CANCER
CYTOPATHOLOGY

Triage of Women With ASCUS and LSIL Cytology

Use of Qualitative Assessment of p16^{INK4a} Positive Cells to Identify Patients With High-Grade Cervical Intraepithelial Neoplasia

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BACKGROUND. The identification of a small percentage of high-grade cervical intraepithelial neoplasias (HGCIN) among patients with minor cytological abnormalities (atypical squamous cells of undetermined significance [ASCUS] and/or low-grade squamous intraepithelial lesions [LSIL] group) is a major problem in cytology-based cervical cancer screening. The authors investigated the efficacy of p16^{INK4a} as a biomarker to identify samples of patients with HGCIN among those with an ASCUS or LSIL result in Papanicolaou cytology.

METHODS. Consecutive liquid-based cytology specimens of 137 ASCUS and 88 LSIL results were selected from gynecologists who adopted a triage regimen with biopsy under colposcopy 2 months later, independent of the p16^{INK4a} result. p16^{INK4a} stained slides were prepared and independently read by 2 observers, who used a recently described score to categorize p16^{INK4a} stained squamous cells. The endpoint of the study was detection of a biopsy-confirmed HGCIN.

RESULTS. The overall sensitivity and specificity of p16^{INK4a} positive cells with a nuclear score >2 for diagnosis of HGCIN in ASCUS and LSIL cases combined was 96% and 83%, respectively. The sensitivity and specificity in the ASCUS group was 95% and 84%, and 100% and 81% in the LSIL group, respectively. Two observers had a high concordance in assessing p16^{INK4a} stained cells (κ value of 0.841).

CONCLUSIONS. These data suggested that the use of p16^{INK4a} as a biomarker combined with nuclear scoring of p16^{INK4a} positive cells in cervical cytology to triage ASCUS and/or LSIL cases allows identification of HGCIN with good sensitivity and specificity. ***Cancer (Cancer Cytopathol)* 2007;111:58–66.**

KEYWORDS: cervical screening, p16^{INK4a}, ASCUS, LSIL, triage, biomarker, liquid-based cytology, human papillomavirus (HPV).

Screening by the cytological test developed by George Papanicolaou has led to a remarkable reduction of incidence and mortality of cervical cancer over the past 40 years.[1] In the United States, 0.6% of all 55 million smears taken each year are being diagnosed as high-grade squamous intraepithelial lesions (HSIL), 2% to 3% are low-grade squamous intraepithelial lesions (LSIL), and about 5% are assigned the diagnosis of atypical squamous cells of undetermined significance (ASCUS).[2] Although ASCUS results describe equivocal tests, LSIL results represent primarily minor cellular aberrations due to acute or transient human papillomavirus (HPV) infections that resolve spontaneously in the majority of cases. However, despite these minor cytological abnormalities, a small number of these patients have high-grade cervical intraepithelial neoplasia (HGCIN) and, thus, require further diagnostic workup and treatment.[3] Because a small percentage of HGCIN are hidden in a large number of

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mildly abnormal smears, substantial expense and effort are required to identify these HGCIN. Three triage strategies have been proposed: 1) Repeat cytology 3-6 months after initial cytology, 2) Direct referral for colposcopy and biopsy, and 3) high-risk HPV testing.[4] The efficacy of these approaches has been analyzed in several epidemiological studies. For ASCUS results, a triage with HPV testing was shown to have higher sensitivity than repeat cytology.[5] As the vast majority of LSILs have an underlying HPV infection, HPV testing was not found to be effective in this indication.[2,6] New biomarkers with a sensitivity similar to HPV testing, but with a higher specificity, may permit more efficient triage and workup of samples with ASCUS and LSIL cytology.

An important event in the development of HGCIN of the cervix is the deregulation of high-risk human papillomavirus (HR-HPV) oncogene expression in basal and parabasal cells that induces major chromosomal instability and initiates clonal selection events that finally may result in malignant transformation.[7] HR-HPV E6 and E7 interfere with several cellular proteins involved in cell-cycle and apoptosis control. E7 binds to pRB and, thereby, releases E2F, a cell cycle activating factor. As a consequence, the cellular tumor suppressor p16^{INK4a} is strongly upregulated in HR-HPV–transformed cells.[8–11]

Several studies have used p16^{INK4a} immunostaining in cervical histopathology.[12,13] A diffuse p16^{INK4a} stain in basal and parabasal cell layers points to HR-HPV–induced transformation.[7,11] p16^{INK4a} positive low-grade cervical intraepithelial neoplasia (LGCIN) cases were shown to have a higher rate of progression to high-grade cervical intraepithelial neoplasia (HGCIN) when they were compared with p16^{INK4a} negative cases.[13–15]

Recently, p16^{INK4a} was used as a biomarker to identify HR-HPV–transformed cells in conventional and liquid-based cytology samples.[16,17] As few nondysplastic cells may also display p16^{INK4a} immunoreactivity,[18] additional criteria that discriminate p16^{INK4a} staining of abnormal cells from atrophic or metaplastic cells may increase the specificity of the p16^{INK4a} based cytology approach.

To overcome this limitation, we have proposed a qualitative score to assess p16^{INK4a} stained cells in liquid-based cytology specimens based on nuclear alterations of p16^{INK4a} positive squamous epithelial cells.[19] In work described in this article, we analyzed, by using the qualitative analysis of p16^{INK4a} stained cells, a series of ASCUS and LSIL specimens that had received colposcopy-guided biopsy 2 months after cytology.

MATERIAL AND METHODS

Patient Samples

A series of 225 consecutive ASCUS (137) and LSIL (88) cytology specimens were selected from the database of the Pasteur-Cerba (Paris, France) cytology laboratory. The laboratory proportions of abnormal cytology results in the year 2005 were: ASCUS, 2.51%; atypical squamous cells—cannot exclude high grade lesion (ASC-H), 0.25%; LSIL, 1.02%; and HSIL/carcinoma 0.49%. All samples were derived from patients of gynecologists who had adopted a triage regimen using colposcopy and biopsy 2 months after ASCUS or LSIL cytology result. There was no previous history of abnormal Papanicolaou smears in these patients.

Routine Cytology and Histology

The collection of cervical material was performed by gynecologists who used a flexible brush and rinsed the specimen directly into 20 mL of CYTO-screen System (C-S; Seroa, Monaco) fixative fluid. Slides were prepared and stained with the Papanicolaou method according to usual laboratory protocol.[20] The screening of cytology slides was first performed by the pool of cytotechnologists, and abnormal results were always confirmed by a pathologist (C.B.). Cytology was performed by using the Bethesda 2001 terminology system. For all cases, paraffin-embedded biopsy material was available obtained 2 months after the initial ASCUS/LSIL results. Biopsies were cut and stained with hematoxylin and eosin and subsequently read by a pathologist at the Pasteur Cerba laboratory and confirmed by a second independent reading at inclusion in the study. The diagnosis of LGCIN was used for disturbance of the architecture, cytological abnormalities, and abnormal mitotic figures limited to the lower third of the squamous epithelium (CIN 1). The diagnosis of HGCIN was used for abnormalities encompassing half (CIN 2) or more (CIN 3) of the squamous epithelium.

p16^{INK4a} Staining and Scoring

p16^{INK4a} staining was performed on a second set of slides prepared from the same C-S samples with the Dako CINtec cytology kit (Dako Cytomation, Glostrup, Denmark) according to the manufacturer's instructions. All slides were counterstained with hematoxylin (Dako Cytomation) to allow for the assessment of the nucleus. p16^{INK4a} stained slides were read and scored as previously described.[19] Only p16^{INK4a} stained squamous cells were taken into account. p16^{INK4a} positive squamous cells without any abnormality were given a score of 1. The reference for shape and structure of nuclei are normal intermediate cells. The following

nuclear abnormalities were assessed and taken into account: 1) Increased nucleocytoplasmic ratio: The nucleus had to be 50% or larger of the whole cell size. 2) Altered chromatin: The nuclear staining was hyperchromatic or hypochromatic compared with normal intermediate cells, or the chromatin distribution was altered and became granular and inhomogenous. 3) Altered nuclear shape and/or membrane structure: The nuclear shape showed irregularities like notches and indentations, or larger protrusions and grooves. 4) Anisonucleosis: $p16^{INK4a}$ stained cell groups and/or sheets showed divergent nuclear size, shape, and structure. When any single nuclear abnormality was present, cells were assigned a score of 2. For a score of 3, an increased nucleocytoplasmic (NC) ratio had to be present plus 1 of the above-mentioned alterations. When more than 1 alteration was observed in addition to the increased NC ratio in the same cell, a score of 4 was given. To identify sparse or single abnormal cells on a slide, by definition the score of the whole sample depended on the highest score given to any cell on the slide.

The $p16^{INK4a}$ stained cytology specimens were read by 2 observers independently. Observer 1 (MD, PhD, board-certified pathologist and cytologist) was a professional cytologist with a long experience in Papanicolaou cytology; Observer 2 (MD) had only little experience in Papanicolaou cytology at the time of the study. Both were trained in reading $p16^{INK4a}$ cytology according to the $p16^{INK4a}$ scoring system. Both observers read all slides blinded to the Papanicolaou diagnoses, blinded to the histology result, and blinded to the other observer's $p16^{INK4a}$ reading. All $p16^{INK4a}$ positive cells found on the slides were counted by Observer 2 during the initial locating of $p16^{INK4a}$ positive cells.

To analyze the correlation between $p16^{INK4a}$ cytology and histology, all biopsies were stained for $p16^{INK4a}$ by using the $p16^{INK4a}$ histology kit (Dako).

Statistics

Calculations of sensitivity and specificity were performed on the basis of the detection of HGCIN histology in ASCUS and LSIL cytology groups by applying $p16^{INK4a}$ cytology with different score cutoffs for both observers. Performance measures with confidence intervals for Observer 1 were calculated by applying the efficient score method with correction for continuity. Unweighted Cohen kappa (κ) statistics were used to calculate the interobserver agreement in the assessment of the $p16^{INK4a}$ scoring. The chi-square test was used to analyze the distribution of $p16^{INK4a}$ cytology and histology. Data analysis was carried out by using the SPSS statistical software package (SPSS, Chicago, Ill). Receiver operator characteristic (ROC) curve analysis was performed by calculating the sensitivity and specificity for different score cutoffs and for different $p16^{INK4a}$ positive cell counts.

RESULTS

Age Distribution and Histology of ASCUS and LSIL Cases

A series of 225 consecutive ASCUS and LSIL cases were included in the current study. The age distribution of these women was from 16 to 76 years (mean, 34 years) for all cases, from 16 to 76 years (mean, 34.6 years) for ASCUS cases, and from 17 to 52 years (mean, 33.1 years) for LSIL cases. The corresponding biopsy diagnoses were negative in 110 cases, LGCIN in 90 cases, and HGCIN in 25 cases. The 137 ASCUS specimens had a normal histology in 78 biopsies, LGCIN in 40 biopsies, and HGCIN in 19 biopsies. The 88 LSIL specimens had a normal histology in 32 biopsies, LGCIN in 50 biopsies, and HGCIN in 6 biopsies.

$p16^{INK4a}$ Positive Squamous Cell Counts

In total, 116 of 225 (52%) ASCUS/LSIL cases contained at least 1 $p16^{INK4a}$ positive squamous cell. The median $p16^{INK4a}$ positive squamous cell count on ASCUS slides was 1 (interquartile range, 0-10 cells), the median count on LSIL slides was 1.5 (interquartile range, 0-17.5 cells). Grouped by underlying histology, the median $p16^{INK4a}$ positive cell count in HGCIN cases was 22 (interquartile range, 9-35 cells), in LGCIN cases 1 (interquartile range, 0-11 cells), and in normal histology cases 0 (interquartile range, 0-5 cells) $p16^{INK4a}$ positive cells per slide. An ROC analysis was performed by using different $p16^{INK4a}$ positive cell count cutoff levels to detect HGCIN. The area under the curve for the quantitative approach to detect HGCIN was 0.808 (Fig. 1). The optimal tradeoff between sensitivity and specificity of $p16^{INK4a}$ cell counting to detect HGCIN was applying a cutoff level of >4 $p16^{INK4a}$ positive cells (Youden index, 0.52). At this cutoff, the sensitivity and specificity for detection of HGCIN were 84% and 68%, respectively.

Detection of HGCIN By using $p16^{INK4a}$ Cytology and Nuclear Scoring

Next we analyzed the performance of a qualitative assessment of $p16^{INK4a}$ positive cells by using the nuclear scoring described above to detect HGCIN in ASCUS/LSIL cytology cases. For both observers, sensitivity and specificity of the scores 1-4 as cutoff to detect HGCIN were determined, and ROC curves were calculated. The curves were very similar for both observers; the area under the curve for Observer

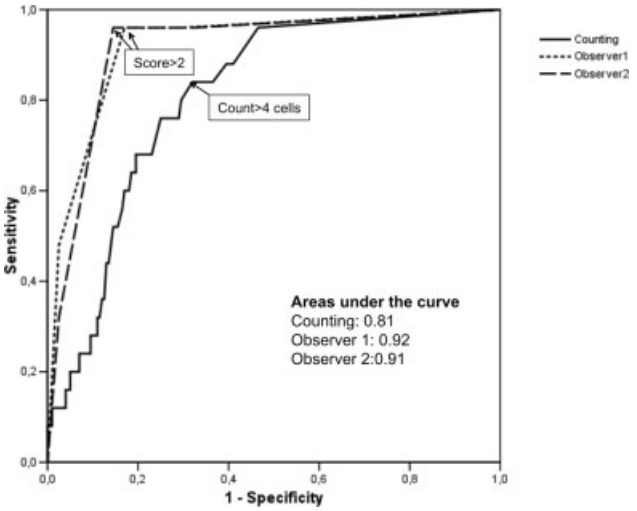

FIGURE 1. Receiver operator curve analysis for counting and scoring of $p16^{INK4a}$ positive cells to detect HGCIN and receiver operator curve analysis for the detection of HGCIN by quantitative and qualitative assessment of $p16^{INK4a}$ positive cells. Count: Sensitivity and specificity for detection of HGCIN were calculated for different $p16^{INK4a}$ positive cell counts. Observer 1 + 2: Performance of qualitative assessment with different score cutoffs for the detection of HGCIN. Areas under the curve were as follows: for counting, 0.81; for Observer1, 0.92; for Observer 2, 0.91. For all curves, the cutoff levels with the highest Youden score are marked on the chart.

1 was 0.918; for Observer 2, it was 0.912 (Fig. 1). For both observers, the best tradeoff between sensitivity and specificity was found when a score >2 was used to detect HGCIN (Youden index of 0.79 and 0.82, respectively). Applying this cutoff for Observer 1, the sensitivity was 95% (95% confidence interval [CI]: 72% to 100%), and the specificity was 84% (95% CI: 76% to 90%); for ASCUS, 100% (95% CI: 52% to 100%); for LSIL, 82% (95% CI: 71% to 89%); and for ASCUS and LSIL combined, 96% (95% CI: 78% to 100%) and 83% (95% CI:77% to 88%), respectively (Table 1). There were no statistically significant differences between observers. The patients included in the study were divided into 2 age groups (<30 years and ≥30 years), and the analysis of the performance of $p16^{INK4a}$ cytology was repeated. When ASCUS and LSIL were combined, a difference in sensitivity (86% vs 100%) was observed that was not statistically significant and only related to the single HGCIN case that was not detected by $p16^{INK4a}$ cytology in the age group <30 years. Further stratification of age-dichotomized data by cytology did not show any significant differences in the performance of $p16^{INK4a}$ cytology; however, the groups were much too small to draw any conclusion from these data (data not shown). There was no correlation between CIN grade 2 and CIN grade 3 and $p16^{INK4a}$ cytology score (Table 2).

TABLE 1
Performance of Observer 1 in Detecting HGCIN by $p16^{INK4a}$ Nuclear Score >2

	Sensitivity	Specificity	Referral	PPV	NPV
ASCUS, n = 137; 19 HGCIN					
$p16^{INK4a}$ >2	94.7	83.9	27	48.6	99
95% CI	71.9-99.7	75.7-89.8	20.0-35.4	32.3-65.3	93.8-99.9
LSIL, n = 88; 6 HGCIN					
$p16^{INK4a}$ >2	100	81.7	23.9	28.6	100
95% CI	51.7-100	71.3-89.1	15.7-34.4	12.2-52.3	93.2-100
Both, n = 225; 25 HGCIN					
$p16^{INK4a}$ >2	96	83	25.8	41.4	99.4
95% CI	77.7-99.8	77.0-87.8	20.3-32.1	28.9-55.0	96.2-99.9

PPV indicates positive predictive value; NPV, negative predictive value; ASCUS, atypical squamous cell of undetermined significance; HGCIN, high-grade cervical intraepithelial neoplasia; CI, confidence interval; LSIL, low-grade squamous intraepithelial lesion.

TABLE 2
Summary of CIN2 and CIN3 Lesions With Cytology Result and $p16^{INK4a}$ Cytology Counts and Scoring

Histology	Cytology	$p16^{INK4a}$ Count	$p16^{INK4a}$ Score*
CIN2	ASCUS	12	3
CIN2	ASCUS	27	3
CIN2	ASCUS	5	4
CIN2	ASCUS	35	3
CIN2	LSIL	25	3
CIN2	LSIL	187	4
CIN2	LSIL	22	3
CIN3	ASCUS	0	0
CIN3	ASCUS	16	4/3
CIN3	ASCUS	25	3
CIN3	ASCUS	1	3
CIN3	ASCUS	1	3
CIN3	ASCUS	20	4/3
CIN3	ASCUS	18	4
CIN3	ASCUS	32	4/3
CIN3	ASCUS	289	3
CIN3	ASCUS	23	4
CIN3	ASC-US	6	3
CIN3	ASC-US	10	3
CIN3	ASCUS	306	4
CIN3	ASCUS	3	4/3
CIN3	LSIL	9	4
CIN3	LSIL	113	3/4
CIN3	LSIL	52	4
CIN3	ASCUS	97	4/3

CIN indicates cervical intraepithelial neoplasia; ASCUS, atypical squamous cell of undetermined significance; LSIL, low-grade squamous intraepithelial lesion.

* In case of disagreement on the score between both observers, both scores are presented in the column (Observer1/Observer2).

Interobserver Variation for the Assessment of $p16^{INK4a}$ Stained Cytology Specimens

To analyze the reproducibility of the qualitative assessment of $p16^{INK4a}$ positive cells, unweighted κ

TABLE 3
Agreement in Qualitative Assessment of $p16^{INK4a}$ Positive Cells Between Both Observers

Observer 2	Observer 1					
Score	0	1	2	3	4	
0	92	16	0	1	0	109
1	8	15	4	1	0	28
2	4	6	18	7	0	35
3	1	0	3	29	7	40
4	0	0	0	3	10	13
Totals	105	37	25	41	17	225

κ for all categories is 0.61; κ for dichotomized scores with a cutoff >2 is 0.84.

values were calculated based on the $p16^{INK4a}$ scores assigned to the samples by both observers (Table 3). By using 5 score categories from 0 (ie, no $p16^{INK4a}$ staining) to a score of 4, the overall κ for agreement on single-score categories was 0.612 (κ standard error, 0.037). With dichotomized categories combined by scores 0–2 and scores 3 + 4, only 13 of 225 slides were scored differently by both observers. The κ value for interobserver agreement on the dichotomized scores was 0.841 (κ standard error, 0.067).

$p16^{INK4a}$ Immunohistochemistry

To analyze the correlation between $p16^{INK4a}$ cytology and histology, all biopsies were stained for $p16^{INK4a}$ and analyzed according to criteria described by Klaes et al.[10] Fifty-six samples showed diffuse staining for $p16^{INK4a}$. The 25 (100%) HGCIN cases displayed diffuse staining in all epithelial layers, 28 of 90 (31%) LGCIN cases and 3 of 110 (3%) normal cases displayed diffuse staining for $p16^{INK4a}$ in the basal and parabasal cell compartment. For the analysis of correlation between $p16^{INK4a}$ cytology and histology, $p16^{INK4a}$ cytology was considered positive when both observers found cells with a score >2 on the respective slides. There was a good correlation between $p16^{INK4a}$ histology and cytology (chi-square test for all cases combined was significant with $P < .001$; Table 4). Figures 2 and 3 display 2 representative HGCIN cases with corresponding abnormal $p16^{INK4a}$ positive cells in cytology specimens that exhibited a nuclear score >2 as well as the initial Papanicolaou slides that were diagnosed as ASCUS.

TABLE 4
Correlation of $p16^{INK4a}$ Histology and Cytology

	Histology	Negative	Total
HGCIN			
Cytology	diffuse positive	—	—
$p16^{INK4a}$ score >2	24	0	24
$p16^{INK4a}$ score ≤2	1	0	1
Total	25	0	25
LGCIN			
Cytology	diffuse positive	—	—
$p16^{INK4a}$ score >2	8	7	15
$p16^{INK4a}$ score ≤2	20	55	75
Total	28	62	90
Normal			
Cytology	diffuse positive	—	—
$p16^{INK4a}$ score >2	1	9	10
$p16^{INK4a}$ score ≤2	3	97	100
Total	4	106	110
All*			
Cytology	diffuse positive	—	—
$p16^{INK4a}$ score >2	33	16	49
$p16^{INK4a}$ score ≤2	24	152	176
Total	57	168	225

HGCIN indicates high-grade cervical intraepithelial neoplasia; LGCIN, low-grade cervical intraepithelial neoplasia.
* $P < .001$.

DISCUSSION

Identifying patients with HGCIN among women with ASCUS/LSIL cytology is an important task that determines the clinical and economical effectiveness of cervical cancer screening programs. Three distinct approaches have been proposed and are being used in different settings: Repeat cytology, HPV testing, and colposcopy-guided biopsy.[6] The impact of these different triage strategies on the detection of HGCIN and the cost efficiency of these models has recently been analyzed in detail by Arbyn et al.[5] Data obtained from this meta-analysis of several cervical cancer screening studies showed that high-risk human papilloma virus testing using the HC2 assay is more accurate in triaging women for HGCIN than repeat cytology with significantly higher sensitivity at similar specificity. Still, the referral rate of women who require further diagnostic evaluation by high-risk human papilloma virus testing is very high (35% to 55% depending on the population) compared with the low percentage of true HGCIN lesions hidden in the ASCUS group.[6] This finding is related to the high prevalence of high-risk human papilloma virus infections in sexually active women and results in low specificity of the human papilloma virus test for diagnosing HGCIN. A biomarker that differentiates transient human papilloma virus infections from persistent infections that have already initiated neoplastic transformation may overcome the current limitations of the ASCUS/LSIL triage.

$p16^{INK4a}$ is induced by aberrant expression of the high-risk human papilloma virus oncogenes in cervical basal and parabasal cells [8–11] and may be a more specific marker for HGCIN than the mere detection

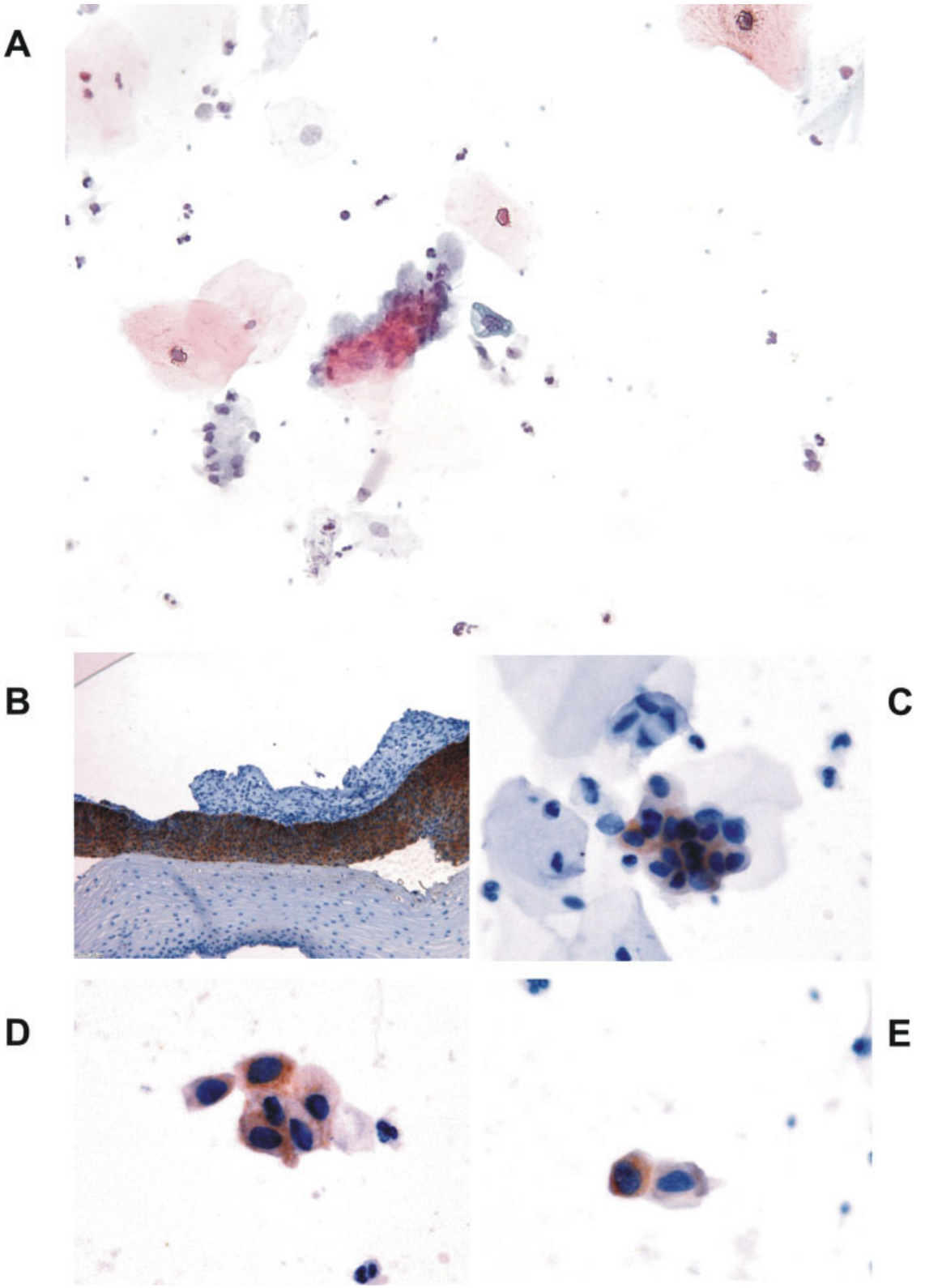

FIGURE 2. Papanicolaou staining, p16^{INK4a} histology, and p16^{INK4a} cytology of HGCIN Case 1. In Figures 2 and 3, two representative HGCIN cases are displayed. Both cases were diagnosed as ASCUS in liquid-based cytology. (A) Papanicolaou cytology, (B) p16^{INK4a} histology, (C-E) and 3 microscopic fields containing abnormal (score >2) p16^{INK4a} positive cells from the initial cytology specimens are shown.

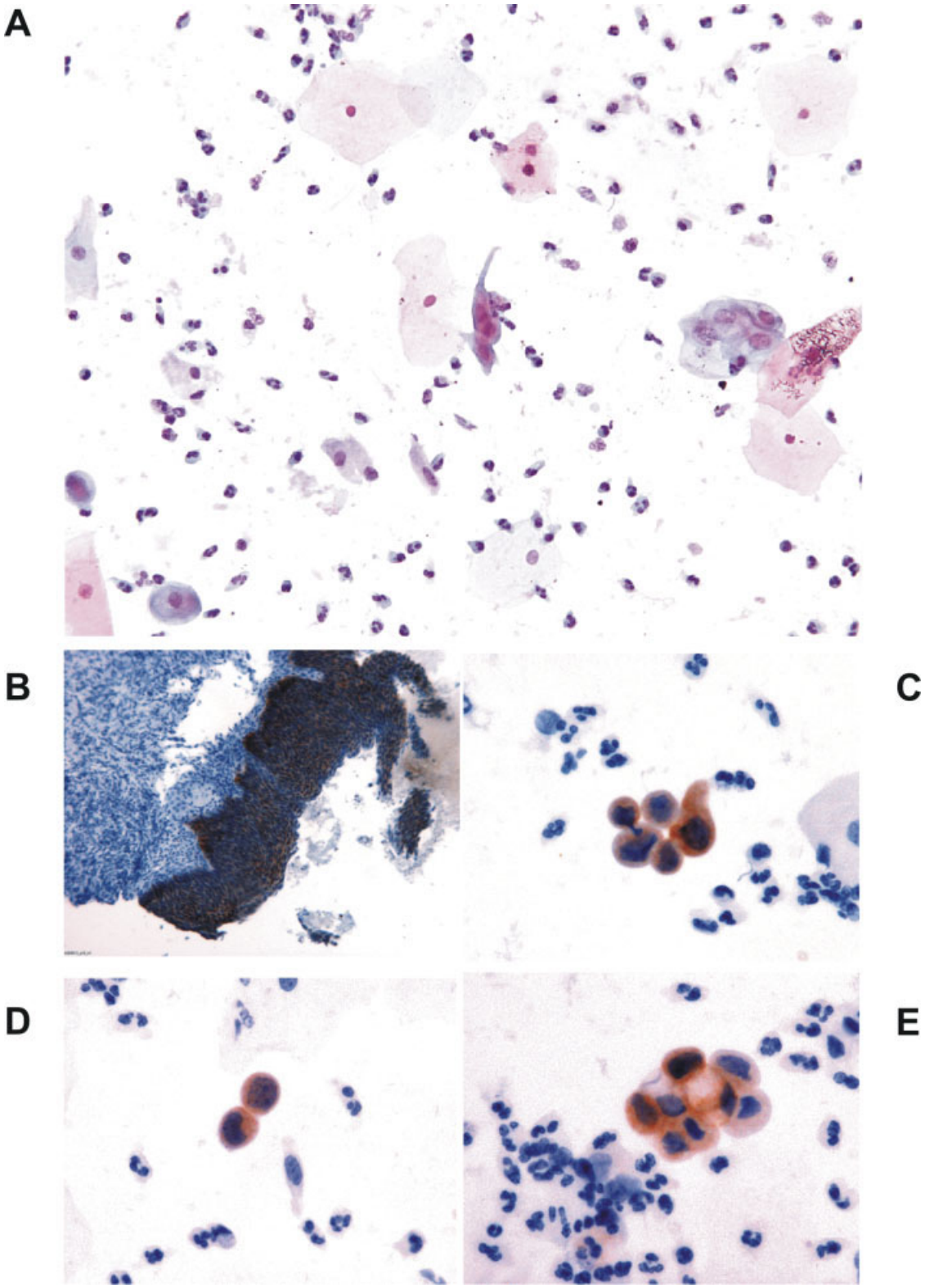

FIGURE 3. Papanicolaou staining, $p16^{INK4a}$ histology, and $p16^{INK4a}$ cytology of HGCIN Case 2. In Figures 2 and 3, two representative HGCIN cases are displayed. Both cases were diagnosed as ASCUS in liquid-based cytology. (A) Papanicolaou cytology, (B) $p16^{INK4a}$ histology, (C-E) and 3 microscopic fields containing abnormal (score >2) $p16^{INK4a}$ positive cells from the initial cytology specimens are shown.

of an high-risk human papilloma virus infection. Two other groups who have previously used p16^{INK4a} cytology to triage patients with ASCUS and/or LSIL cytology results for HGCIN have found a better performance of p16^{INK4a} cytology to detect HGCIN compared with human papilloma virus testing.[21,22] Carozzi et al have recently published a study that assessed p16^{INK4a} cytology in women with positive human papilloma virus results after inconclusive cytology. The combined triage approach had a higher positive predictive value than human papilloma virus testing alone, but it had lower sensitivity and was associated with a substantially higher cost.[23] In these initial studies, p16^{INK4a} cytology was quantitatively evaluated by counting p16^{INK4a} positive cells. Because few atrophic or metaplastic cells may express increased levels of p16^{INK4a}, we used qualitative criteria on the basis of nuclear aberrations of p16^{INK4a} stained cells to detect underlying HGCIN.[19] By applying the nuclear score on p16^{INK4a} positive cells, we noted that a statistically significant increase in specificity (from around 50% to 80%) was achieved. In addition, by using the qualitative approach, even single abnormal cells highlighted by p16^{INK4a} were sufficient to detect HGCIN.

In the current study, 1 observer was an experienced cytologist, whereas the other observer was less experienced. Despite their differing experience, both observers had a high agreement in independent readings. This suggests that identification and interpretation of p16^{INK4a} positive cells in cytological specimens may be more reproducible and may result in higher interobserver concordance of cytological diagnoses. A detailed interobserver variation analysis including a larger number of experts and less experienced observers is currently being performed.

The data obtained in this study indicate that p16^{INK4a} cytology may be an alternative to current triage strategies. Because the analyzed cases were triaged by colposcopy referral only, a direct comparison with human papilloma virus testing was not possible within the frame of this study. Human papilloma virus testing performed in a similar population by the same cytology laboratory 5 years ago[24] showed 86% sensitivity and 41% specificity for the detection of HGCIN by HC2 and 95% sensitivity and 40% specificity by high-risk human papilloma virus-specific polymerase chain reaction. To obtain further data on the performance of p16^{INK4a} cytology, a study that directly compares p16^{INK4a} and human papilloma virus testing needs to be performed.

The gold standard used in this study was colposcopy performed 2 months after cytology. It has been discussed that immediate colposcopy-guided biopsy could miss a number of prevalent HGCIN. This seems to be largely dependent on the size of the lesion and the experience of the colposcopist. In the ASCUS-LSIL Triage Study (ALTS) trial, immediate colposcopy detected only 53% of patients who developed a CIN3 lesion in 2 years of follow-up.[25] It is not clear whether the remaining lesions were missed during colposcopy or developed during the follow-up time.

To further assess the performance of the p16^{INK4a} based cytology approach to triage women with abnormal Papanicolaou results described in this study, studies with longer follow-up times are required and are currently being performed.

In conclusion, the p16^{INK4a} based morphological evaluation of cervical smears may be performed with a higher interobserver agreement than Papanicolaou cytology and may detect HGCIN cases with high sensitivity and specificity. It could help reduce the number of patients with ASCUS or LSIL results that require colposcopy and further clinical workup. In addition, p16^{INK4a} cytology may allow for improved automated preselection of suspicious slides compared with current automated Papanicolaou-based analysis.

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