

Detection of Human Papillomavirus 16, 18, and 45 in Women With ASC-US Cytology and the Risk of Cervical Precancer

Results From the CLEAR HPV Study

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ABSTRACT

Objectives: The Aptima human papillomavirus (HPV) 16 18/45 Genotype (GT) assay (AHPV-GT) is a qualitative E6/E7 oncogene messenger RNA test that detects HPV 16 and a pool of HPV 18 and 45. The CLEAR (Clinical Evaluation of APTIMA mRNA) study was the pivotal, prospective, multicenter US clinical study to validate the Aptima HPV (AHPV) assays.

Methods: In this analysis, we evaluated the clinical performance of AHPV and AHPV-GT assays for detection of cervical intraepithelial neoplasia grade 2 or more severe (CIN2+) and grade 3 (CIN3) or adenocarcinoma in situ in 912 women with atypical squamous cells of undetermined significance (ASC-US) Papanicolaou result. The AHPV-GT assay was performed on high-risk HPV (hrHPV) positives as determined by the AHPV assay.

Results: Overall, the percent positive for hrHPV was 38.8% (354/912), of which 34.2% (121/354) were GT positive. Among hrHPV-positive women, the risks of CIN2+ were 37.0% for HPV 16 positives, 15.9% for HPV 18/45 positives, 14.3% for other hrHPV positives, and 2.2% for AHPV negatives. The risks of CIN3+ were 20.5% for HPV 16 positives, 9.1% for HPV 18/45 positives, 4.3% for other hrHPV positives, and 0.7% for HPV negatives.

Conclusions: We demonstrated that AHPV-GT is a reliable and effective test for cervical cancer risk stratification in women with an ASC-US cytology diagnosis.

High-risk human papillomavirus (hrHPV) testing is now recommended for use with Papanicolaou (Pap) testing (“cotesting”) for routine cervical cancer screening¹ and for the management of women with atypical squamous cells of undetermined significance (ASC-US) Pap results, postcolposcopy surveillance, and as test of cure following excisional treatment.² There are currently four US Food and Drug Administration (FDA)–approved screening tests for hrHPV, including Hybrid Capture 2 (Qiagen, Gaithersburg, MD), Cervista HPV HR (Hologic, Bedford, MA), cobas4800 (Roche Molecular Systems, Pleasanton, CA), and Aptima HPV and Aptima HPV 16 18/45 Genotype (Hologic). The CLEAR (Clinical Evaluation of APTIMA mRNA) study was the pivotal, prospective, multicenter US clinical study to validate the Aptima HPV assay (AHPV), a qualitative E6/E7 oncogene messenger RNA (mRNA) test for a pool of 13 hrHPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and one possible hrHPV type (HPV 66). The CLEAR study led to FDA approval³ of AHPV as an adjunctive test for women with normal for intraepithelial lesion and malignancy Pap results and as a triage test for women with ASC-US Pap results.

The clinical performance of AHPV in ASC-US triage has been reported previously.⁴ For this analysis, samples from the CLEAR study with ASC-US cytology and positive AHPV results were reflex tested with the Aptima HPV 16 18/45 Genotype assay (AHPV-GT) for HPV 16, and a pool of HPV 18 and HPV 45 (HPV 18/45) for risk stratification. Here, we report the results of AHPV-GT testing in a population of women with ASC-US Pap results.

Materials and Methods

Study Design, Conduct, and Participants

The CLEAR clinical study consisted of two parts: the ASC-US study and the adjunct study (Figure 1). For the data presented here, eligible women invited to participate were 21 years or older who were undergoing routine Pap testing and who had an ASC-US cytology result. Women were recruited from 19 US family planning and obstetric/gynecologic clinics (private and academic), family practice medical groups, and clinical research centers encompassing a wide geographic area representative of the US population. Informed consent was obtained prior to enrollment of participants. The study protocol was approved by institutional review boards at the participating centers, and the study was conducted in accordance with applicable regulatory requirements and good clinical practices.

Women were excluded from the study if they were pregnant, reported prior vaccination against HPV, had a history of cervical disease (cancer or precancerous) or an abnormal (ASC-US, low-grade squamous intraepithelial lesion [LSIL], or worse) cytology test result in the previous 12 months, or had a history of illness that could interfere with the study or create an unacceptable risk to the participant. Demographic information and relevant medical information (cervical cancer history, prior HPV diagnosis, and any abnormal cytology history) were collected from each participant.

Cytology (Referral Pap)

Cervical specimens were collected with a broom-like device (Papette; Wallach Surgical Devices, Orange, CT) or an endocervical brush and spatula (Cytobrush Plus GT and Pap Perfect Plastic Spatula; Medscand, Trumbull, CT) and placed into a ThinPrep Pap test vial containing PreservCyt Solution ("referral Pap" specimen) (Hologic). Referral Pap specimens were processed locally using the ThinPrep 2000 System (Hologic) and evaluated for routine screening cytology. Cytology results were classified using the 2001 Bethesda System for reporting cervical cytology.⁵ Participants meeting study selection criteria with ASC-US cytology results were referred for a colposcopy examination (Figure 1).

Disease Ascertainment (Colposcopy and Biopsy/Consensus Histology)

Most colposcopy visits (>98%) were completed within 12 weeks from the initial visit (median, 4 weeks; interquartile range, 3 weeks). Colposcopists were masked to HPV results and were instructed to obtain four cervical punch biopsy specimens (one specimen from each of four quadrants) and an endocervical curettage (ECC) biopsy specimen. Quadrants with visible lesion(s) were biopsied

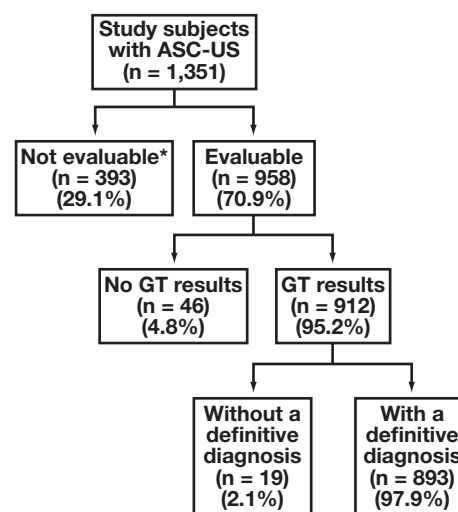


Figure 1 CONSORT diagram for women with an atypical squamous cells of undetermined significance (ASC-US) result enrolled in the CLEAR (Clinical Evaluation of APTIMA mRNA) trial and included in this analysis. *Not evaluable for the following reasons: younger than age 21 years (n = 99), did not meet eligibility criteria (n = 31), insufficient Papanicolaou (Pap) volume for Aptima human papillomavirus assay testing (n = 11), specimen expired or unsuitable for testing (n = 34), Pap specimen lost (n = 9), no colposcopy visit (n = 62), noncompliant study site (n = 142), patient participation terminated (n = 3), and other (n = 2). GT, genotype.

at the most severe area of any lesion; quadrant(s) without a visible lesion were biopsied at the squamocolumnar junction ("random biopsy"). Thus, each participant had five specimens for disease ascertainment. The biopsy specimens were processed according to each site's normal procedures to produce H&E-stained specimen slides. After local pathologist review, slides were reviewed by two central pathologists (M.H.S. and T.C.W.) using the three-tiered cervical intraepithelial neoplasia (CIN) terminology.⁶ Slides with discordant central diagnoses were reviewed by a third central pathologist to reach a consensus diagnosis (two of three agreements). If agreement was not achieved, the three panel pathologists reviewed the slides in conference to reach consensus. A patient's cervical disease status was based on the highest grade consensus histology result from colposcopy biopsy (clinical reference). Review pathologists were masked to all other pathologists' diagnoses, the punch biopsy method, the patients' clinical status, enrollment status (ASC-US or adjunct study), and HPV test results.

HPV Testing

After the colposcopy visit was completed, PreservCyt specimens (1-mL aliquot) were tested with the AHPV and the AHPV-GT (both from Hologic) on the automated Tigris

DTS System (Hologic). AHPV and AHPV-GT are isothermal target amplification assays that use transcription-mediated amplification to detect mRNAs transcribed from the E6/E7 oncogenes of hrHPV genotypes. AHPV detects mRNA qualitatively from HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. AHPV-GT uses the same technology as AHPV for detection of mRNA from hrHPV genotypes 16, 18, and 45; the assay differentiates genotype 16 from 18 and 45 but does not differentiate between 18 and 45. Samples positive with AHPV were reflex tested with AHPV-GT, volume permitting, under a separate protocol. Three clinical laboratories each tested approximately one-third of all samples with the Aptima tests. Testing and results interpretation of both Aptima HPV tests were done according to the manufacturer's instructions. These results were not used for clinical decisions for the management of women with ASC-US cytology enrolled in CLEAR.

Statistical Analysis

Patients with positive AHPV results (hrHPV positive) had further testing with AHPV-GT and were categorized as GT positive if they had positive AHPV-GT results and other 11 hrHPV (HPV types 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68) positive if they had negative AHPV-GT results. For some analyses, patients were further categorized based on the presence of genotype 16 and/or genotypes 18/45. Patients with negative AHPV results did not have further testing and were categorized as hrHPV negative.

For CIN grade 2 (CIN2) or more severe (CIN2+) risk calculations, patients with a consensus histology result of CIN2+ were classified positive for cervical disease, and those with CIN grade 1 (CIN1) or normal consensus histology results were classified negative for cervical disease. For CIN grade 3 (CIN3) or more severe (CIN3+) risk calculations, patients with a consensus histology result of CIN3 or CIN3+ were classified positive for cervical disease, and those with CIN2, CIN1, or normal histology were classified negative for cervical disease. Patients who attended the colposcopy visit were classified as indeterminate for cervical disease status if biopsy specimens were not collected or were lost or if the slides were inadequate to determine disease status.

General sociodemographics were described. The impact of age group (21-29, 30-39, and 40 years and older) on percent test positive for hrHPV, GT, other 11 hrHPV, and the individual 16 or 18/45 genotypes by age group was tested for significance using a nonparametric test of trend.⁷ The relationship of percent test positive by testing sites was tested for significance using either Pearson χ^2 or Fisher exact test. The absolute risks, relative risks, and positive likelihood ratios with 95% confidence intervals (CIs) for CIN2+ and CIN3+ were calculated for all women and stratified by age group (21-24, 25-29, and 30 years and older).

Results

Of the 958 women with an ASC-US Pap result enrolled into the CLEAR trial who had evaluable AHPV results, 912 (95.2%) also had AHPV-GT results available and defined the analytic group. The mean, median, and range of ages of the 912 women included in this analysis were 34.2, 32, and 21 to 85 years, respectively; women who were hrHPV-positive ASC-US were younger than women who were hrHPV-negative ASC-US (median age, 29.5 years vs 37.1 years, respectively; $P < .001$). The distribution for race/ethnicity was 46.7% white (non-Hispanic), 21.9% black, 11.7% white (Hispanic), and 20.1% other or unknown. Most women had never smoked (58.7%), had at least one live birth (63.6%), and had ever used oral contraceptives (76.2%) (data not shown).

Table 1 reports the hrHPV and GT results overall, stratified by age, and stratified by site in the 912 women with ASC-US included in this analysis. The percent hrHPV positive was 38.8% overall and decreased in older age groups: 57.0% for 21 to 29 years to 16.7% for 40 years and older ($P_{\text{trend}} < .001$). The percent hrHPV positive did not vary significantly by testing site.

While the percentage of GT positive among hrHPV positives did not vary greatly by age (32.7% for 21-29 years, 37.1% for 30-39 years, and 35.6% for ≥ 40 years), GT-positive women aged 21 to 29 years were more likely to be HPV 16 positive, while women 30 years and older were more likely to be HPV 18/45 positive (odds ratio, 3.8; 95% CI, 1.6-8.8). By comparison, there was little variation in the percent GT positive among hrHPV positives and for which HPV type (16 or 18/45) by site. It was rare to test positive for both HPV 16 and HPV 18/45 ($n = 3$).

The percent positive for GT, other 11 hrHPV, individual 16 or 18/45 HPV genotypes, and hrHPV positive as well as the percent hrHPV negative with increasing severity of histologically confirmed diagnoses are shown in **Table 2**. Among hrHPV positives, the high-grade disease (CIN2+) found among GT positive was marginally more likely to be CIN3/adenocarcinoma in situ (AIS) than among other 11 hrHPV positives (55.8% vs 30.3%, respectively; $P = .05$). Fifteen of the 33 (45.4%) CIN3/AIS and 12 of the 46 CIN2 (26.1%) were HPV 16 positive.

Of the 893 (93.2%) of 958 women who had a certain histologic diagnosis, absolute risk, relative risk, and positive likelihood ratio were calculated for CIN2+ **Table 3** and CIN3/AIS **Table 4**. Women who tested HPV 16 positive were at the highest absolute risk of CIN2+ (37.0%) and CIN3/AIS (20.5%). Women who were GT positive were at 29.1% risk of CIN2+ and 16.2% risk of CIN3/AIS, which was significantly higher than the risk of CIN2+ (14.3%, $P = .001$) and risk of CIN3/AIS (4.3%, $P < .001$) among women who were positive for other 11 hrHPVs.

Table 1
Percent Positive Results for 912 Women With an ASC-US Papanicolaou Result Enrolled in the CLEAR Trial^a

Characteristic	Genotype Status			Genotype Positive		
	hrHPV+	GT- (16/18/45-)	GT+ (16/18/45+)	16+, 18/45-	16-, 18/45+	16+, 18/45+
Overall (N = 912)						
n (positive)	354	233	121	71	47	3
% Positive	38.8	25.5	13.3	7.8	5.2	0.3
% of hrHPV positive	—	65.8	34.2	20.1	13.3	0.8
Age group, y						
21-29 (n = 386)						
n (positive)	220	148	72	51	19	2
% Positive	57.0	38.3	18.7	13.2	4.9	0.5
% of hrHPV positive	—	67.3	32.7	23.2	8.6	0.9
30-39 (n = 257)						
n (positive)	89	56	33	14	18	1
% Positive	34.6	21.8	12.8	5.4	7.0	0.4
% of hrHPV positive	—	62.9	37.1	15.7	20.2	1.1
≥40 (n = 269)						
n (positive)	45	29	16	6	10	0
% Positive	16.7	10.8	5.9	2.2	3.7	0.0
% of hrHPV positive	—	64.4	35.6	13.3	22.2	0.0
<i>P</i> _{trend} (all women)	<.001	<.001	<.001	<.001	.658	.273
<i>P</i> _{trend} (hrHPV women)	—	.496	.496	.059	.001	.760
Testing site						
Site 1 (n = 301)						
n (positive)	117	75	42	27	13	2
% Positive	38.9	24.9	14.0	9.0	4.3	0.7
% of hrHPV positive	—	64.1	35.9	23.1	11.1	1.7
Site 2 (n = 310)						
n (positive)	124	82	42	23	19	0
% Positive	40.0	26.5	13.5	7.4	6.1	0.0
% of hrHPV positive	—	66.1	33.9	18.5	15.3	0.0
Site 3 (n = 301)						
n (positive)	113	76	37	21	15	1
% Positive	37.5	25.2	12.3	7.0	5.0	0.3
% of hrHPV positive	—	67.3	32.7	18.6	13.3	0.9
<i>P</i> (all women)	.823	.914	.835	.637	.598	.326
<i>P</i> (hrHPV women)	—	.874	.874	.621	.630	.422

ASC-US, atypical squamous cells of undetermined significance; CLEAR, Clinical Evaluation of APTIMA mRNA; GT, genotype; hrHPV, high-risk human papillomavirus; —, not calculated.

^a The results are shown for all patients and stratified by age group and by testing site. The percent positives for each category overall (% Positive) and among hrHPV positive (% of hrHPV positive) are shown. A nonparametric test of trend was used to test for age trends. Fisher exact test was used to test for differences across testing sites.

Finally, the absolute risks of CIN2+ and CIN3/AIS by HPV status for each age group (21-24, 25-29, and 30 years and older) is shown in **Figure 2**. For all age groups, testing GT positive denoted a subgroup of women who were at higher risk of CIN3/AIS than those who tested other 11 hrHPV positive (16.7% vs 4.8%, *P* = .04 for 21-24 years; 14.3% vs 6.3%, *P* = .2 for 25-29 years; and 17.4% vs 2.4%, *P* = .004 for 30 years and older). Similar differences were noted for CIN2+ except for women aged 25 to 29 years, in whom there was a higher proportion of CIN2 than in other subgroups.

Discussion

Here we present the first report on performance of the AHPV-GT assay for detection of HPV 16 and HPV 18/45.

Our results demonstrate that the AHPV-GT assay has utility in stratifying low and high risk of CIN2+ and CIN3/AIS among women with hrHPV-positive ASC-US. While HPV 18/45 testing was no more predictive for CIN2 than the 11 other hrHPV types, it detected one-fourth of the CIN3/AIS cases in this study, with a relative risk approximately twice that seen for the other 11 hrHPV types for this end point. Notably, among those subjects with CIN2+, detection of both HPV 16 and 18/45 identified proportionally more CIN3/AIS cases than CIN2 cases, compared with detection of other hrHPV genotypes. In addition, detection of HPV 16 correlated well with the highest relative risk for CIN3/AIS lesions compared with detection of all other HPV genotypes.

The general patterns of risk stratification by the AHPV-GT assay in the CLEAR population were similar to those observed in the National Cancer Institute-sponsored

Table 2
Distribution of Histopathologic Diagnoses for 912 Women With an ASC-US Papanicolaou Result Enrolled in the CLEAR Trial by hrHPV Status^a

	Total No.	No. (Row %)				
		Undetermined	Negative	CIN1	CIN2	CIN3/AIS
HPV 16+	74	1 (1.4)	28 (37.8)	18 (24.3)	12 (16.2)	15 (20.3)
HPV 18/45+	47	3 (6.4)	23 (48.9)	14 (29.8)	3 (6.4)	4 (8.5)
HPV 16/18/45+ (GT+)	121	4 (3.3)	51 (42.1)	32 (26.4)	15 (12.4)	19 (15.7)
Other hrHPV+ (GT-)	233	2 (0.9)	125 (53.6)	73 (31.3)	23 (9.9)	10 (4.3)
hrHPV+	354	6 (1.7)	176 (49.7)	105 (29.7)	38 (10.7)	29 (8.2)
hrHPV-	558	13 (2.3)	458 (82.1)	75 (13.4)	8 (1.4)	4 (0.7)
Total	912	19 (2.1)	634 (69.5)	180 (19.7)	46 (5.0)	33 (3.6)

AIS, adenocarcinoma in situ; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; CLEAR, Clinical Evaluation of APTIMA mRNA; GT, genotype; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus.

^a Includes HPV 16 positive (with or without HPV 18 and/or HPV 45), HPV 18 and/or HPV 45 (HPV 18/45) positive, HPV 16 and/or HPV 18 and/or 45 (HPV16/18/45) or GT positive, GT negative but hrHPV positive, or hrHPV negative.

Table 3
Absolute Risk and RR for CIN2+ for 893 Women With an ASC-US Papanicolaou Result Enrolled in the CLEAR Trial by hrHPV Status

Characteristic	Total, No. (Column %)	CIN2+, No. (Column %)	Absolute Risk, % (95% CI)	RR, Value (95% CI)
HPV 16+	73 (8.2)	27 (34.2)	37.0 (27.5-46.9)	16.8 (8.9-31.7)
HPV 18/45+	44 (4.9)	7 (8.9)	15.9 (7.2-28.3)	7.2 (3.0-17.4)
HPV 16/18/45+ (GT+)	117 (13.1)	34 (43.0)	29.1 (22.4-36.0)	13.2 (7.0-24.7)
Other hrHPV+ (GT-)	231 (25.9)	33 (41.8)	14.3 (10.9-17.9)	6.5 (3.4-12.3)
hrHPV+	348 (39.0)	67 (84.8)	19.3 (17.1-21.3)	8.7 (4.8-15.9)
hrHPV-	545 (61.0)	12 (15.2)	2.2 (1.2- 3.5)	1 [reference]
Total	893 (100)	79 (100)	8.8 (7.2-10.9)	—

ASC-US, atypical squamous cells of undetermined significance; CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or more severe; CLEAR, Clinical Evaluation of APTIMA mRNA; GT, genotype; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; RR, relative risk; —, not calculated.

^a Includes HPV 16 positive (with or without HPV 18 and/or HPV 45), HPV 18 and/or HPV 45 (HPV 18/45) positive, HPV 16 and/or HPV 18 and/or 45 (HPV16/18/45) or GT positive, GT negative but hrHPV positive, or hrHPV negative.

Table 4
Absolute Risks and RR for CIN3+ or Adenocarcinoma In Situ for 893 Women With an ASC-US Papanicolaou Result Enrolled in the CLEAR Trial by hrHPV Status

Characteristic	Total, No. (Column %)	CIN3+, No. (Column %)	Absolute Risk, % (95% CI)	RR, Value (95% CI)
HPV 16+	73 (8.2)	15 (45.5)	20.5 (13.3-28.3)	28.0 (9.6-82.1)
HPV 18/45+	44 (4.9)	4 (12.1)	9.1 (2.9-19.5)	12.4 (3.2-47.8)
HPV 16/18/45+ (GT+)	117 (13.1)	19 (57.6)	16.2 (11.4-21.1)	22.1 (7.7-63.8)
Other hrHPV+ (GT-)	231 (25.9)	10 (30.3)	4.3 (2.4-6.8)	5.9 (1.9-18.6)
hrHPV+	348 (39.0)	29 (87.9)	8.3 (6.9-9.4)	11.4 (4.0-32.0)
hrHPV-	545 (61.0)	4 (12.1)	0.7 (0.2-1.6)	1 [reference]
Total	893 (100)	33 (100)	3.7 (2.6-5.1)	—

ASC-US, atypical squamous cells of undetermined significance; CI, confidence interval; CIN3+, cervical intraepithelial neoplasia grade 3 or more severe; CLEAR, Clinical Evaluation of APTIMA mRNA; GT, genotype; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; RR, relative risk; —, not calculated.

^a Includes HPV 16 positive (with or without HPV 18 and/or HPV 45), HPV 18 and/or HPV 45 (HPV 18/45) positive, HPV 16 and/or HPV 18 and/or 45 (HPV 16/18/45) or GT positive, GT negative but hrHPV positive, or hrHPV negative.

ASC-US LSIL Triage Study (ALTS), although the absolute and relative risks found in CLEAR were somewhat less than those in the ALTS.⁸ There may be several possible explanations for these differences. First, the ALTS was conducted 15 years ago,⁹ and the prevalence of CIN2/3 over the intervening time may have decreased. Second, the ALTS analysis of risk stratification by HPV 16 included the cumulative 2-year incidence of CIN2+⁸ and CIN3/AIS, whereas the CLEAR analysis only considers disease found at the enrollment period. Differences in total disease ascertainment may result in differences in risk. Finally, women in the ALTS

were enrolled with ASC-US, a cytologic diagnosis based on the 1992 Bethesda System¹⁰ that was inclusive of the higher risk diagnosis of atypical squamous cells—cannot rule out high-grade squamous intraepithelial lesion (ASC-H). Women were enrolled in CLEAR with ASC-US, as defined by the 2001 Bethesda System,⁵ which does not include ASC-H.

We also compared the CLEAR results with a more contemporary clinical trial for ASC-US triage, cobas HPV 4800 testing in ATHENA (Addressing the Need for Advanced HPV Diagnostics).¹¹ The cobas HPV 4800 tests separately

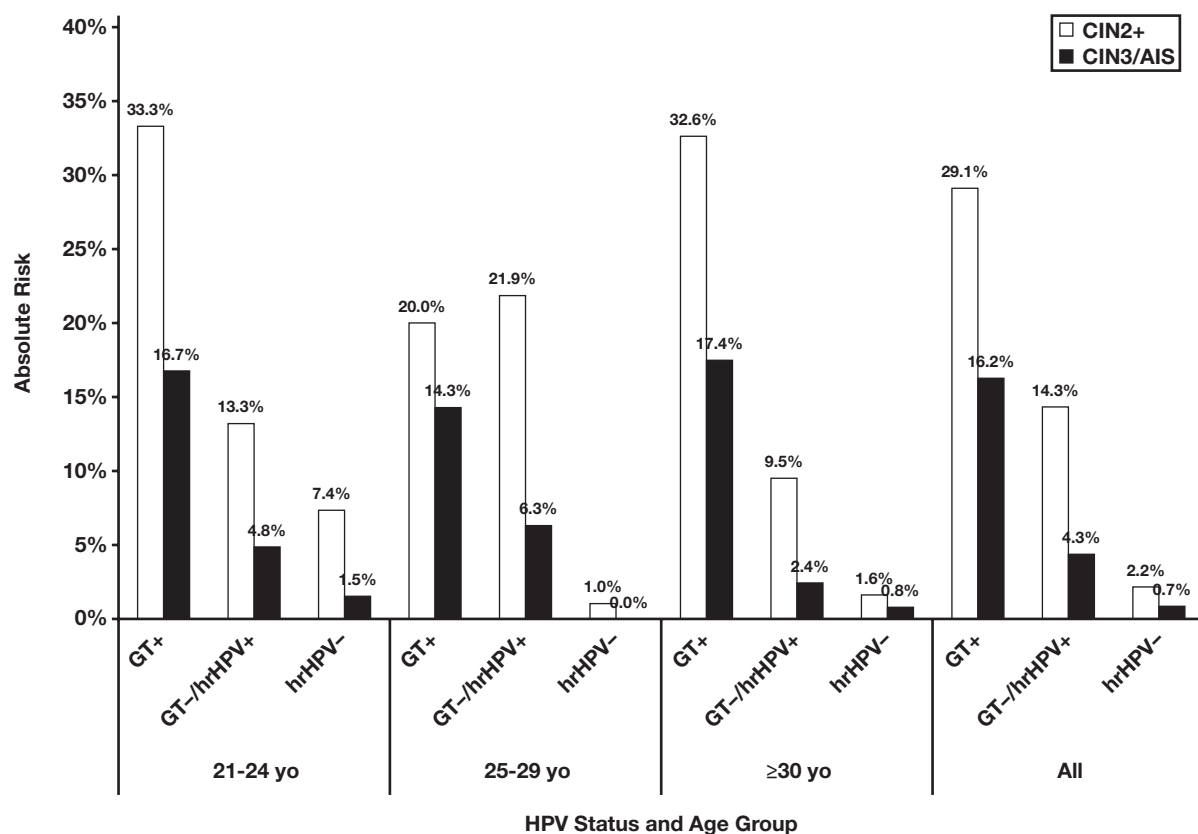


Figure 2 Absolute risks of cervical intraepithelial neoplasia grade 2 (CIN2) or more severe (CIN2+) and cervical intraepithelial neoplasia grade 3 (CIN3) or adenocarcinoma in situ (AIS) (CIN3/AIS) among 893 women with an atypical squamous cells of undetermined significance Papanicolaou result by high-risk human papillomavirus (hrHPV) status: positive for HPV 16, 18, and/or 45 genotype (GT); GT negative but positive for other hrHPV; or hrHPV negative.

for HPV 16 and HPV 18, whereas the AHPV-GT enables pooled testing for HPV 18/45. HPV 45, genetically closely related to HPV 18, is the HPV genotype that causes the third most cervical cancer¹² and is found in precursors of cervical cancer with a pattern that most resembles that caused by HPV 18, albeit less commonly.¹³ The inclusion of HPV 45 with HPV 18 should increase the sensitivity for disease detection while slightly decreasing the positive predictive value. As shown in Supplemental Table 1 (available at <http://www.ascp.org/docs/default-source/pdf/press/castlefeb15.pdf>), the absolute risks for CIN2+ observed in CLEAR were greater than those in ATHENA. The differences in the absolute risks are not likely attributable to interpathologist variability since the same pathologists reviewed the histology in both studies. And given that both trials had many clinical sites throughout the United States, it also seems unlikely that there were significant differences in the underlying risk of CIN2/3 in the two study populations.

Instead, the absolute risk differences are likely due to differences in disease ascertainment between the studies, resulting in higher population prevalence of CIN2+ in CLEAR vs ATHENA (9% vs 5%, respectively). In CLEAR,

a four-quadrant biopsy with ECC was performed, with random biopsy specimens obtained in quadrants with no visible lesions at colposcopy, while ATHENA relied chiefly on directed biopsy of visible lesions with only a random biopsy if no lesions were visible.¹¹ If analysis of CIN2+ ascertainment in CLEAR is limited to disease identified from directed biopsies only (Supplemental Table 1), the prevalence of CIN2+ decreases to 6.4%, and the absolute risk results between the two studies become very similar. Specifically, the absolute risk of CIN2+ among hrHPV-negative women in CLEAR decreased from 2.2% using combined random/ECC and directed biopsy results to 0.7% using directed biopsy results alone, close to the value of 0.8% obtained in ATHENA for this end point.

Finally, the finding of low risk of CIN3 among women with ASC-US who are positive for other 11 hrHPV and negative for HPV 16 and HPV 18 or HPV 18/45 raises two considerations. First, these data highlight the potential challenges of screening populations of women who have been previously vaccinated against HPV 16 and HPV 18. Following HPV 16 and HPV 18 vaccination, the positive predictive values of hrHPV-positive ASC-US for CIN2+ and CIN3 are

predicted to decrease approximately 25% and 50%, respectively, so that only one in 14 referred to colposcopy will have CIN2+ and one in 25 will have CIN3. It will be critical to introduce new, more specific biomarkers or algorithms to minimize the harms of screening in these lower risk populations. Second, these results raise the question of whether some of these women, especially younger women who are still intending to have children, might benefit from clinical management involving repeat testing in a year rather than being referred to colposcopy. There is some evidence from a meta-analysis to suggest that excision treatment increases the risk of preterm delivery,¹⁴ although a recent report did not confirm this association.¹⁵ Current management guidelines² recommend that women aged 21 to 24 years with an hrHPV-positive ASC-US diagnosis undergo repeat Pap testing in a year because of the low risk of cancer and the high likelihood of a transient HPV infection. Thus, women aged 25 to 29 years with hrHPV-positive ASC-US and negative for the riskiest HPV genotypes would be expected to have a similarly low risk of cervical cancer,¹⁶ and many of the HPV infections would be expected to clear in a year. Future studies focusing on the relative benefits of employing newer HPV tests with improved specificity for detection of CIN3 may provide clarity as to the most effective cervical cancer screening strategy in younger women.

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CLEAR Study Central Pathology Review Panel: Thomas C. Wright Jr, MD, Department of Pathology, Columbia University School of Medicine, New York, NY; Mark Stoler, MD, Department of Pathology, University of Virginia Health System, Charlottesville, VA; and Alex Ferenczy MD, BioVision, Outremont, Quebec, Canada.

The CLEAR study testing sites and participants are as follows: laboratory testing sites: B. A. Body, Laboratory Corporation of America, Burlington, NC; B. Yen-Lieberman, Cleveland Clinic Foundation, Cleveland, OH; C. Ginocchio, North Shore Long Island Jewish Health System Laboratory, Lake Success, NY; and T. Davis, Eskenazi Health Services, Indianapolis, IN.

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