing provides consistent DNA extraction and purification. Moreover, unlike the widely utilized hc2 hrHPV assay, the cobas HPV test includes an internal human Beta-globin control that gives confidence of a negative result in the setting of specimen types known to contain PCR inhibitory substances or those that demonstrate low cellularity. Also unlike hc2, the cobas HPV test does not have an "equivocal zone" which eliminates the requirement for retesting of samples whose results are considered "equivocal". This eliminates both the time and costs associated with the retesting samples. Since retesting is infrequently called for and the assay requires only 1 ml of residual ThinPrep fluid, use of the cobas HPV test will greatly reduce the frequency at which laboratories have to inform both clinicians and patients that a specimen is insufficient for testing.

cobas HPV Test Specifications

The cobas HPV Test is a qualitative *in vitro* test for the detection of the human Papillomavirus in patient specimens. The test, which can be used to screen up to 240 or 960 individual tests, contains a four component kit, as follows: a System Sample Preparation Kit, an HPV Amplification/ Detection Kit, an HPV Controls Kit, a System Liquid Cytology Preparation Kit, and a System Wash Buffer Kit.

The cobas HPV Test utilizes amplification of target DNA by PCR and nucleic acid hybridization for the detection of 14 hrHPV types in a single analysis. The test specifically identifies the HPV16 and HPV18 strains while concurrently detecting the rest of the high risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

When assessing the clinical data from the ATHENA Trial, the cobas HPV Test demonstrated acceptable sensitivity and specificity, meeting FDA required thresholds for performance. False positive and false negative results may occur with low frequency with screening assays, but frequently can be attributed to sample collection, processing, stability, and the subjective nature of correlation with cytology and biopsy interpretation.

Disclosures

Dr. Wright is a paid consultant to Roche Molecular Diagnostics and has spoken for Roche Molecular Diagnostics at national and international meetings.

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