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Risk detection for high-grade cervical disease using Onclarity HPV extended genotyping in women, ≥21 years of age, with ASC-US or LSIL cytology

Thomas C. Wright Jr [a,*], Mark H. Stoler [b], Valentin Parvu [c], Karen Yanson [c], Charles Cooper [c], Jeffrey Andrews [c]

[a] Columbia University, New York, NY 10032, USA
[b] University of Virginia Health System, Charlottesville, VA 22908, USA
[c] Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, 7 Loveton Circle, Sparks, MD 21152, USA

HIGHLIGHTS

- HPV genotyping stratifies risk of ≥CIN2 or ≥CIN3 in women ≥21 years with ASC-US or LSIL cytology.
- Women with HPV 16 had the greatest risk of ≥CIN2 or ≥CIN3 which is followed by those with HPV 31 in most instances.
- HPV genotypes such as 51, 35/39/68, and 56/59/66 have a lower risk of ≥CIN2 or ≥CIN3 and may not require colposcopy.
- These results support the use of HPV genotyping in risk-based management algorithms for women with either ASC-US or LSIL

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ABSTRACT

Objectives. There is growing interest in using human papillomavirus (HPV) genotyping as a risk-based triage approach for women with atypical squamous cells-undetermined significance (ASC-US) and low-grade squamous intraepithelial lesions (LSIL) cytology.

Methods. This analysis includes 2807 subjects with ASC-US or LSIL cytology, ≥21 years, from the baseline phase of the Onclarity HPV trial. All women were referred to colposcopy/biopsy. Hierarchical-ranked prevalence and risk values, associated with high-grade cervical disease, were calculated based on extended genotyping.

Results. HPV 16 carried the highest risk for cervical intraepithelial neoplasia grade 2 or worse (≥CIN2) in both the ASC-US and LSIL populations. Risk of ≥CIN3 and ≥CIN2 associated with the other 13 genotypes varied somewhat for women with ASC-US and LSIL, however, HPV 31, 18, 33/58, 51 and 52 appear to comprise an intermediate risk band. Risk associated with HPV 35/39/68, 45, and 56/59/66, in either cytology population, was relatively low and beneath the benchmark threshold risk for immediate colposcopy. Restricting the analysis to women 21–24 years, ≥25 years, or ≥30 years produced similar results.

Conclusions. HPV genotyping identified multiple risk bands for ≥CIN3 and ≥CIN2 in the ≥21 year-old ASC-US and LSIL populations. These results support a 1-year follow-up period to preclude immediate colposcopy for ASC-US or LSIL women positive for the lowest-risk HPV genotypes.

1. Introduction

Atypical squamous cells of undetermined significance (ASC-US) is reported in approximately 5% of all women in the United States (U.S.) undergoing cytologic screening [1]. Pooled high-risk human papillomavirus (HPV) testing is routinely utilized to risk-stratify (i.e., "triage") women with ASC-US into two groups. HPV positive women with ASC-US are referred to colposcopy whereas HPV negative women are followed-up with repeat cytology and HPV testing in three years [2]. This approach reduces the number of colposcopies that are performed by approximately 50%, however, most HPV positive women with ASC-US who are referred to colposcopy do not have ≥CIN3. Five U.S. regulatory studies of different HPV diagnostic tests have reported that risk stratification of women with ASC-US by HPV testing (pooled high-risk genotypes) refers 32.6%–57.1% to colposcopy and identifies 87.5%–100% of ≥CIN3 lesions [4–8]. The positive predictive value for ≥CIN3 of HPV testing (pooled high-risk genotypes) ranged from 2.9%–9.4%. Therefore, there is a clinical need to develop alternative risk-

* Correspondence to: T.C. Wright, Jr, Department of Pathology, Room 16-404 P&S Bld, Columbia University, 630 West 168 Street, New York, NY 10032, USA.
E-mail address: tcw1@columbia.edu (T.C. Wright).

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stratification approaches that would further reduce the number of unnecessary colposcopy exams for women with ASC-US while ensuring that most women with ≥CIN3 receive colposcopy. Because there are such wide differences in risk of ≥CIN3 associated with the individual high-risk HPV genotypes, one potential alternative risk-stratification approach would be to refer only women with selected high-risk HPV genotypes to colposcopy [9–11].

An additional 2.5% of all cytologic results in the U.S. are low-grade squamous intraepithelial lesion (LSIL) [1]. The prevalence of HPV (pooled high-risk) is much higher in women with LSIL than in those with ASC-US. Eighty-one (81) percent of LSIL women, 30–65 years of age, in Kaiser Permanente Northern California (KPNC) were HPV positive [12]. A similar prevalence (83%) was found in a somewhat younger cohort of women with LSIL in the National Cancer Institute's ASC-US/LSIL Triage Study (ALTS) [13]. Because of the high-prevalence of HPV (pooled high-risk genotypes) in women with LSIL it is not recommended that women with LSIL undergo "reflex" HPV testing and if ≥25 years colposcopy is recommended. [2] The 5-year cumulative risk of ≥CIN3 was only 5.2% in women 30 years and older with LSIL in KPNC [12]. Therefore as with HPV positive ASC-US, most women with LSIL who undergo colposcopy do not have high-grade neoplasia and there is a clinical need to develop risk-stratification approaches that reduce the number colposcopy exams for women with LSIL while ensuring that most women with ≥CIN3 receive colposcopy.

In this study we analyze data from the baseline phase of the Onclarity clinical trial to investigate whether the use of specific HPV genotyping information could provide improved risk-stratification for women with ASC-US or LSIL cytology. In order to evaluate the efficacy of genotyping-based risk-stratification, the baseline risk values for ≥CIN3 and ≥CIN2, obtained using the Onclarity assay, were compared with the consensus benchmark risk values for referral to colposcopy that have been previously established by the American Society of Colposcopy and Cervical Pathology (ASCCP) Consensus Guidelines [2].

2. Materials and methods

2.1. Study design and enrollment visit

The design of the Onclarity HPV trial has been described previously in depth [8,15,16]. In brief, 33,858 subjects, ≥21 years, undergoing routine cervical cancer screening were recruited at 31 clinical sites in 17 states. Sample size was determined by the number of subjects needed to obtain ≥70 subjects with ≥CIN2 histology from the ASC-US population. Cervical samples were collected in a SurePath™ vial (TriPath Imaging, Inc., Burlington, NC) and were used for both cervical cytology and HPV testing with the BD Onclarity™ HPV assay (Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, Sparks, MD), which is a real-time, multiplex PCR assay that detects 14 high-risk HPV genotypes [17]. The assay results include individual genotyping results for 16, 18, 31, 45, 51, and 52. The remaining HPV types are pooled into three groups: 33/58, 35/39/68 and 56/59/66. The specific details of the assay have been described previously [18]. Cytology is reported here according to the 2001 Bethesda System [19]. Computer-assisted imaging was not used to evaluate cytology. Henceforth in this report, HPV refers to pooled, high-risk HPV genotypes—unless otherwise noted.

2.2. Colposcopy visit

There were 2816 subjects, ≥21 years, with ASC-US or LSIL cytology results at enrollment, of which, 9 were excluded due to non-evaluable Onclarity results, 312 were lost to follow-up, 135 withdrew consent, 33 were pregnant, 5 had a hysterectomy, 5 were excluded due to a specimen collection error, and 1 was removed due to decision by the Principal Investigator. All others were referred for colposcopy with biopsy. 2316 subjects with ASC-US or LSIL underwent colposcopy with 5 excluded from this analysis due to unsatisfactory histopathology—leaving 2311 subjects with evaluable cytology and HPV results for these analyses. Women referred for colposcopy had to undergo the procedure within 84 days of enrollment, as previously reported [15]. Colposcopists were blinded to cytology and HPV results; biopsy specimens included any lesion or acetowhite area and an endocervical curettage (ECC). A random biopsy was performed at the squamocolumnar junction and an ECC was collected in the absence of lesions or acetowhite areas. Biopsies were evaluated using a consensus pathology review procedure with 3 gynecologic pathologists (2 of the pathologists were authors—TCW and MHS) who were masked to all study information except patient age as previously described [15]. A three-tiered CIN terminology was employed during histology adjudication [20]. When at least one pathologist diagnosed a specimen as CIN2, or when one pathologist diagnosed a specimen as ≥CIN2, with a second pathologist scoring the same specimen as <CIN2, $p16^{INKK4A}$ immunostaining was employed to adjudicate a final diagnosis.

2.3. Statistical analysis

Prevalence estimates were restricted to subjects with key demographic information, evaluable liquid-based cytology results, and valid HPV assay results for all genotypes. For risk determination, values were determined hierarchically using an order established previously by Stoler and colleagues [16]. In that report, a Bayesian model (derived by Markov-Chain Monte Carlo methods) was utilized to hierarchically rank the nine different genotype Onclarity channels based on ≥CIN3 baseline risk (for subjects with co-infections, the model assigned the ≥CIN3 risk as equal to the highest-risk genotype). Uninformative prior uniform distributions were used for estimation of all risk values. The established order there (HPV 16, else 31, else 18, else 33/58, else 52, else 45, else 35/39/68, else 51, else 56/59/66) was used in all analyses in this study to maintain consistency across extended genotyping result reporting from the Onclarity HPV trial. When the Bayesian model is restricted to specific cytologic groups such as NILM or ASC-US/LSIL some subtle reshuffling of risk hierarchy does occur. However, HPV 16 always has the highest risk, followed by HPV 31, and then HPV 18. The lower 3 genotypes (HPV56/59/66) always have a lower risk than any other genotype, regardless of cytology. Fig. 1 provides the Bayesian model results restricted to ASC-US/LSIL subjects. Simple proportions were used to generate risk values using standard statistical methods to generate 95% confidence intervals (CI). Data analyses were accomplished using SAS/STAT® and R software.

3. Results

Table 1 reports the demographics and histologic outcomes of the 1953 subjects with ASC-US and 854 with LSIL included in this analysis. The mean and median ages of the subjects with ASC-US were somewhat higher than those with LSIL. HPV vaccination (self-reported) was more common in subjects with ASC-US. As expected, histologic CIN1 and CIN2 were more prevalent in subjects with LSIL compared to those with ASC-US, as was ≥CIN3. The prevalence of CIN2 was 4.3% and 7.8% in subjects with ASC-US and LSIL having a colposcopy, respectively; and the prevalence of ≥CIN3 for subjects having a colposcopy from the two cytology groups was 2.2% and 3.1%, respectively.

HPV genotyping results by histologic outcome for subjects with ASC-US and LSIL are shown for subjects ≥21 years in Table 2. As expected, the prevalence of HPV in subjects with <CIN2 was more than twice that for LSIL compared to ASC-US. In contrast, the prevalence of HPV was similar for subjects with CIN2 and ≥CIN3 in both LSIL and ASC-US. The relative prevalence of the specific genotypes by histologic outcome was also similar for ASC-US and LSIL. The prevalence of HPV 16 increased with higher grades of disease in both cytology groups and over 50% of ≥CIN3 lesions were associated with HPV 16. HPV 31 was the second most prevalent genotype detected in ≥CIN3 lesions in the LSIL group and was tied with HPV 52 as the second-most prevalent genotype

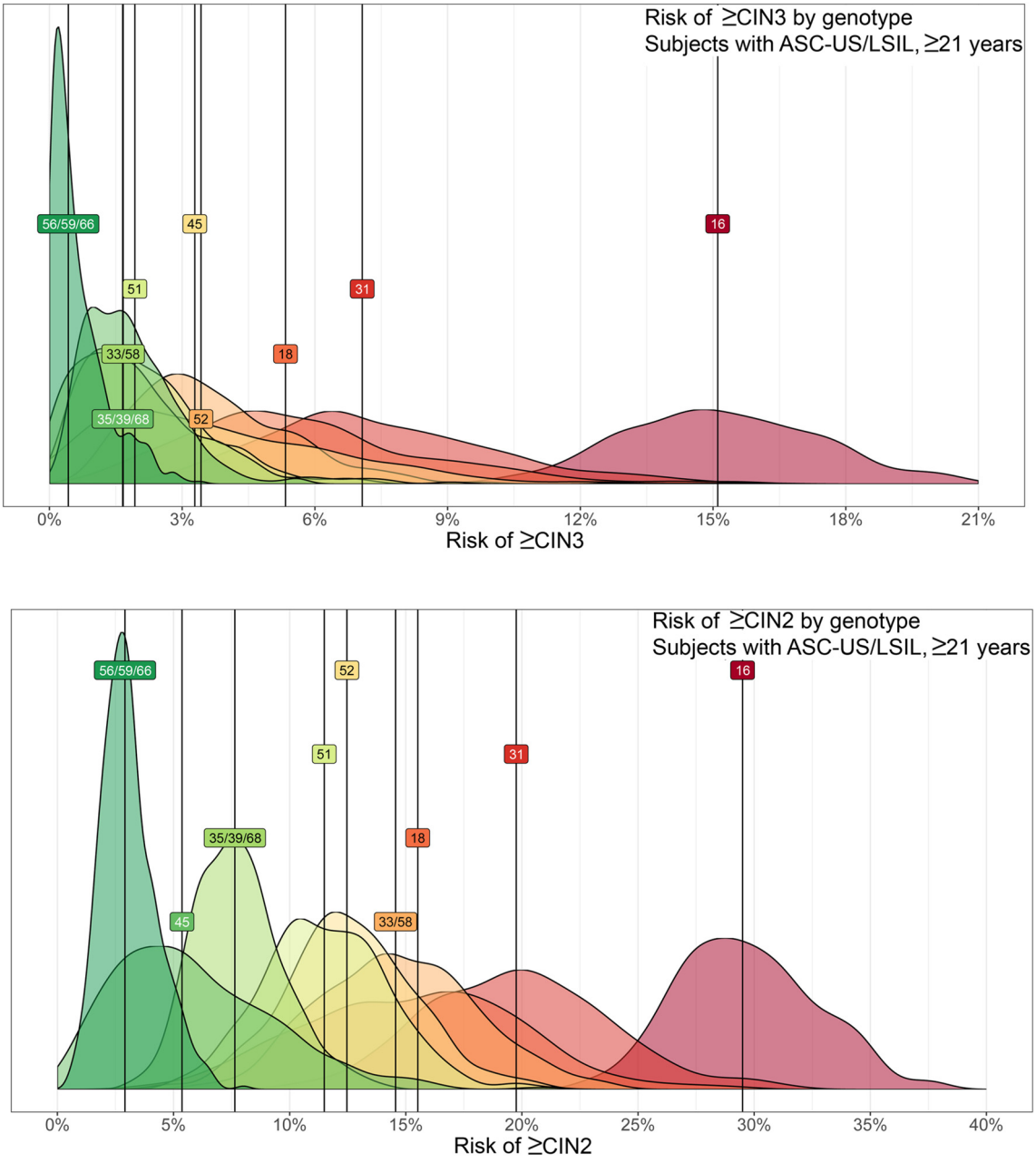

Fig. 1. Bayesian model for risk of ≥CIN3 and ≥CIN2 by HPV genotype using Markov-Chain Monte Carlo methods and including 95% confidence intervals in the ASC-US/LSIL combined cytology population. Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; ASC-US, atypical cells-undetermined significance; LSIL, low-grade squamous intraepithelial lesion.

detected in ≥CIN3 lesions in the ASC-US group. Although HPV 16 was prevalent in both groups of women with <CIN2, HPV 52, HPV 35/59/66, and HPV 56/59/66 were more prevalent than HPV 16 in <CIN2 in the ASC-US group and HPV 35/59/66 and HPV 56/59/66 were more prevalent than HPV 16 in <CIN2 in the LSIL group. This same data stratified into 3 age groups (21–24 years, ≥25 years, and ≥30 years) is provided in Table S1.

Two cases of adenocarcinoma in-situ (ACIS) were identified in the ASC-US population and one case of invasive squamous cell carcinoma (SCC) case was identified in the LSIL population. Of the two ACIS cases, one (age 30 years) was associated with HPV 16 (single infection) and the other (age 33 years) was associated with HPV 16, 31, and 35/39/68 (mixed infection). The SCC case (age 39 years) was associated with HPV 18 (Table S1).

The risk of histologic ≥CIN3 and ≥CIN2 in subjects, ≥21 years, with ASC-US is reported in Table 3. The risk for HPV 31 includes all subjects with HPV 31 or a lower risk genotype and subjects with HPV 16 are excluded. Similarly, the risk for HPV 18 includes all subjects with HPV 18 or a lower risk genotype and subjects with HPV 16 or HPV 31 are excluded. Risk determination is also hierarchical for an HPV result when specific genotypes are negative. The risk if negative for HPV 18, for example, excludes not only subjects with HPV 18, but also subjects with HPV 16 or HPV 31.

For women with ASC-US cytology, the traditional determinant for referral to colposcopy is HPV positivity. In the current study, HPV positivity in the setting of ASC-US cytology, conferred a baseline (year 0) risk of 5.1% (95% CI: 3.6–7.1) for ≥CIN3 and 14.2% (95% CI: 11.7–17.1) for ≥CIN2. The 5.1% ≥CIN3 risk value (and corresponding 14.2% ≥CIN2 risk value) here is above the baseline risk associated with LSIL cytology (with unknown HPV result) reported by Katki et al. [3,12,14,21] in the KPNC cohort (which is 2.5% for ≥CIN3 and corresponds to an 8.7% risk for ≥CIN2). In addition, it is similar to the 5-year

Table 1
Baseline age, Onclarity HPV result, and histology for subjects with ASC-US and LSIL cytology[a,b].

	ASC-US ($n = 1953$)	LSIL ($n = 854$)
Age		
Mean (SD)	36.1 (11.5)	32.0 (10.2)
Median	34	28
Min	21	21
Max	82	66
Race		
Asian	1.0% (20)	1.1% (9)
African American	23.3% (456)	28.7% (245)
White	73.8% (1442)	68.5% (585)
Other[c]	1.8% (35)	1.8% (15)
Ethnicity		
Hispanic or Latino	15.5% (303)	20.7% (177)
Not Hispanic or Latino	84.5% (1650)	79.3% (677)
Smoking history		
Nonsmoker	66.6% (1300)	61.1% (522)
Current	15.7% (307)	22.2% (190)
Past	17.7% (346)	16.6% (142)
HPV vaccinated		
Yes	11.3% (221)	15.5% (132)
No	86.7% (1694)	83.3% (711)
Unknown	1.9% (38)	1.3% (11)
Postmenopausal	12.6% (247)	6.9% (59)
Immunocompromised	1.9% (37)	2.5% (21)
Abnormal cytology (past 5 years)	26.5% (517)	32.6% (278)
Colposcopy (past 5 years)	16.2% (317)	22.6% (193)
Histopathology		
No colposcopy	17.6% (343)	17.5% (148)
Colposcopy	82.4% (1610)	83.5% (706)
Negative	81.4% (1311)	64.6% (456)
CIN1	11.9% (191)	24.2% (171)
CIN2	4.3% (70)	7.8% (55)
≥CIN3	2.2% (35)	3.1% (22)
UNSAT	0.2% (3)	0.3% (2)

Abbreviations: HPV, human papillomavirus; ASC-US, atypical cells-undetermined significance; LSIL, low-grade squamous intraepithelial lesion; SD, standard deviation; Min, minimum age; Max, maximum age; CIN, cervical intraepithelial neoplasia; UNSAT, unsatisfactory.

[a] Only includes subjects with valid (negative or positive) HPV results (9 non-evaluable Onclarity results were excluded).

[b] HPV genotype prevalence determined hierarchically.

[c] Includes American Indian, Alaska Native, Native Hawaiian, or other Pacific Islander.

Table 2
Genotyping prevalence results in subjects ≥21 years with ASC-US and LSIL stratified by histopathologic outcome.

Women with ASC-US						
	<CIN2 ($n = 1502$)		CIN2 ($n = 70$)		≥CIN3 ($n = 35$)	
Genotype[a]	N⁰	%	N⁰	%	N⁰	%
Any HPV	539	35.9%	58	82.9%	32	91.4%
HPV 16	79	5.3%	15	21.4%	18	51.4%
HPV 31	41	2.7%	10	14.3%	4	11.4%
HPV 18	29	1.9%	5	7.1%	1	2.9%
HPV 33/58	42	2.8%	8	11.4%	1	2.9%
HPV 52	85	5.7%	9	12.9%	4	11.4%
HPV 45	31	2.1%	1	1.4%	0	0.0%
HPV 35/59/66	106	7.1%	3	4.3%	3	8.6%
HPV 51	42	2.8%	5	7.1%	1	2.9%
HPV 56/59/66	84	5.6%	2	2.9%	0	0.0%
HPV negative	963	64.1%	12	17.1%	3	8.6%
Women with LSIL						
	<CIN2 ($n = 627$)		CIN2 ($n = 55$)		≥CIN3 ($n = 22$)	
Genotype[a]	N⁰	%	N⁰	%	N⁰	%
Any HPV	477	76.1%	49	89.1%	20	90.9%
HPV 16	72	11.5%	15	27.3%	13	59.1%
HPV 31	41	6.5%	4	7.3%	3	13.6%
HPV 18	27	4.3%	2	3.6%	2	9.1%
HPV 33/58	46	7.3%	5	9.1%	1	4.5%
HPV 52	43	6.9%	5	9.1%	0	0.0%
HPV 45	19	3.0%	0	0.0%	1	4.5%
HPV 35/59/66	84	13.4%	9	16.4%	0	0.0%
HPV 51	39	6.2%	6	10.9%	0	0.0%
HPV 56/59/66	106	16.9%	3	5.5%	0	0.0%
HPV negative	150	23.9%	6	10.9%	2	9.1%

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

[a] Without other higher risk genotypes and ranked by risk of ≥CIN3.

benchmark clinical action threshold for colposcopy referral (for LSIL cytology and an unknown HPV result) in the U.S.A. (5.2% for ≥CIN3 risk and a corresponding ≥CIN2 risk of 16.0%) [12,14]. HPV 16 conferred the highest risk of both ≥CIN3 and ≥CIN2 associated with ASC-US cytology. The risk of ≥CIN3 and ≥CIN2 associated with HPV 16 in ASC-US subjects was 16.1% (95% CI: 10.4–24.0) and 29.5% (95% CI: 21.8–38.5), respectively. At baseline, the ≥CIN3 risk (and corresponding ≥CIN2 risk) associated with HPV 16 was 2–3 times higher than the established 5-year benchmark threshold for colposcopy.

For patients with ASC-US cytology and an HPV 31 result (in the absence of HPV 16), the baseline risk for ≥CIN3 was 7.4% (95% CI: 2.9–17.5), which was higher than the 5-year benchmark risk threshold (5.2%) for a colposcopy referral—although the lower bound of the 95% CI was beneath the threshold. However, the lower-bound of the 95% CI for HPV 31 (2.9%) was still above the baseline LSIL cytology value (2.5%) demonstrated in the KPNC cohort. Similar to HPV 31, HPV 52 had a baseline ≥CIN3 risk of 4.1% (95% CI: 1.6–10.0), which (although lower than the 5-year benchmark, 5.2%, threshold) is higher than the baseline risk value for LSIL (2.5%). All other genotypes were associated with a risk of ≥CIN3 that was considerably beneath the benchmark risk threshold for immediate colposcopy referral.

If ≥CIN2 is used as the endpoint, the baseline risk of HPV 16, 31, 18, and 33/58 exceed the KPNC ≥CIN2 5-year clinical action threshold values for colposcopy referral reported by KPNC (16.0%). The ≥CIN2 risk values for HPV 52, and 51 here exceeded the baseline ≥CIN2 risk associated with LSIL (8.7%) reported by KPNC. However, the ≥CIN2 risk for 45, 35/59/66, and 56/59/66 was only 3.1%, 5.4%, and 2.3%, respectively (although the 95% CI for these risk estimates are relatively wide).

Because of its prevalence and associated risk for ≥CIN3, HPV 16 is the major determinant of risk in women with ASC-US. If HPV 16 is removed, risk for ≥CIN3 in ASC-US drops to 2.7% (95% CI: 1.6–4.5), which is still above the KPNC baseline risk for LSIL (2.5%) (Table 3). However, removal of HPV 31 lowers the risk of ≥CIN3 (2.2%) to below the KPNC baseline LSIL risk value. Removal of subsequent genotypes results in modest reductions in risk until the top 7 genotypes are removed (HPV 16, 31, 18, 33/58, 52, and 45). This same data, stratified into 3 age groups (21–24 years, ≥25 years, and ≥30 years), is provided in Table S2.

The baseline risk values for ≥CIN3 and ≥CIN2, regardless of HPV result, were 3.1 (95% CI: 2.1–4.7) and 10.9 (95% CI: 8.8–13.5), respectively, for LSIL cytology (Table 4). HPV 16 conferred the greatest risk for ≥CIN3 (13.0%; 95% CI: 7.8–21.0) and ≥CIN2 (28.0%; 95% CI: 20.1–37.5); both of which exceeded the 5-year ≥CIN3 (5.2%; and corresponding 16.0% value for ≥CIN2) colposcopy referral threshold. HPV 31 (6.2%; 95% CI: 0.0–14.6) and HPV 18 (6.5%; 95% CI: 0.0–17.4) carried the highest risk values after HPV16 within the LSIL group for ≥CIN3 risk. HPV 31, 18, 33/58, 52, 51 and 35/59/66 conferred an intermediate level of risk (14.6%–9.7%) for ≥CIN2 risk. HPV 45 and 56/59/66 conferred the lowest level of risk for ≥CIN2 (2.8%–5.0%).

Within the LSIL group, removal of only HPV 16 was sufficient to reduce the point estimate ≥CIN3 risk value below the KPNC baseline LSIL risk value (2.5%), whereas, removal of HPV 16 and 31 from the HPV-positive LSIL group resulted in a ≥CIN2 risk point estimate (8.5%) that was below the KPNC baseline LSIL risk value (8.7%). However, 95% CI were wide for many of these point estimate results associated with both ≥CIN3 and ≥CIN2. The data for women with LSIL, stratified into 3 age groups (21–24 years, ≥25 years, and ≥30 years), is provided in

Table 3
Risk of disease stratified by HPV genotype[a] (hierarchical) in subjects ≥21 years with ASC-US.

Risk of ≥CIN3 (35 cases)						
	Positive[b]			Negative[c]		
Genotype[d]	Nº	Risk if positive	95% CI	Nº	Risk if negative	95% CI
HPV 16	18	16.1%	10.4–24.0	14	2.7%	1.6–4.5
HPV 31	4	7.4%	2.9–17.5	10	2.2%	1.2–4.0
HPV 18	1	2.9%	0.5–14.5	9	2.1%	1.1–4.0
HPV 33/58	1	2.0%	0.4–10.3	8	2.1%	1.1–4.2
HPV 52	4	4.1%	1.6–10.0	4	1.4%	0.6–3.6
HPV 45	0	0.0%	0.0–10.7	4	1.6%	0.6–4.1
HPV 35/59/66	3	2.7%	0.9–7.6	1	0.7%	0.1–4.1
HPV 51	1	2.1%	0.4–10.9	0	0.0%	0.0–4.3
HPV 56/59/66	0	0.0%	0.0–4.3			
Any HPV	32	5.1%	3.6–7.1			
HPV negative	3	0.3%	0.1–0.9			
Risk of ≥CIN2 (105 cases)						
	Positive[b]			Negative[c]		
Genotype[d]	Nº	Risk if positive	95% CI	Nº	Risk if negative	95% CI
HPV 16	33	29.5%	21.8–38.5	57	10.9%	8.5–13.9
HPV 31	14	24.1%	14.6–37.0	43	9.3%	7.0–12.3
HPV 18	6	17.1%	8.1–32.7	37	8.7%	6.4–11.7
HPV 33/58	9	17.6%	9.6–30.2	28	7.5%	5.2–10.6
HPV 52	13	13.3%	7.9–21.4	15	5.4%	3.3–8.7
HPV 45	1	3.1%	0.5–15.7	14	5.7%	3.4–9.4
HPV 35/59/66	6	5.4%	2.5–11.3	8	6.0%	3.1–11.3
HPV 51	6	12.5%	5.9–24.7	2	2.3%	0.6–8.1
HPV 56/59/66	2	2.3%	0.6–8.1			
Any HPV	90	14.2%	11.7–17.1			
HPV negative	15	1.5%	0.9–2.5			

Abbreviations: HPV, human papillomavirus; ASC-US, atypical squamous cells-undetermined significance; CIN, cervical intraepithelial neoplasia; CI, confidence interval.
[a] Only includes cases with reportable HPV results and satisfactory histology.
[b] Genotypes were assigned hierarchically (16, else 31, else 18, else 33/58, else 52, else 45, else 39/68/35, else 51, else 59/56/66, else hrHPV neg).
[c] Includes subjects with given genotype or with genotypes ranked lower in the hierarchical order (e.g., 52 row includes subjects with 52/45/39/68/35/51/59/56/66 infections).
[d] Without other higher risk genotypes and ranked by risk of ≥CIN3.

Table S3. In addition, Analysis of these data for ASC-US and LSIL cytology groups, combined, reveals similar risk stratification by genotype result for ≥CIN3 and ≥CIN2 as shown for either cytology group alone (Fig. 2 and Table S4).

4. Discussion

The current results indicate that HPV genotyping could add value to risk-based management algorithms for women with either ASC-US or LSIL. The baseline risk (3.1%) associated with LSIL cytology (with any HPV result), reported here from Onclarity results, was very close to year 0 risks for LSIL cytology reported previously by the benchmark study by KPNC. For comparison of extended genotyping results here, the internal LSIL risk value (3.1%) might have precluded the use of the KPNC 2.5% with minor differences in interpretation of the overall findings. However, in the absence of demonstrable differences between this population and the population of over 1 million subjects in KPNC, it is more appropriate to refer back to the KPNC benchmark study to evaluate the "universal" application of extended genotyping. We found an approximately 5-fold range in risk values for high-grade cervical disease in ASC-US and LSIL cytology groups, depending on respective HPV genotype results. Not surprisingly, HPV 16 had a uniquely high risk. Risk of ≥CIN3 and ≥CIN2 was almost identical in HPV 16 positive women, ≥21 years, with either ASC-US or LSIL. Risks associated with other genotypes varied depending on lesion grade and whether risk is determined in women with ASC-US or LSIL. In general, HPV 31 tended to confer the next highest risk; it was followed by HPV 18, 33/58, 52, and 51.

Table 4
Risk of disease stratified by HPV genotype[a] (hierarchical) in subjects ≥21 years with LSIL.

Risk of ≥CIN3 (22 cases)						
	Positive[b]			Negative[c]		
Genotype[d,e]	Nº	Risk if positive	95% CI	Nº	Risk if positive	95% CI
HPV 16	13	13.0%	7.8–21.0	7	1.6%	0.8–3.2
HPV 31	3	6.2%	2.1–16.8	4	1.0%	0.4–2.6
HPV 18	2	6.5%	1.8–20.7	2	0.5%	0.1–2.0
HPV 33/58	1	1.9%	0.3–10.1	1	0.3%	0.1–1.8
HPV 52	0	0.0%	0.0–7.4	1	0.4%	0.1–2.1
HPV 45	1	5.0%	0.9–23.6	0	0.0%	0.0–1.5
HPV 35/59/66	0	0.0%	0.0–4.0	0	0.0%	0.0–2.4
HPV 51	0	0.0%	0.0–7.9	0	0.0%	0.0–3.4
HPV 56/59/66	0	0.0%	0.0–3.4			
Any HPV	20	3.7%	2.4–5.6			
HPV negative	2	1.3%	0.4–4.5			
Risk of ≥CIN2 (77 cases)						
	Positive[b]			Negative[c]		
Genotype[d,f]	Nº	Risk if positive	95% CI	Nº	Risk if positive	95% CI
HPV 16	28	28.0%	20.1–37.5	41	9.2%	6.8–12.2
HPV 31	7	14.6%	7.2–27.2	34	8.5%	6.2–11.7
HPV 18	4	12.9%	5.1–28.8	30	8.2%	5.8–11.4
HPV 33/58	6	11.5%	5.4–23.0	24	7.6%	5.2–11.1
HPV 52	5	10.4%	4.5–22.2	19	7.1%	4.6–10.8
HPV 45	1	5.0%	0.9–23.6	18	7.3%	4.7–11.2
HPV 35/59/66	9	9.7%	5.2–17.4	9	5.8%	3.1–10.7
HPV 51	6	13.3%	6.3–26.2	3	2.8%	0.9–7.8
HPV 56/59/66	3	2.8%	0.9–7.8			
Any HPV	69	12.6%	10.1–15.7			
HPV negative	8	5.1%	2.6–9.7			

Abbreviations: HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; CI, confidence interval.
[a] Only includes cases with reportable HPV results and satisfactory histology.
[b] Genotypes were assigned hierarchically (16, else 31, else 18, else 33/58, else 52, else 45, else 39/68/35, else 51, else 59/56/66, else hrHPV neg).
[c] Includes subjects with given genotype or with genotypes ranked lower in the hierarchical order (e.g., 52 row includes subjects with 52/45/39/68/35/51/59/56/66 infections).
[d] Without other higher risk genotypes and ranked by risk of ≥CIN3.
[e] The baseline risk for ≥CIN3 associated with LSIL and an unknown HPV result for this age group was 3.1; 95% CI: (2.1, 4.7).
[f] The baseline risk for ≥CIN2 associated with LSIL and an unknown HPV result for this age group was 10.9; 95% CI: (8.8, 13.5).

Management guidelines in the U.S.A. for abnormal cervical cancer screening results are based on the concept that women with equivalent risk for ≥CIN3 should be managed in a similar fashion. Risks are based on outcomes from the various screening test combinations; in general, four management options are possible. Women at very low risk can exit follow-up and return to routine screening. It is recommended that women with a slightly elevated ≥CIN3 risk be referred to repeat screening in 12 months using a combination of cytology and HPV testing (cotesting). This includes HPV negative women with ASC-US who, according to our data, have a ≥CIN3 risk value of approximately 0.3% (95% CI: 0.0–0.7). The purpose of surveillance by repeat testing at a shortened interval is to identify women with persistent HPV infection. According to the benchmarking study by KPNC [12], women in this risk tier include those who have a baseline ≥CIN3 risk between 0.5%–2.5% (corresponding ≥CIN2 risk between 2.1%–8.7%) or a 5-year ≥CIN3 risk between 2.0%–5.2% (corresponding ≥CIN2 risk between 5.1%–16%); the lower threshold value for this band is set by a risk associated with LSIL cytology and an HPV-negative result, whereas the upper threshold value is set by a risk associated with LSIL cytology and an unknown HPV result. Of note, LSIL cytology and HPV-negative subjects in this analysis carried a ≥CIN3 risk of approximately 1.3% (95% CI: 0.0–3.2). According to recommendations from current guidelines, the third risk tier includes women at a high-enough risk to warrant immediate colposcopy. This would apply to HPV-positive women with ASC-US, who had a ≥CIN3 risk of approximately 5.1% (95% CI: 3.6–7.1) here; it would also apply to women of unknown HPV status with LSIL,

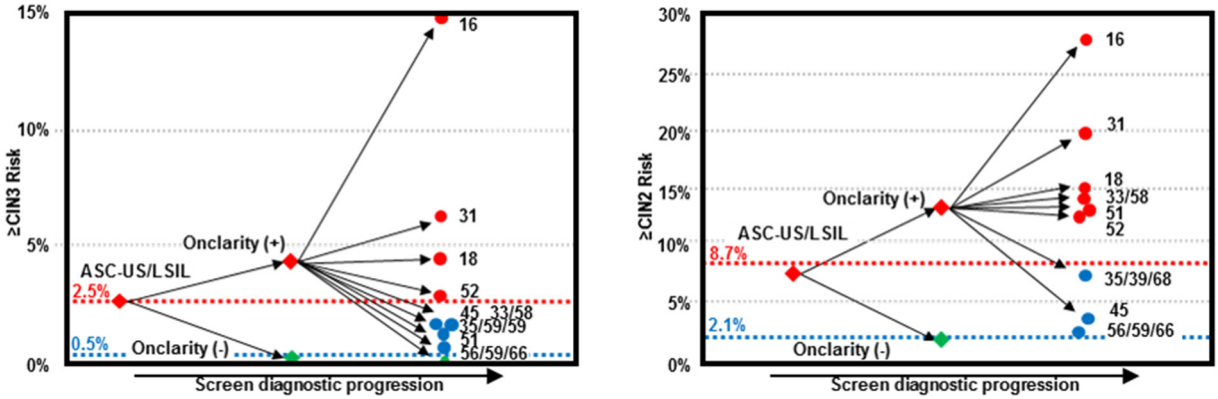

Fig. 2. Baseline ≥CIN3 and ≥CIN2 risk values, stratified by HPV result, in ASC-US and LSIL subjects (groups combined) in relation to KPNC baseline risk thresholds for clinical management during cervical cancer screening. Risk values for ≥CIN3 (left) and ≥CIN2 (right) are plotted for subject groups composed of ASC-US and LSIL cytology subjects (combined)—depending on their corresponding HPV status. The far left diamond in both plots represents CIN risk in subjects with unknown HPV status from the Onclarity trial. These subjects are then stratified by overall HPV status (positive or negative; diamonds in the middle of the plots). Further stratification, via Onclarity assay genotyping, demonstrates the range of associated risk values for genotype results. The hashed red lines represent the year 0 (baseline) risk for LSIL (any HPV result) previously published (2.5% and 8.7% for ≥CIN3 and ≥CIN2, respectively) by KPNC. The hashed blue lines represent the year 0 (baseline) risk for LSIL (with an HPV negative result) previously published (0.5% and 2.1% for ≥CIN3 and ≥CIN2, respectively) by KPNC [12]. The red and blue lines are superimposed on the figure to represent risk values that would correspond to those directing a patient to colposcopy and 12 month repeat for cotesting, respectively. [a]The red, blue, and green circles indicate that the values support immediate colposcopy, repeat cotesting at a shortened duration, or a return to routine screening, respectively. Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; ASC-US, atypical squamous cells-undetermined significance; LSIL, low-grade squamous intraepithelial lesion; KPNC, Kaiser-Permanente Northern California. [a]Although the actually clinical action thresholds were set by KPNC [12] as 5 year risk values, the baseline values are shown here so for comparison to baseline Onclarity trial data.

who had a ≥CIN3 risk of 3.1% (95% CI: 2.1–6.3; data not shown) in the Onclarity trial—both results are above the KPNC baseline LSIL risk value (2.5%) established by KPNC [3,12]. Finally, excisional therapy is considered appropriate for women at greatest risk—such as those with a cytology result of HSIL and HPV16 positive. In KPNC, a single cytology result of HSIL in HPV positive women had a 5-year cumulative risk of 71% and 49% for ≥CIN2 and ≥CIN3, respectively [21]. Irrespective of associated HPV genotype, none of the women with ASC-US or LSIL in this study had a high enough risk of ≥CIN3 to merit immediate treatment.

Because current guidelines suggest that all HPV-positive women with ASC-US be referred for colposcopy [22,23], the potential role of HPV genotyping in ASC-US would be to identify a subset of HPV positive women at low enough risk that they could be safely diverted from immediate colposcopy to surveillance cotesting at 12 months. In the current study there were only 35 and 22 cases of ≥CIN3 among women with ASC-US and LSIL, respectively. Therefore, we utilized ≥CIN2 risk as a surrogate for risk of ≥CIN3, where appropriate, to identify the risk band that might safely be switched from immediate colposcopy to cotesting in 12 months. The data here is consistent with previous work that support clinical management according to this risk threshold [3,12] —the ≥CIN2 risk here for HPV-negative LSIL was 5.1%, which supports a clinical action of cotesting at 12 months; the ≥CIN2 risk here for HPV-positive ASC-US (14.2%) would justify immediate colposcopy.

When assessing risk, two approaches can be utilized. The first is to identify the risk that an individual woman has for ≥CIN2. When viewed from this perspective, women with ASC-US who have HPV 45, HPV 35/59/66, and HPV 56/59/66 are at low enough risk that they do not require colposcopy and could safely undergo surveillance cotesting in 12 months. This would reduce colposcopies by 41% among HPV positive ASC-US. An alternative approach to assessing risk is to determine the residual risk that remains when women with specific genotypes are removed from the population. If women with HPV 16 were identified and referred to colposcopy, the residual risk of ≥CIN2 in HPV positive ASC-US drops to 10.9% (95% CI 8.4–13.6). This may be sufficiently low to allow follow-up in 12 months. If so, this would have a profound impact on ASC-US management since only 7% of women with ASC-US had HPV 16. A recent National Cancer Institute study of women with ASC-US in KPNC came to a similar conclusion [11]. It should be noted, however, that an earlier evaluation of women with ASC-US enrolled in ALTS found that although genotyping for HPV 16 and 18 identified women at highest risk for ≥CIN2 or ≥CIN3, HPV positive women with other genotypes were considered to be at high enough risk to warrant colposcopy—although in ALTS, part of the clinical decision-making regarding HPV positive ASC-US was driven by the 2-year longitudinal follow up [9]. If specific genotypes in addition to HPV 16 are referred to colposcopy, risk in the remaining HPV-positive women with ASC-US drops even further. For example if women with HPV 16, 31, 18 and 33/58 are referred to colposcopy, risk for ≥CIN2 in the remaining HPV positive women drops to only approximately 5.4%. Ultimately, the tradeoff for minimizing residual risk is an increase in the number of colposcopy exams. In another study, Lee et al. evaluated the use of similar genotyping in women with ASC-US and found that referring only those with HPV 16, 18, 31, 33, 52 and 58 to colposcopy reduced colposcopy referrals by 37% at the expense of delaying detection of 8% of ≥CIN2 lesions [10]. Separate analysis of the current data stratified by two additional age groups, ≥25 years (HPV primary screening population) and ≥30 years (cotesting population) showed similar results.

This study and the conclusions have limitations. One limitation is that although 33,858 subjects were enrolled in the Onclarity trial, the number of cases of ≥CIN3 identified among women with ASC-US and LSIL is limited. Thus the risk estimate CI are wide and we chose to use risk of ≥CIN2 as a surrogate for risk of ≥CIN3 in certain cases. Only 2 cases of ACIS and 1 case of invasive cancer were identified in this population and the study can draw no conclusions with respect to how HPV genotyping will perform for the detection invasive cervical cancer. This will impact conclusions regarding the importance of specific genotypes such as HPV 45 that are relatively common in invasive cervical cancers, but less common in CIN3 lesions [24]. A second limitation is that although the Onclarity trial includes a 3-year follow up of women referred to colposcopy at baseline, the analysis here is restricted to baseline data. A third limitation is that the Onclarity HPV assay groups some genotypes together (33/58, 35/59/66, and 56/59/66). This may obscure important differences in risk associated with specific genotypes—although the relative lack of risk associated with 56/59/66, in particular, mitigates this limitation to a degree. In both the New Mexico Pap registry and a British colposcopy clinic study, risks associated with HPV 33 were the same as—or higher than—that for HPV 31; in the current study, however, the risk associated with HPV 33/58 was modest compared to HPV 31 [25,26]. Pooling HPV 66 together with HPV 56 and 59 could also impact risk estimates since HPV 66 is no longer considered

a "carcinogenic" genotype [27]. Additional limitations include the relatively low vaccination rate (11.3% in ASC-US and 15.5% in LSIL) and the fact this analysis does not take into account the number of prior screens that the subjects had had. Although a larger number of vaccinated subjects would reduce the prevalence of vaccine targeted genotypes and of HPV 16/18 associated ≥CIN2, it would not be expected to change the actual risk hierarchy [28]. Similarly, although subjects with a history of multiple prior negative screens would be expected to have a lower prevalence of ≥CIN2, such a history would not be expected to change the risk hierarchy [29]. In interpreting our data, we are limited by a paucity of management options and levels of risk stratification—the risk bands used for clinical management are relatively wide and it is unclear what the precise cut-offs should be. For example, current management guidelines recommend that an HPV-negative woman with LSIL (≥CIN3 risk of 2.0%) do not require colposcopy and that an HPV-positive woman with ASC-US does (≥CIN3 risk of 6.8%) [3,12]; but how should a woman with a risk of ≥CIN3 that lies in between these established cutoffs be managed? As additional triage options become available it will be necessary for expert opinion leaders to consider guidelines that identify more precise clinical action thresholds.

In summary, our results support the concept that specific HPV genotyping may provide clinical benefit for women with ASC-US and LSIL regarding treatment and follow-up decisions.

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Declaration of Competing Interest

MHS serves as a consultant in clinical trial design and as an expert pathologist for HPV vaccine and/or diagnostic trials for Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, Roche, Inovio Pharmaceuticals and Merck and as a speaker for Roche and Becton, Dickinson and Company, BD Life Sciences.

TCW serves as a consultant in clinical trial design and as an expert pathologist for HPV vaccine and/or diagnostic trials for Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, Roche, and Inovio Pharmaceuticals and as a speaker for Roche and Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems.

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All authors contributed to the interpretation of the data, critically revised the manuscript for important intellectual content, and approved the final version to be published. Becton, Dickinson and Co. employees that are also authors, played the following roles during the study and development of the paper: VP facilitated data analyses and revision of the manuscript; KY facilitated conception and design of the study, data acquisition and interpretation, and drafting and revision of the manuscript; CC and JA facilitated study conception and design, and manuscript revision. All authors provided final approval of the manuscript and agree to be accountable for the accuracy and integrity of this work.

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