

# Adjunctive Human Papillomavirus DNA Testing Is a Useful Option in Some Clinical Settings for Disease Risk Assessment and Triage of Females With ASC-H Papanicolaou Test Results

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• **Context.**—Recent guidelines recommend colposcopy for women with atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H).

**Objective.**—To determine whether adjunctive high-risk human papillomavirus (hrHPV) testing is useful for disease risk assessment in females with ASC-H Papanicolaou (Pap) test results.

**Design.**—High-risk HPV prevalence and histopathologic follow-up data on 1187 females with ASC-H ThinPrep Pap test (TPPT) and hrHPV test results were analyzed.

**Results.**—ASC-H was reported in 1646 (0.006%) of 277 400 (270 338 TPPT and 7062 conventional) Pap test results. The difference in ASC-H detection rates between TPPTs and conventional Pap smears was statistically significant (0.60% vs 0.38%;  $P = .02$ ). High-risk HPV was detected in 589 (49.6%) of 1187 females with ASC-H TPPT and hrHPV testing. The hrHPV DNA-positive rate in females younger than 40 years was 54.7%, significantly high-

er than the 36.5% in women 40 years and older. Among 505 females with histopathologic follow-up, cervical intraepithelial neoplasia 2/3 was identified in 32.7% of hrHPV-positive females compared with 1.2% in hrHPV-negative females. The sensitivity, specificity, positive predictive value, and negative predictive value of ASC-H cytology in conjunction with hrHPV DNA testing results for detection of cervical intraepithelial neoplasia 2/3 were 96.1% versus 100.0%, 54.0% versus 68.4%, 35.8% versus 20.8%, and 98.1% versus 100.0% in females younger than 40 years and women 40 years and older, respectively.

**Conclusions.**—Our data suggest that reflex hrHPV testing is a highly useful option for women with ASC-H Pap tests. Females with ASC-H and negative hrHPV testing may be more efficiently managed by follow-up with regular Pap and hrHPV testing rather than universal colposcopy, especially for women 40 years and older.

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The abnormal Papanicolaou (Pap) test result category atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), is a relatively recently recognized subcategory of atypical squamous cells (ASCs) formally introduced in the 2001 Bethesda System (TBS2001).<sup>1</sup> ASC-H Pap tests include a spectrum of abnormal cytomorphologic changes with features suggestive of a high-grade squamous intraepithelial lesion (HSIL) but judged to lack sufficient criteria for a definitive interpretation of HSIL. Therefore, ASC-H is often regarded as equivocal HSIL.<sup>2</sup> Inevitably included in the ASC-H category, however, are also benign mimics of high-grade dysplastic cells. This category reflects a mixture of true HSIL and its mimics.<sup>3</sup> ASC-H is a relatively uncommon Pap test interpretation and represents an interpretive challenge for

both cytotechnologists and cytopathologists. The reported incidence of cases interpreted as ASC-H varies significantly among laboratories and has ranged from 0.22% to 1.09% of all Pap test results; the reported rate of subsequent histologic diagnoses of cervical intraepithelial neoplasia (CIN) 2/3 on follow-up tissue studies also varies widely and has ranged from 12.2% to 68.2%.<sup>4–17</sup> In addition, the reported rates of high-risk human papillomavirus (hrHPV) DNA detection in ASC-H cases also have ranged widely, from 33.3% to 85.6%.<sup>2,4,5,7–11,14,18–34</sup>

Despite the reported variability in ASC-H reporting rates, histologic outcomes, and association with hrHPV DNA, Consensus Follow-up Guidelines from the American Society for Colposcopy and Cervical Pathology continue to suggest universal colposcopic referral to women with a Pap interpretation of ASC-H.<sup>35,36</sup> This recommendation was based primarily on data generated from the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion (ASC-US/LSIL) Triage Study (ALTS), in which ASC-H interpretations were rendered only on follow-up thin-layer cytology slides interpreted by a small group of 4 pathologists designated the Pathology Quality Control Group.<sup>2</sup> This group identified 110 ASC-H cases during internal quality control reviews and reported that 84% tested positive for hrHPV DNA.

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Today's general practice settings, however, differ in a number of important ways from the ALTS trial, a highly selective scenario in which all enrolled patients had abnormal ASC-US or LSIL Pap findings based on community conventional Pap smears interpreted according to the Bethesda System 1991 (TBS1991).<sup>37</sup> Enrollment and referral ALTS slides interpreted as ASC-US based on 1991 terminology could now be easily reclassified using current TBS2001 terminology as: (1) negative if formerly ASC-US, favor reactive; (2) ASC-US if suggestive of low-grade or intermediate-grade squamous intraepithelial lesion; and (3) ASC-H if suggestive of HSIL.<sup>2</sup> Furthermore, the small number of internal ASC-H interpretations in ALTS were rendered only by 4 designated, closely interacting review pathologists. Also, ALTS data reflect findings in a younger-than-average patient age population (median age, 24 years).<sup>18</sup> More diverse studies reflecting larger numbers of reviewing pathologists consistently show that ASC-H interpretations are highly subjective and have limited reproducibility, even when using standardized published criteria.<sup>17</sup> Other studies, even studies using similar cytologic and hrHPV DNA test methods as ALTS, have demonstrated a range of significantly lower rates of hrHPV DNA detection for women with ASC-H Pap tests than reported in ALTS.\*

The 2001 American Society for Colposcopy and Cervical Pathology guidelines recommended reflex hrHPV DNA testing as the preferred approach for managing women with a liquid-based cytology Pap test result of ASC-US, and it has been estimated recently that 85% of all ASC-US Pap tests in the United States are now routinely referred for reflex hrHPV DNA testing.<sup>38</sup> A number of smaller studies have presented data suggesting that hrHPV DNA testing for triage of ASC-H in a number of practice settings can also reduce substantially the number of women who are referred for colposcopy without increased risk for failure to detect high-grade squamous lesions.† Authors in other small studies have presented data supporting the American Society for Colposcopy and Cervical Pathology Guideline position,<sup>10,19,33</sup> reflecting the substantial diversity of laboratory experience.

Given the variability of reported findings and the limited number of patients in most studies, larger studies are clearly needed for further consideration of a possible role for hrHPV DNA in the management of selected populations of women with ASC-H Pap test results. In this study, we report results from the largest number of females studied to date with ASC-H Pap and concurrent hrHPV DNA test results. Histologic outcomes reflect actual reported results from a large, subspecialized, academic women's hospital practice. The impact of the presence or absence of an endocervical/transformation zone sample (EC/TZS) in females with ASC-H Pap test results is also reported for the first time.

## MATERIALS AND METHODS

### Case Selection and Cytology Screening

A retrospective study was designed and initiated after obtaining approval from the institutional review board at the University of Pittsburgh Medical Center. A computer-based search was carried out to retrieve Pap tests with ASC-H interpretations and

with concomitant hrHPV DNA testing during a 30-month period from July 1, 2005, to December 31, 2007. All specimens were processed at Magee-Womens Hospital of the University of Pittsburgh Medical Center pathology laboratory and reported using the current TBS2001 terminology.<sup>1</sup> The Magee-Womens Hospital/University of Pittsburgh Medical Center cytopathology laboratory is a large, subspecialized, academic hospital laboratory which consistently reports more than 110 000 Pap tests per year from a large, integrated, 20-hospital health system and which serves a metropolitan area with a significantly older age profile than the national average.<sup>39</sup> The presence or absence of an EC/TZS was documented from the laboratory information system files. ThinPrep Pap tests (TPPTs) were prepared according to manufacturer's specifications from PreservCyt samples using an automated processor (ThinPrep 3000, Cytyc Corp, Marlborough, Mass). Staining of slides was done on a Sakura Tissue Tek Automated Slide Stainer according to a Food and Drug Administration-approved manufacturer's protocol. Location-guided, computer-assisted screening of TPPT slides was accomplished using the ThinPrep Imaging System (TIS; Cytyc).<sup>40</sup> The TIS performed analysis of batches of up to 250 TPPT slides with specialized image software. The protocol was the same as that described in our previous reports.<sup>41,42</sup> In general, an ASC-H interpretation was rendered when small, rounded squamous cells with dense limited cytoplasm, enlarged and euchromatic or hyperchromatic nuclei, and some degree of nuclear membrane irregularities were identified. The EC/TZS status was determined according to the method described in TBS2001; that is, at least 10 well-preserved endocervical or squamous metaplastic cells, singly or in clusters. In all cases in which the 22 fields did not contain enough endocervical or squamous metaplastic cells, the cytotechnologists manually rescreened the entire TPPT slide to determine EC/TZS status using the same criteria.

### High-Risk HPV DNA Detection

High-risk HPV DNA testing was ordered by clinicians according to several ordering options: reflex testing triggered by indeterminate abnormal ASC Pap test results, routine cotesting with Pap testing in women 30 years and older, and hrHPV DNA cotesting regardless of age or Pap test result (HPV regardless). High-risk HPV DNA detection was performed by the commercially available Food and Drug Administration-approved Hybrid Capture 2 (HC2) System (Digene Corp, Gaithersburg, Md). The results of hrHPV testing were either positive or negative. Patients who had equivocal results or residual samples with quantities insufficient for hrHPV DNA testing were excluded from this study.

### Histopathologic Follow-up

Histopathologic follow-up included endocervical curettage, cervical biopsy, cervical conization by loop electrosurgical excision procedure or cold knife conization. Time elapsed from Pap testing until colposcopic examination and cervical biopsy, follow-up procedures, and histologic findings were abstracted from record reviews. The surgical pathology slides of all cases with CIN were reviewed by 2 surgical pathologists to confirm the diagnoses. In this study, CIN terminology refers exclusively to histopathology results. The degree of CIN was categorized as CIN 2 or higher (CIN 2/3) and CIN 1. For patients undergoing 2 or more procedures during the follow-up period, only the most abnormal histologic diagnosis was recorded. All outcomes were stratified by age and presence or absence of an EC/TZS.

### Statistical Analysis

The 95% confidence intervals (CIs) for the frequency of hrHPV DNA detection were obtained by a Wald test. Comparison of categorical data was performed using standard contingency table analyses based on the  $\chi^2$  test or Fisher exact test for small number using the SAS 9.1 system (SAS Institute Inc, Cary, NC). *P* values of <.05 were considered statistically significant.

\* References 9, 11, 20–22, 25, 26, 28–32, 34.

† References 7, 9, 11, 20, 22, 24–26, 28, 32.

**Table 1. High-Risk Human Papillomavirus (hrHPV) Prevalence in Females With Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion ThinPrep Papanicolaou Test Results With and Without an Endocervical/Transformation Zone Sample (EC/TZS): 10-Year Interval**

Age, y	Total				EC/TZS Present			EC/TZS Absent			P
	No. hrHPV Tested	Positive		95% Confidence Intervals	No. hrHPV Tested	Positive		No. hrHPV Tested	Positive		
		No.	%			No.	%		No.	%	
10–19	68	56	82.4	73.3–91.5	64	52	81.3	4	4	100.0	>.99*
20–29	488	284	58.2	53.8–62.6	453	263	58.1	35	21	60.0	.86
30–39	302	129	42.7	37.1–48.3	282	118	41.8	20	11	55.0	.35
40–49	195	68	34.9	28.2–41.6	184	64	34.8	11	4	36.4	.92
50–59	89	36	40.4	30.2–50.6	75	29	38.7	14	7	50.0	.43
60–69	30	13	43.3	25.6–61.0	26	11	42.3	4	2	50.0	>.99*
70–79	15	3	20.0	0–40.2	15	3	20.0	0	0	0	
<b>Total</b>	<b>1187</b>	<b>589</b>	<b>49.6</b>	<b>46.8–52.4</b>	<b>1099</b>	<b>540</b>	<b>49.1</b>	<b>88</b>	<b>49</b>	<b>55.7</b>	<b>.24</b>

\* Fisher exact test.

**Table 2. High-Risk Human Papillomavirus (hrHPV) Prevalence in Older and Younger Females With Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion ThinPrep Papanicolaou Test Results (2 Age Groups)**

Age Groups, y	No. hrHPV Tested	Positive		95% Confidence Intervals	P
		No.	%		
<30	556	340	61.2	57.1–65.3	<.001
≥30	631	249	39.5	35.7–43.3	
<40	858	469	54.7	51.4–58.0	<.001
≥40	329	120	36.5	31.3–41.7	

## RESULTS

### Prevalence of hrHPV in Females With ASC-H Pap Tests

During the July 1, 2005, to December 31, 2007, 30-month study period, 277 400 Pap tests were reported, including 270 338 (97.5%) TPPT and 7062 (2.5%) conventional Pap smears. The number of ASC-H interpretations during this period was 1646 (1619 TPPT and 27 conventional Pap smears), 0.59% of all Pap results. The ASC-H detection rate was 0.60% in TPPT and 0.38% in conventional Pap smears. The difference in ASC-H detection rates between TPPT and conventional Pap smears was statistically significant ( $P = .02$ ). Of 1619 females with an ASC-H TPPT result, 1187 patients (73.3%) had a concurrent hrHPV DNA test result. The age of females with ASC-H TPPT and reflex HC2 hrHPV DNA test results ranged from 14 to 88 years, with a mean age of 34 years and a median age of 30 years. A positive hrHPV DNA result was obtained in 589 (49.6%) of a total of 1187 females with ASC-H Pap results. The age-related hrHPV DNA prevalence analyzed in 10-year intervals and the 95% CIs are shown in Table 1. Patients with ASC-H Pap results who tested positive for hrHPV DNA were younger than those with negative hrHPV DNA results, with a peak infection rate in females younger than 20 years (82.4%). There was a clear decline in the hrHPV DNA prevalence in older age groups. When females were divided into 2 age groups, younger than 30 years and 30 years and older, the hrHPV DNA rate in 556 females younger than 30 years was 61.2%, significantly higher than the 39.5% hrHPV DNA rate in 631 women 30 years and older (Table 2). The difference in hrHPV DNA-positive rates was significant between females younger than 40 years and women 40 years and

older (54.7% vs 36.5%;  $P < .001$ ; Table 2). No statistically significant variation in hrHPV DNA detection rates was identified when comparisons were made between females with ASC-H Pap results with and without an EC/TZS in all age groups (Table 1).

### Histopathologic Follow-up Results

Histopathologic follow-up results were assessed for women with ASC-H TPPT and concurrent hrHPV DNA test results between July 2005 and June 2007. Among 926 women with ASC-H Pap results and concurrent hrHPV DNA testing results, 421 patients had either no histologic follow-up data, repeat cytology results only, or endocervical curettage only. These patients with limited follow-up were excluded from our histopathologic follow-up analyses. A total of 505 females with ASC-H Pap test and concurrent hrHPV DNA test results had at least 1 follow-up cervical biopsy result in our system. These follow-up biopsy results included 257 females (50.9%) with positive hrHPV DNA results and 248 females (49.1%) with negative hrHPV DNA results. The 505 females with subsequent histopathologic follow-up ranged in age from 15 to 87 years (mean, 34 years; median, 31 years). The average follow-up duration (period between the ASC-H Pap test and the last cervical biopsy) was 75 days, ranging from 0 days (few cases with biopsies obtained synchronously) to 19 months.

A total of 101 females had 2 or more biopsies, including 66 loop electrosurgical excision procedure/cone biopsies and 6 hysterectomies. The detailed histopathologic follow-up results, correlated with hrHPV DNA test results and stratified by age, are presented in Table 3. Cervical intraepithelial neoplasia 2/3 was identified in 87 (17.2%) of 505 females with ASC-H during the follow-up period. A total of 90.8% (79/87) of the initial histologic diagnoses of CIN 2/3 were on the first cervical biopsy, 6.9% (6/87) on the second cervical biopsy, and 2.3% (2/87) on the loop electrosurgical excision procedure/cone biopsy. The average period between the ASC-H Pap test and the initial diagnosis of CIN 2/3 was 63 days, ranging from 6 days to 13 months. A total of 76 (87.4%) of 87 cases of histologic CIN 2/3 were diagnosed within 3 months, and 80 (92.0%) were within 4 months.

### Correlation Between hrHPV DNA Results and Subsequent Histopathologic CIN

The comparison of the histopathologic outcomes in women with positive and negative hrHPV DNA results

*HPV Testing Is Useful in Females With ASC-H—Bandyopadhyay et al*

**Table 3. Histologic Follow-up Results in Females With Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion Comparing High-Risk Human Papillomavirus (hrHPV)-Positive and -Negative Groups Stratified by Ages\***

Age, y	hrHPV Positive			hrHPV Negative			P (CIN 2/3)	P (CIN 1)
	F/U, No.	CIN 2/3, No. (%)	CIN 1, No. (%)	F/U, No.	CIN 2/3, No. (%)	CIN 1, No. (%)		
10-19	19	2 (10.5)	6 (31.6)	5	0	0		
20-29	118	40 (33.9)	37 (31.4)	79	0	19 (24.1)	<.001	.27
30-39	67	31 (46.3)	18 (26.9)	73	3 (4.1)	7 (9.6)	<.001	.008
40-49	33	8 (24.2)	10 (30.3)	52	0	2 (3.8)	<.001†	.001†
50-59	12	3 (25.0)	4 (33.3)	21	0	4 (19.0)		
60-69	6	0	1 (16.7)	10	0	0		
70-79	2	0	0	8	0	0		
<b>Total</b>	<b>257</b>	<b>84 (32.7)</b>	<b>76 (29.2)</b>	<b>248</b>	<b>3 (1.2)</b>	<b>32 (12.9)</b>	<b>&lt;.001</b>	<b>&lt;.001</b>

\* F/U indicates follow-up; CIN, cervical intraepithelial neoplasm.

† Fisher exact test.

**Table 4. Histologic Follow-up Results Comparing Older and Younger Females With Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion ThinPrep Papanicolaou Test Results\***

Age Groups, y	F/U, No.	CIN 2/3				CIN 1			
		No.	%	95% Confidence Intervals	P	No.	%	95% Confidence Intervals	P
<40	361	76	21.1	16.9-25.3	<.001	87	24.1	19.7-28.5	.02
≥40	144	11	7.6	3.3-11.9		21	14.6	8.8-20.4	
<b>Total</b>	<b>505</b>	<b>87</b>	<b>17.2</b>	<b>13.9-20.5</b>		<b>108</b>	<b>21.4</b>	<b>17.8-25.0</b>	

\* F/U indicates follow-up; CIN, cervical intraepithelial neoplasm.

stratified by patients' ages is shown in Table 3. Both CIN 2/3 and CIN 1 were identified on histologic follow-up in a significantly greater proportion of females who were hrHPV DNA positive than in females who were hrHPV DNA negative. With any grade of CIN as the clinical endpoint, the cumulative CIN detection rate was 38.6% (195/505). The CIN detection rate was 62.3% (160/257) in females with positive hrHPV DNA testing, significantly higher than the 14.1% (35/248) in females with negative hrHPV DNA testing ( $P < .001$ ). In the hrHPV DNA-positive group, 84 (32.7%) of 257 patients (95% CI, 27.0-38.4) had a subsequent histologic diagnosis of CIN 2/3. In comparison, only 3 (1.2%) of 248 females (95% CI, 0-2.6) in the hrHPV DNA-negative group had a follow-up tissue diagnosis of CIN 2/3. This difference was statistically significant, with a  $P$  value of  $<.001$ . Females 30 to 39 years of age in the hrHPV DNA-positive group had the greatest risk (46.3%; 95% CI, 34.4-58.2) for CIN 2/3 on follow-up. Adenocarcinoma in situ was histologically diagnosed in 4 females with ASC-H, 1 in her 20s, 2 in the 30s age range, and 1 in her 70s. Two of four cases coexisted CIN 2/3. All 4 females with adenocarcinoma in situ tested positive for hrHPV DNA.

#### Relationship Between Age and Histopathologic Follow-up

When stratified into 2 age groups irrespective of hrHPV DNA test results, 76 (21.1%) of 361 females in the younger age group (<40 years) had a histologic diagnosis of CIN 2/3. In comparison, 11 (7.6%) of 144 cases in the older (≥40 years) group had a follow-up diagnosis of CIN 2/3. This difference was statistically significant (Table 4). When stratified into 2 age groups and including only hrHPV DNA-positive females, 73 (35.8%) of 204 females younger than 40 years had CIN 2/3 in follow-up biopsies, and only 11 (20.8%) of 53 women 40 years and older had

CIN 2/3. The difference was statistically significant ( $P < .001$ ). The difference in CIN 1 detection rates was statistically significant between females with positive and negative hrHPV DNA test results (Table 3) and also between females younger than 40 years and women 40 years and older age groups (Table 4).

#### Positive Predictive Value, Negative Predictive Value, Sensitivity, and Specificity of hrHPV DNA Testing Results for CIN 2/3

When an ASC-H cytology result was considered by itself, it had a positive predictive value (PPV) of 17.2% for detection of CIN 2/3. However, when the ASC-H cytology result was used in conjunction with a positive hrHPV DNA test result, the PPV for CIN2/3 detection almost doubled (32.7%). A negative hrHPV DNA test had a negative predictive value of 98.8% in predicting nondetection of CIN 2/3. The sensitivity and specificity for reflex hrHPV DNA testing to detect CIN 2/3 in females with ASC-H were 96.6% and 58.6%, respectively. When stratified into 2 age groups (<40 years and ≥40 years), the sensitivity, specificity, PPV, and negative predictive value of ASC-H cytology in conjunction with reflex hrHPV DNA testing results for detection of CIN 2/3 were 96.1% versus 100.0%, 54.0% versus 68.4%, 35.8% versus 20.8%, and 98.1% versus 100.0%, in females younger than 40 years and women 40 years and older, respectively.

#### EC/TZ Sampling and CIN Detection

The presence or absence of an EC/TZS did not affect the subsequent rates of diagnoses of CIN 2/3 or CIN 1 in the younger or older age groups (Table 5).

#### COMMENT

The category of ASC-H is a relatively new abnormal result category and was first included in TBS2001 nomen-

**Table 5. Histologic Follow-up Results in Females With Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion ThinPrep Papanicolaou Test Results With and Without Endocervical/Transformation Zone Sample (EC/TZS)\***

Age, y	EC/TZS Present			EC/TZS Absent			P	
	Total No.	CIN 2/3, No. (%)	CIN 1, No. (%)	Total No.	CIN 2/3, No. (%)	CIN 1, No. (%)	CIN 2/3	CIN 1
<30	203	38 (18.7)	54 (26.6)	18	4 (22.2)	8 (44.4)	.75	.11
≥30	267	41 (15.4)	43 (16.1)	17	4 (23.5)	3 (17.6)	.37	.74†
<b>Total</b>	<b>470</b>	<b>79 (16.8)</b>	<b>97 (20.6)</b>	<b>35</b>	<b>8 (22.9)</b>	<b>11 (31.4)</b>	.36	.13

\* CIN indicates cervical intraepithelial neoplasia.

† Fisher exact test.

**Table 6. Accumulated Data on Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion Prevalence in Women With Liquid-Based Cytology Papanicolaou (Pap) Test (Literature Review)**

Reference	Total No.	Positive	
		No.	%
Lee and Ng, <sup>4</sup> 2007	10 745	36	0.34
Taraif et al, <sup>5</sup> 2005	107 021	257	0.24
Geisinger et al, <sup>6</sup> 2007	20 354	222	1.09
Gupta et al, <sup>7</sup> 2007	29 475	60	0.22
McHale et al, <sup>8</sup> 2007	83 667	488	0.58
Wu et al, <sup>9</sup> 2006	119 659	377	0.32
Duncan and Jacob, <sup>10</sup> 2005	60 390	414	0.69
Srodon et al, <sup>11</sup> 2005	30 658	96	0.31
Stany et al, <sup>12</sup> 2006	76 675	223	0.29
Saad et al, <sup>13</sup> 2006	152 495	800	0.52
Selvaggi, <sup>14</sup> 2003	9214	25	0.27
Shidham et al, <sup>15</sup> 2007	77 979	255	0.33
<b>Total</b>	<b>778 332</b>	<b>3253</b>	<b>0.42</b>
Current study	270 338	1619	0.60
Davey et al, <sup>17</sup> 2004			0.57*

\* Mean rate for ThinPrep Pap tests from 122 laboratories.

clature.<sup>1,3</sup> This interpretation is usually based on presence of small metaplastic squamous cells, arranged in clusters or present singly, with a high nucleus to cytoplasmic ratio, nuclear granularity, hyperchromasia, and nuclear membrane irregularities and thickening. Although cytomorphic criteria have been described for this category, there is a significant degree of variability and subjectivity in its application, resulting in interobserver and even intraobserver variability and limited reproducibility. In a study by Quddus et al,<sup>43</sup> the overall agreement among reviewers for ASC-H was 20%, with a poor interobserver  $\kappa$  value of 0.11. Another study reported a concordance rate of only 14% for an interpretation of ASC-H.<sup>44</sup> The detection rates for ASC-H have varied significantly in the literature, ranging from 0.22% to 1.09% with a calculated mean of 0.42% in liquid-based cytology (Table 6). Bethesda 2001 implementation and reporting rates from the College of American Pathologists interlaboratory comparison programs in cervicovaginal cytology indicate that the mean rate of ASC-H was 0.57% for TPPT results from 122 laboratories.<sup>17</sup> In our large series, the ASC-H rate was 0.60%, similar to the mean rate for ASC-H results identified in TPPT specimens in the College of American Pathologists study, and slightly higher than the calculated mean rate (0.42%) from the general cytology literature.

At Magee-Womens Hospital, location-guided, computer-assisted screening of TPPT slides has been used since 2005 using the TIS. Two Cleveland Clinic abstracts report

that ASC-H interpretations significantly increased after introduction of the TIS and conclude that the TIS appears to be especially effective in detecting rare small atypical metaplastic cells.<sup>30,31</sup> Modest decreases in rates of hrHPV DNA detection and CIN 2/3 biopsy findings are also reported in the abstracts, suggesting that a significant portion of the previously undetected rare small atypical cells are not precancerous cells. Other investigators have reported increased detection of abnormal cases with the TIS, including ASC-H, with little or no changes in hrHPV DNA detection rates, suggesting that diagnostic thresholds and disease prevalence can be significant variables.<sup>45</sup> The impact of employing different ASC thresholds on verification bias-adjusted sensitivity and specificity are largely unknown.<sup>46,47</sup>

As noted earlier, current follow-up guidelines for patients with ASC-H results are for direct referral to colposcopy, largely based on data from 110 cases identified by the Pathology Quality Control Panel in the ALTS trial. In the ALTS data set, approximately 40% of patients with an ASC-H result were subsequently diagnosed with CIN 2/3, further contributing to the American Society for Colposcopy and Cervical Pathology follow-up recommendation. A total of 84% of 110 females with ASC-H results in ALTS were reportedly positive for hrHPV DNA, suggesting limited efficacy for testing.<sup>2,18</sup> The ALTS trial, as noted earlier, was a limited, highly selected clinical trial in which all entry Pap tests were abnormal conventional smears reported using TBS1991 terminology. Moreover, ALTS reflected a relatively young age population (median age, 24 years). Additional studies utilizing liquid-based cytology have indicated a lower hrHPV-positive rate for ASC-H women compared with the ALTS study, as low as 33.3% (Table 7).

The purpose of this study was to evaluate the histologic outcomes in patients with an ASC-H result and the possible utility of hrHPV DNA testing in stratifying risk for a subsequent histologic diagnosis of high-grade dysplasia on biopsy in routine practice (ie, single-pathologist interpretations). We examined the prevalence of hrHPV DNA in a series of 1187 TPPTs with an ASC-H interpretation. To the best of our knowledge, this is the largest study to assess CIN 2/3 risk and hrHPV DNA detection rates associated with a Pap interpretation of ASC-H. In our ASC-H study population, the detection rate for hrHPV DNA was approximately 50% (49.6%; 95% CI, 46.8%–52.4%). Although this detection rate was lower than that reported in the ALTS trial (84%–85.6%)<sup>2,18</sup> and some other studies, this rate was higher than that reported in a number of other reports (Table 7). We combined data on hrHPV DNA test results with HC2, including most larger ASC-H studies in the literature, including ALTS results

**Table 7. Accumulated Data on Hybrid Capture II High-Risk Human Papillomavirus (hrHPV)-Positive Rates in Women With Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion Liquid-Based Cytology Results and Histopathologic Follow-up (Literature Review)\***

hrHPV Test		Histologic CIN 2/3							Panel Review/ Reclassification	Year of Publication	Reference
		hrHPV Positive			hrHPV Negative						
No. Tested	Positive		No. Tested	CIN 2/3		No. Tested	CIN 2/3				
	No.	%	No.	No.	%	No.	No.	%			
23	17	73.9							Yes	2005	Duncan and Jacob <sup>10</sup>
110	92	83.6	92	54	58.7	18	2	11.1	Yes	2001, 2006	Sherman et al <sup>2,18</sup>
48	38	79.2	38	22	57.9	10	0	0	Yes	2005	Liman et al <sup>19</sup>
101	82	81.2	33	16	48.5	9	0	0	Yes	2007	Nguyen et al <sup>33</sup>
96	64	66.7	45	18	40.0	12	1	4.5	No	2006	Srodon et al <sup>11</sup>
95	54	56.8	31	13	41.9	13	1	7.7	No	2007	Reid-Nicholson et al <sup>20</sup>
88	59	67.0	35	15	42.9	7	0	0	No	2006	Wu et al <sup>9</sup>
16	6	37.5							No	2004	Rowe et al <sup>21</sup>
48	16	33.3	10	3	30.0	6	0	0	No	2005	Palma <sup>22</sup>
33	21	63.6	15	4	26.7	4	0	0	No	2006	Chivukula and Shidham <sup>24</sup>
73	43	58.9	43	14	32.6	30	1	3.3	No	2007	Owens et al <sup>25</sup>
109	58	53.2	58	19	32.8	51	1	2.0	No	2007	You et al <sup>26</sup>
88	60	68.2	60	15	25.0	28	0	0	No	2004	El-Fakharany et al <sup>28</sup>
40	21	52.5	21	11	52.4	13	1	7.7	No	2004	Hoschar et al <sup>29</sup>
257	151	58.8	87	33	37.9				No	2005	Taraif et al <sup>5</sup>
187	86	46.0							No	2006	O'Brien et al <sup>30</sup>
209	105	50.2							No	2007	Howard et al <sup>31</sup>
138	53	38.4	36	11	30.6	45	2	4.4	No	2006	Christal et al <sup>32</sup>
115	54	47.0	54	23	42.6	19	4	21.1	No	2007	Sullivan et al <sup>34</sup>
1874	1080	57.6	658	271	41.2	265	13	4.9			In total
1187	589	49.6	257	84	32.7	248	3	1.2	No	2008	Current

\* CIN indicates cervical intraepithelial neoplasia.

(1874 women with ASC-H) and found an overall hrHPV DNA detection rate of 57.6% (95% CI, 55.4%–59.8%; Table 7). This average hrHPV rate is significantly lower than that reported in ALTS.

There are a number of potential factors to explain the significant variation in hrHPV DNA detection rates for women with ASC-H Pap test results (33.3%–85.6%). Significant differences in cytologic interpretation thresholds for ASC-H interpretations are inevitably present between different laboratories and also exist within laboratories. Geisinger et al<sup>6</sup> reported that hrHPV DNA rates in women with ASC-H among 5 pathologists in one laboratory ranged from 54% to 83%. Higher hrHPV DNA rates in women with ASC-H could indicate many things; for example, that pathologists effectively differentiate HPV-associated ASC-H cytomorphic changes from cell changes of ASC-US or LSIL or that pathologists tend to undercall true HSIL cases. On the other hand, lower hrHPV DNA rates in women with ASC-H could indicate that pathologists overcall ASC-H as HSIL or report more cases with metaplastic cells and other mimics as ASC-H.

The ALTS trial represents a limited multi-institutional study with reassessment of all Pap smear interpretations by a panel consisting of 1 gynecologic surgical pathologist and 3 cytopathologists. Accumulated data from studies where ASC-H interpretations were based on adjudication panel reviews or reclassification reveal that the hrHPV DNA-positive rate was 81.2% (229/282), with a 95% CI of 76.6% to 85.8%.<sup>2,10,19,33</sup> On the other hand, accumulated data from studies (not including current data) without adjudication reviews reveals that the hrHPV DNA detection rate was 53.5% (851/1592), with a 95% CI of 51.0% to 56.0% (Table 7),<sup>‡</sup> similar to our current findings. The dif-

ferences in hrHPV DNA detection rates between the 2 groups was statistically significant ( $P < .001$ ). Interestingly, the hrHPV DNA detection rate in all 4 studies with reassessments of ASC-H Pap interpretations was more than 70%, but was lower than 70% in all studies without reassessment. With increased recognition of the potential significance of atypical metaplastic squamous cells as a possible reflection of underlying CIN 2/3 and an adverse medicolegal climate, thresholds for abnormal interpretations may be lowered. Pathologists understand that retrospectively reviewed small metaplastic cells with a high nuclear to cytoplasm ratio and any suggestion of nuclear abnormality are quite likely to be interpreted in outcome-biased retrospective reviews as examples of “missed” ASC-H or HSIL.<sup>48</sup> Therefore, specimens that are classified routinely as ASC-H through single-pathologist readings can be expected to have a lower detection rate for hrHPV DNA compared with specimens subjected to broader consensus reviews.<sup>11</sup>

Another plausible explanation for reported variations might be that different hrHPV DNA detection rates reflect different demographic population profiles with different HPV prevalences. The prevalence of hrHPV DNA detected by the Food and Drug Administration–approved HC2 test method in general screening groups of North American and European women with manually screened, cytologically negative conventional and liquid-based cytology Pap test results has been reported to vary from 5.4% to 32.7%.<sup>49</sup> A low hrHPV DNA rate of 3.9% was also reported in 1000 cytology-normal women aged 30 years and older (30–45) who had been screened with TIS-imaged TPPT.<sup>50</sup> Our recent study confirmed and extended those observations with a larger dataset of more than 8000 cytologically negative patients with HC2 hrHPV DNA test results after TIS location-guided, computer-assisted screening of

‡ References 5, 9, 11, 20–22, 24–26, 28–32, 34.

TPPT, showing a very low hrHPV DNA rate of 2.9% in the same population as our current study.<sup>41</sup> The hrHPV DNA detection rate in our ASC-H cases would logically have been influenced in part by hrHPV DNA infection rates in our overall older-than-average, low-risk population. High-risk HPV DNA detection rates in cytology negative women have been reported to be as much as 11 times higher in the ALTS study population compared with our current study population (32.7% vs 2.9%).<sup>41,49,51</sup>

In this study, the hrHPV DNA detection rate in females with ASC-H showed a peak infection rate in females younger than 20 years (82.4%) and declined significantly in women 30 years and older, as noted in numerous other studies.<sup>2,9,23,52</sup> When stratified by age (<30 years and ≥30 years), the prevalence of hrHPV DNA in the older age group was significantly lower (39.5%) than in the younger age group (61.2%). The median age of the 193 females with ASC-H was 24 years in ALTS,<sup>18</sup> but was 30 years, with a mean age of 34 years, in our study. Morphologic mimics of ASC-H include atrophic changes, reactive and reparative changes, naked nuclei, parakeratosis, and immature metaplastic cells.<sup>7,24,53</sup> Many of these changes played a role in hrHPV DNA-negative ASC-H cases, especially in women 40 years and older.

ASC-H interpretations associated with follow-up histologic diagnoses of CIN 2/3 have also varied significantly in the literature. Two relatively large studies, for example, reflect different extremes. McHale et al<sup>8</sup> reported a cumulative detection rate for CIN 2/3 of 12.2% in 229 ASC-H women after 12 months of follow-up. Barreth et al,<sup>16</sup> in contrast, reported that 70.2% of 517 ASC-H women had CIN 2+, including 2.9% cervical cancer and 1.7% adenocarcinoma in situ, in a shorter period of follow-up. Neither study reported hrHPV DNA detection data for these patients with ASC-H Pap test results. The CIN 2/3 histologic follow-up in women with hrHPV DNA-positive ASC-H has ranged from 25% to 58.7% in available reports (Table 7). The accumulated mean risk of biopsy-proven CIN 2/3 for 658 hrHPV DNA-positive ASC-H cases was 41.2%, compared with 4.9% for 265 hrHPV-negative ASC-H cases.

In the present study, histologic follow-up was available in 505 females with ASC-H. The CIN detection rate in women with positive hrHPV DNA testing was 62.3% (160/257), significantly higher than 14.1% (35/248) in females with negative hrHPV DNA testing ( $P < .001$ ). The PPV for biopsy-confirmed CIN 2/3 was 32.7% in hrHPV DNA-positive ASC-H cases, almost double the 17.2% PPV for ASC-H results when hrHPV DNA test results were unavailable. The negative predictive value of a negative hrHPV DNA test with ASC-H results was calculated at 98.8% in all cases, and 100% in women 40 years and older.

The highest CIN 2/3 detection rate was 46.3% in women 30 to 39 years old with hrHPV DNA-positive ASC-H results. A follow-up histologic diagnosis of CIN 2/3 was made in 21.1% of ASC-H cases from females younger than 40 years compared with a significantly lower rate of 7.6% in women 40 years and older (Table 3). The lower prevalence of hrHPV DNA infection in the older age group would increase the specificity of hrHPV DNA testing in the older age group. Additionally, the negative predictive value of a negative hrHPV DNA test in this age group (≥40 years) increased to 100%, implying that adjunctive hrHPV DNA testing after ASC-H Pap results would be useful in minimizing risk of high-grade dysplasia, espe-

cially in the older population. Even more limited ALTS data suggest that hrHPV DNA testing may be more useful in evaluating older women with ASC-H Pap results.<sup>2</sup>

The high negative predictive value of hrHPV DNA testing further suggests that women with a Pap result of ASC-H and a negative hrHPV DNA test may only need regular screening for follow-up as opposed to any more extensive workup. On the other hand, the low specificity of a positive result for hrHPV DNA-positive ASC-H cases indicates the need for caution in interpreting these results to patients.

There are different opinions regarding the utility of hrHPV DNA testing for triage of women with ASC-H. Combining the 2 screening tests (Pap testing and hrHPV DNA testing) allows stratification of the patients into low- and high-risk groups before deciding on referral to colposcopy. Colposcopy is a more invasive procedure, more expensive, and requires an additional patient visit. Many authors suggest that reflex hrHPV testing for the triage of women with ASC-H Pap tests would serve as a better method for selecting patients with ASC-H Pap results who should undergo immediate colposcopic examination.<sup>7,9,11,19,20,32,33</sup>

In our study, a small subset of hrHPV DNA-positive ASC-H cases had CIN 1 on subsequent histologic examination. This result is similar to findings reported by Srodon et al,<sup>11</sup> who found that 24.4% of women with hrHPV DNA-positive ASC-H had follow-up biopsy findings of low-grade dysplasia. In addition, approximately 12.9% of the females who had hrHPV DNA-negative ASC-H results were diagnosed with CIN 1 on follow-up colposcopic biopsy. A significant number of these females likely harbored the low-risk HPV subtypes, which are not detected by the hrHPV DNA HC2 test.

Our study also documents that the presence or absence of an EC/TZS in ASC-H cases did not affect either hrHPV DNA detection rates or histologic follow-up results. This finding is consistent with our recent report that HC2 hrHPV DNA detection in females with negative, LSIL, or HSIL TPPT results is independent of cytologic sampling of the transformation zone.<sup>42</sup>

In summary, results from our current, large ASC-H study indicate that hrHPV DNA testing has an extremely high negative predictive value for histologic CIN 2/3, reaching 100% in women 40 years and older, and almost doubles the PPV of an ASC-H Pap test result without hrHPV DNA testing. The recommendation for colposcopic referral for all females with ASC-H is reasonable if the laboratory has an associated hrHPV DNA detection rate similar to that noted in ALTS. Otherwise, reflex hrHPV DNA testing is a highly useful option for females with ASC-H Pap results. Routine testing for hrHPV DNA in conjunction with an ASC-H Pap test result appears to be useful and justified in formulating treatment options for these patients. Our data and other studies' results suggest that females with ASC-H TPPT results and negative HC2 hrHPV testing can be managed by follow-up with regular Pap and hrHPV DNA testing rather than universal colposcopy. The attractiveness of reflex hrHPV DNA testing and triage is especially striking for patients 40 years and older.

#### References

1. Solomon D, Nayar R. *The Bethesda System for Reporting Cervical Cytology*. 2nd ed. New York, NY: Springer-Verlag Publishers; 2004.
2. Sherman ME, Castle PE, Solomon D. Cervical cytology of atypical squamous

cells cannot exclude high grade squamous intra-epithelial lesion (ASC-H): characteristics and histologic outcomes. *Cancer (Cytopathol)*. 2006;108:298–305.

3. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*. 2002;287:2114–2119.

4. Lee CY, Ng WK. A follow-up study of atypical squamous cells in gynecologic cytology using conventional Papanicolaou smears and liquid-based preparations: the impact of the Bethesda System 2001. *Am J Clin Pathol*. 2007;127:548–555.

5. Tarai S, Siddiqui MT, West AM, et al. ASC-H correlation with reflex HC2 HPV test and biopsy findings [abstract]. *Mod Pathol*. 2005;18:79A.

6. Geisinger KR, Vrbin C, Grzybicki DM, Wagner P, Garvin AJ, Rabb SS. Interobserver variability in human papillomavirus test results in cervicovaginal cytologic specimens interpreted as atypical squamous cells. *Am J Clin Pathol*. 2007;128:1010–1014.

7. Gupta S, Sodhani P, Chachra KL, Singh V, Sehgal A. Outcome of “atypical squamous cells” in a cervical cytology screening program: implications for follow up in resource limited settings. *Diagn Cytopathol*. 2007;35:677–690.

8. McHale MT, Soutjer J, Elkas JC, Monk BJ, Harrison TA. Is atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesion clinically significant? *J Low Genit Tract Dis*. 2007;11:86–89.

9. Wu HH, Allen SL, Kirkpatrick JL, Elsheikh TM. Reflex high-risk human papilloma virus DNA test is useful in the triage of women with atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion. *Diagn Cytopathol*. 2006;34:707–710.

10. Duncan LD, Jacob SV. Atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion: the practice experience of a hospital-based reference laboratory with this new Bethesda system diagnostic category. *Diagn Cytopathol*. 2005;32:243–246.

11. Srodon M, Parry Dilworth H, Ronnett BM. Atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion: diagnostic performance, human papillomavirus testing and follow up results. *Cancer (Cytopathol)*. 2005;108:32–38.

12. Stany MP, Bidus MA, Reed EJ, et al. The prevalence of HR-HPV DNA in ASC-US Pap smears: a military population study. *Gynecol Oncol*. 2006;101:82–85.

13. Saad RS, Dabbs DJ, Kordunsky L, Kanbour-Shakir A, Silverman JF, Liu Y. Clinical significance of cytologic diagnosis of atypical squamous cells, cannot exclude high grade, in perimenopausal and postmenopausal women. *Am J Clin Pathol*. 2006;126:381–388.

14. Selvaggi SM. Reporting of atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion (ASC-H) on cervical samples: is it significant? *Diagn Cytopathol*. 2003;29:38–41.

15. Shidham VB, Kumar N, Narayan R, Brozman GL. Should LSIL with ASC-H (LSIL-H) in cervical smears be an independent category?: a study on Surepath specimens with review of literature. *Cytojournal*. 2007;4:7.

16. Barreth D, Schepansky A, Capstick V, Johnson G, Steed H, Faught W. Atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (ASC-H): a result not to be ignored. *J Obstet Gynaecol Can*. 2006;28:1095–1098.

17. Davey DD, Neal MH, Wilbur DC, Colgan TJ, Styer PE, Mody DR. Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology. *Arch Pathol Lab Med*. 2004;128:1224–1229.

18. Sherman ME, Solomon D, Schiffman M. Qualification of ASCUS: a comparison of equivocal LSIL and equivocal HSIL cervical cytology in the ASCUS/LSIL Triage Study. *Am J Clin Pathol*. 2001;116:386–394.

19. Liman AK, Giampolli EJ, Bonfiglio TA. Should women with atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion, receive reflex human papillomavirus-DNA testing? *Cancer (Cytopathol)*. 2005;105:457–460.

20. Reid-Nicholson M, Gatscha RM, Riedel ER, Lin O. Atypical squamous cells, cannot exclude high grade intraepithelial lesion (ASC-H): does HPV matter? *Diagn Cytopathol*. 2007;35:1–5.

21. Rowe LR, Aldeen W, Bentz JS. Prevalence and typing of HPV DNA by Hybrid Capture II in women with ASCUS, ASC-H, LSIL and AGC on ThinPrep Pap Tests. *Diagn Cytopathol*. 2004;30:426–432.

22. Palma PD, Pojer A, Girlando S. HPV triage of women with atypical squamous cells of undetermined significance: a 3-years experience in an Italian organized programme. *Cytopathol*. 2005;16:22–26.

23. Evans MF, Adamson CS-C, Papillo JL, St. John TL, Leiman G, Cooper K. Distribution of human papillomavirus types in ThinPrep Papanicolaou tests classified according to the Bethesda 2001 terminology and correlations with patient age and biopsy outcomes. *Cancer (Cytopathol)*. 2006;106:1054–1064.

24. Chivukula M, Shidham VB. ASC-H in Pap test-definitive categorization of cytomorphological spectrum. *Cytojournal*. 2006;3:14.

25. Owens CL, Moats DR, Burroughs FH, Gustafson KS. “Low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion” is a distinct cytologic category. *Am J Clin Pathol*. 2007;128:398–403.

26. You K, Liang X, Qin F, Guo Y, Geng L. High-risk human papillomavirus DNA testing and high grade cervical intraepithelial lesions. *Aust N Z J Obstet Gynaecol*. 2007;47:141–144.

27. Bonvicino A, Huitron S, Fadare O. Papanicolaou test interpretations of

“atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion.” *Cancer (Cytopathol)*. 2007;111:477–481.

28. El-Fakharany M, Al-Khafaji B, El-Dean MS. Atypical squamous cells, cannot exclude HSIL (ASC-H): the new term on the block: a year in review [abstract]. *Acta Cytol*. 2004;48:672.

29. Hoschar A, Kink I, Dawson AE. Atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion (ASC-H): correlation of morphologic features, reflex HPV testing and biopsy follow-up [abstract]. *Acta Cytol*. 2004;48:672.

30. O'Brien D, Brainard J, Chen L, Prok A, Booth C. Comparison of HPV and follow-up data for ASC-H before and after implementation of the ThinPrep imaging system [abstract]. *Cancer (Cytopathol)*. 2006;108(suppl):342.

31. Howard MT, Cohen DW, Underwood DL, Deeds DA, Booth CN. Utilization of ASC-H, LSIL, cannot exclude HSIL (LSIL-H) and HSIL terminology following implementation of the ThinPrep imaging system [abstract]. *Mod Pathol*. 2007;20(suppl 2):71A.

32. Christal JL, Avery D, Valente PT. Atypical squamous cells, cannot exclude HSIL (ASC-H); Is Digene Hybrid Capture a useful ancillary test? [abstract]. *Cancer (Cytopathol)*. 2006;108(suppl):360.

33. Nguyen C, Parveen Z, Pragasam P, Masood S, Zhang F. Potential role of age-directed human papillomavirus reflex testing in managing patients with atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion on cervical Pap smears [abstract]. *Cancer (Cytopathol)*. 2007;111(suppl):355.

34. Sullivan PS, Vu T, Lim SS, Hirschowitz SL, Rao J. Atypical squamous cells, cannot exclude HSIL (ASC-H): follow-up biopsy and HPV DNA testing [abstract]. *Cancer (Cytopathol)*. 2007;111(suppl):388–389.

35. Wright TC, Cox JT, Massad LS, Twigg LB, Wilkinson EJ. 2001 Consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA*. 2002;287:2120–2129.

36. Wright TC, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. *Am J Obstet Gynecol*. 2007;197:346–355.

37. The Bethesda System for reporting cervical/vaginal cytologic diagnoses. *Acta Cytol*. 1993;37:115–124.

38. Solomon D, Breen N, McNeel T. Cervical cancer screening rates in the United States and the potential impact of implementation of screening guidelines. *CA Cancer J Clin*. 2007;57:105–111.

39. 2006 U.S. Census Bureau American Community Survey (ACS). Available at: <http://www.census.gov/acs/www/>. Accessed April 3, 2008.

40. Dzura B, Quinn S, Richard K. Performance of an imaging system vs. manual screening in the detection of squamous intraepithelial lesions of the cervix. *Acta Cytol*. 2006;50:309–311.

41. Zhao C, Elishaev E, Yuan KH, Yu J, Austin RM. Very low human papillomavirus DNA prevalence in mature women with negative computer-imaged liquid-based Pap tests. *Cancer (Cytopathol)*. 2007;111:292–297.

42. Zhao C, Austin RM. Human papillomavirus DNA detection in ThinPrep Pap test vials is independent of cytologic sampling of the transformation zone. *Gynecol Oncol*. 2007;107:231–235.

43. Qudus MR, Sung CJ, Steinhoff MM, Lauchlan MB, Singer DB, Hutchinson ML. Atypical squamous metaplastic cells; reproducibility, outcome and diagnostic features on ThinPrep Pap test. *Cancer (Cytopathol)*. 2001;93:16–22.

44. Saad RS, Kanbour AI, Mauser N, Modery J, Dabbs DJ. Atypical squamous cells-cannot exclude high grade squamous intraepithelial lesion (ASC-H): diagnostic reproducibility, HPV positive rates and clinical implications [abstract]. *Mod Pathol*. 2005;18(suppl):76A.

45. Papillo JL, St. John TL, Lehman G. Effectiveness of the ThinPrep Imaging System: clinical experience in a low risk screening population. *Diagn Cytopathol*. 2008;36:155–160.

46. Belinson J, Quao YL, Pretorius R, et al. Shanxi Province cervical cancer screening study: a cross-sectional comparative trial of multiple techniques to detect cervical neoplasia. *Gynecol Oncol*. 2001;83:439–444.

47. Pan Q, Belinson JL, Li L, et al. A thin-layer, liquid-based Pap test for mass screening in an area of China with a high incidence of cervical carcinoma: a cross-sectional, comparative study. *Acta Cytol*. 2003;47:45–50.

48. Frable WJ. Litigation in gynecologic cytology. *Pathol Case Rev*. 2005;10:106–114.

49. Tworek JA, Jones BA, Rabb S, Clary KM, Walsh MK. The value of monitoring human papillomavirus DNA results for Papanicolaou tests diagnosed as atypical squamous cells of undetermined significance. *Arch Pathol Lab Med*. 2007;131:1525–1531.

50. Cibas ES, Hong X, Crum CP, Feldman S. Age-specific detection of high risk HPV in cytologically normal, computer-imaged ThinPrep Pap samples. *Gynecol Oncol*. 2007;104:702–706.

51. Solomon D, Schiffman M, Tarone R, for the ALTS Group. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline result from a randomized trial. *J Natl Cancer Inst*. 2001;93:293–299.

52. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer*. 2006;119:1095–1101.

53. Cibas ES, Browne TJ, Bassichis MH, Lee KR. Enlarged squamous nuclei in cervical cytologic specimens from perimenopausal women (“PM cells”): a cause of ASC overdiagnosis. *Am J Clin Pathol*. 2005;124:58–61.