Cytologic Patterns of Cervical Adenocarcinomas With Emphasis on Factors Associated With Underdiagnosis

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BACKGROUND: New cervical cancers continue to be diagnosed despite the success of Papanicolaou (Pap) tests. In an effort to identify pitfalls that limit the diagnosis of adenocarcinoma, the authors reviewed the cytologic characteristics of endocervical adenocarcinomas in their patient population. **METHODS:** Liquid-based cytology slides from 45 women who had concurrent, histologically confirmed cervical adenocarcinomas were reviewed retrospectively and semiquantitatively for 25 key cytologic traits. The original sign-out diagnosis, available clinical findings, and high-risk human papillomavirus (HR HPV) results also were noted. **RESULTS:** Abundant tumor cellularity, nuclear size from 3 to 6 times normal, abundant 3-dimensional tumor cell groups, round cell shape, and cytoplasmic neutrophils characterized the 23 cases that were identified correctly as adenocarcinomas. Key reasons for undercalls included low tumor cellularity and low-grade columnar morphology; these also tended to correlate with low-grade or unusual adenocarcinoma variants on histology. Overall, 73% of adenocarcinomas had a concurrent positive HR HPV test. **CONCLUSIONS:** Most endocervical adenocarcinomas can be diagnosed accurately in cases with classical features, but some cases continue to be problematic when evaluated based on cytologic features alone. Reflex HPV testing may help increase Pap test sensitivity for challenging cases that have atypical glandular cells of undetermined significance. Occasional cases with negative HR HPV test results remain of concern. ***Cancer Cytopathol* 2018;126:950-958.** © 2018 The Authors. *Cancer Cytopathology* published by Wiley Periodicals, Inc. on behalf of American Cancer Society.

KEYWORDS: adenocarcinoma, cancer screening, cytodiagnosis, Papanicolaou test, uterine cervical neoplasia.

INTRODUCTION

Despite the success of Papanicolaou (Pap) smear screening and the promise of human papillomavirus (HPV) vaccination, approximately 12,990 new cervical cancers were identified in the United States in 2016.[1] In an effort to improve our ability to recognize malignant cases in routine screening, we previously reported our experience with Pap test interpretation in a large population of women who were referred for workup of abnormal cervical cytology, including women with cervical carcinoma,[2] and observed an increase in suboptimal interpretations (ie, qualified adequacy and unsatisfactory) among women who had cancers compared with those who had less significant diagnoses. This was particularly true for women who had squamous cancers. We also observed that Pap tests from women who had adenocarcinomas were less likely to be recognized as

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malignant compared with those from women who had squamous cancers, although most were recognized with some degree of abnormality.

Subsequently, we compared the cellular patterns of squamous cancers and adenocarcinomas[3] and observed that squamous cancers had more pronounced tumor diathesis, which, with ThinPrep Pap tests (Hologic Inc, Marlborough, MA), was accompanied by differences in tumor cellularity. Specifically, the prominent tumor diathesis associated with squamous cancers was accompanied by tumor cells and fewer cells overall. Conversely, adenocarcinomas had less diathesis and had a larger population of normal-appearing endocervical cells.

Because the rates of cervical adenocarcinoma diagnoses appear to be increasing relative to squamous cancer diagnoses,[4,5] these findings led us to focus on the cytologic characteristics of adenocarcinomas in our patient population in an effort to identify features that may be associated with underdiagnosis of this lesion.

MATERIALS AND METHODS

The ThinPrep Pap tests originally were collected from 45 women who had concurrent histologic diagnoses of cervical adenocarcinoma as part of the Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED)[6–8] and the National Cancer Institute-University of Oklahoma Health Sciences Center Biopsy Studies.[9] Each woman provided informed consent, and the studies were approved by the institutional review boards of the University of Oklahoma Health Sciences Center and the National Cancer Institute. The 3015 study participants were women aged 18 to 85 years who were referred for follow-up of abnormal Pap tests or of a diagnosis of cancer between 2002 and 2010. Women who had undergone previous surgery for cervical neoplasia, those with human immunodeficiency virus infections, or those who were pregnant were excluded. At the time of study entry, participants provided informed consent and underwent ThinPrep Pap testing and colposcopy, with further treatment according to routine patient care guidelines. The ThinPrep Pap tests were originally evaluated in a routine fashion by staff cytotechnologists and study cytopathologists at the University of Oklahoma Medical Center laboratories according to the 2001 Bethesda guidelines.[10] An aliquot of each cytologic sample underwent HPV genotyping for 37 HPV genotypes using the Linear Array HPV Genotyping Test (Roche Molecular Diagnostics, Pleasanton, CA), as previously described.[8] HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered to be carcinogenic (high-risk [HR] HPV types). Adenocarcinoma cases that were interpreted as primary from the endometrium at hysterectomy were excluded from this study.

The 45 adenocarcinoma cases described here were extracted from our previous descriptions of this population.[2,3] The de-identified ThinPrep slides were retrospectively reviewed for key cytologic elements (see below) without knowledge of the original cytologic interpretation. For each case, each element was assessed semiquantitatively and scored as 0 (little or none), 1+ (some), 2+ (many/much), or 3+ (abundant).

We conducted chi-square tests to evaluate distributions of categorical variables. All statistical tests were 2-sided and considered to be significant at $P < .05$.

RESULTS

The key clinical characteristics of the patients with adenocarcinoma who were included in this study are provided in Table 1. It is noteworthy that 73.3% the patients were positive for HR HPV genotypes. The cytologic features analyzed retrospectively in our review of these slides are listed in Table 2.

The relative distribution of the various cellular characteristics observed in these cases of cervical adenocarcinoma is provided in Table 2. In aggregate, the characteristics most frequently recognized in this population of cervical adenocarcinomas include: abundant cells, including tumor cells; abundant 3-dimensional tumor cell groups; nuclear size 3 to 6 times normal; tumor diathesis; and small, definite nucleoli. Conversely, psammoma bodies and histiocytes rarely were noted. The remaining traits were variably present in this group of cases.

Although all cases in this study were histologically confirmed adenocarcinomas coincident with the Pap test, there were various interpretations at the time of original cytology sign-out. These included 23 cases correctly diagnosed as adenocarcinoma (CA); 15 cases as high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells (AGCs) or adenocarcinoma in situ (AIS) (high grade), and 6 were diagnosed as low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells (ASC), ASC cannot rule

TABLE 1. Key Clinicopathologic Features of 45 Patients With Cervical Adenocarcinoma

Trait	No. of Patients (%)
Age, y	
≤30	6 (13.3)
31-40	14 (31.1)
1-50	12 (26.7)
51-60	9 (20.0)
≥61	4 (8.9)
Stage	
IA	2 (4.4)
IB1	25 (55.6)
IB2	5 (11.1)
II	7 (15.5)
III	1 (2.2)
IV	2 (4.4)
Not done	3 (6.7)
FIGO grade	
1	10 (22.2)
2	14 (31.1)
3	15 (33.3)
Not done	6 (13.3)
HPV status	
High risk	33 (73.3)
Low risk	1 (2.2)
Negative	10 (22.2)
Not done	1 (2.2)
Original impression	
Adenocarcinoma	24 (53.3)
Squamous carcinoma	0 (0.0)
AGCs	11 (24.4)
HSIL	4 (8.9)
ASC-H	1 (2.2)
ASCUS	2 (4.4)
Negative	2 (4.4)
Unsatisfactory	1 (2.2)
Adequacy	
Adequate	44 (97.8)
Unsatisfactory	1 (2.2)
Limiting factors	
Bloody	4 (8.9)
Hypocellular unsatisfactory	1 (2.2)
No T-zone	6 (13.3)
Inflammation	1 (2.2)
None	34 (75.5)

Abbreviations: AGCs, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion.

out HSIL (ASC-H), or negative for intraepithelial lesion or malignancy (less than high grade). The latter cases were interpreted as undercalls for the purposes of the current study. One case was unsatisfactory because of hypocellularity.

In an effort to identify factors that facilitated or hampered the identification of adenocarcinoma, we compared the various cytologic characteristics in cases with the various diagnostic interpretations. The key findings from these comparisons are provided in Table 3.

Cases Correctly Identified as Adenocarcinoma

When comparing findings from the 23 cases that were correctly identified as adenocarcinoma at the time of original sign-out compared with findings from lesser diagnoses, most cases (19 of 23 cases; 82.6%) that were interpreted correctly as cancer had *many* or *abundant* tumor cells compared with cases that had lesser diagnoses ($P = .02$).

Similarly, most cases that were interpreted correctly as adenocarcinoma (17 of 23 cases; 73.9%) had *many* or *abundant* tumor cells with a round/oval appearance, possibly indicating a higher grade compared with 6 of 22 cases (27.3%) that had lesser diagnoses ($P = .002$).

Among the 23 cases that were identified correctly as adenocarcinoma at the time of original sign-out, compared with those of lesser diagnoses, the dominant, key cytologic features included overall greater tumor cellularity (19 of 23 cases; 82.6%); cohesive, 3-dimensional cell groups (19 of 23 cases; 73.9%); and round-oval tumor cell shape (17 of 23 cases; 73.9%) (Table 3). In contrast, the cases that were interpreted correctly as cancer had fewer normal-appearing endocervical cells and fewer tumor cells with a columnar configuration (Table 3). Overall, 19 of these cases (82.6%) harbored HR HPV genotypes.

Cases Interpreted as Less Than HSIL

Six cases were interpreted originally as less than high grade and, for the purposes of the current study, are considered undercalls. The results from a comparison between these 6 cases and cases that were diagnosed as cancer are provided in Table 3. The features that most clearly distinguished the cases interpreted as adenocarcinoma from these 6 cases were increased tumor cellularity; increased presence of cohesive, 3-dimensional cell groups; round-oval tumor cell shape; and cell groups with frayed edges. The cases with lesser diagnoses were noteworthy for having fewer tumor cells and for having columnar-shaped tumor cells.

An additional case that was interpreted as unsatisfactory for cytologic diagnosis because of insufficient cellularity was not included in the above comparison. Four of these 7 cases (57%) were positive for HR HPV DNA, although 2 were unusual histologic types that did not appear to be associated with HPV (Table 4).

We re-reviewed the ThinPrep slides from these 7 cases after the analysis in an effort to understand the

TABLE 2. Relative Distribution of Cytologic Features in 45 Cases of Cervical Adenocarcinoma

	Score (%)			
Trait	0: Few/None	1+: Some	2+: Many	3+: Abundant
Overall tumor cells	0 (0.0)	15 (33.3)	12 (26.7)	18 (40.0)
Normal endocervical cells	14 (31.1)	21 (46.7)	10 (22.2)	0 (0.0)
Background				
Blood	14 (31.1)	15 (35.6)	12 (26.7)	3 (6.7)
RBCs	13 (28.9)	14 (31.1)	15 (33.3)	3 (6.7)
Diathesis	5 (11.1)	13 (28.9)	9 (20.0)	18 (40.0)
Inflammation	4 (8.9)	14 (31.1)	19 (42.2)	8 (17.8)
Histiocytes	31 (68.9)	12 (26.7)	2 (4.4)	0 (0.0)
Psammoma bodies	45 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tumor cell pattern				
3-D groups	2 (4.4)	11 (24.4)	19 (42.2)	13 (28.9)
Single atypical cells	10 (22.2)	26 (57.8)	9 (20.0)	0 (0.0)
Tumor cell morphology				
Columnar	16 (35.6)	11 (24.4)	12 (26.7)	6 (13.3)
Round/oval	2 (22.2)	20 (44.4)	14 (31.1)	9 (20.0)
Frayed edges	10 (22.2)	18 (40.0)	13 (28.9)	4 (8.9)
Loss of polarity	3 (6.7)	18 (40.0)	17 (37.8)	7 (15.6)
Abundant cytoplasm	7 (15.6)	21 (46.7)	14 (31.1)	3 (6.7)
Cytoplasmic mucin	34 (75.6)	9 (20.0)	2 (4.4)	0 (0.0)
Polys in cytoplasm	25 (55.6)	15 (33.3)	4 (8.9)	1 (2.2)
Nuclear size				
Nearly normal	19 (42.2)	19 (42.2)	5 (11.1)	2 (4.4)
4-6 Times normal	2 (4.4)	9 (20.0)	22 (48.9)	12 (26.7)
>6 Times normal	9 (20.0)	21 (46.7)	9 (20.0)	6 (13.3)
Mitotic figures	23 (51.1)	16 (35.6)	6 (13.3)	0 (0.0)
Nucleoli				
Large single	15 (33.3)	14 (31.1)	11 (24.4)	5 (11.1)
Multiple	22 (48.9)	18 (40.0)	5 (11.1)	0 (0.0)
Small, definite	4 (8.9)	13 (28.9)	16 (35.6)	12 (26.7)
Inconspicuous	24 (53.3)	17 (37.8)	4 (8.9)	0 (0.0)

Abbreviations: 3D, 3-dimensional; RBCs, red blood cells.

reasons for the undercalls, and the summaries are provided in Table 4 along with representative images in Figure 1. Overall, there were 2 major reasons for the undercalls: 1) few tumor cells and 2) low tumor grade with bland-appearing tumor cells. Both problems were present in some of these cases.

The pattern of hypocellularity in malignant ThinPrep Pap tests with dense rings of diathesis demarcating an empty central portion has been well described.[11,12] We highlighted this as a particular problem for squamous cell cancer cases.[3] We observed that this was a lesser problem for ThinPrep slides from adenocarcinoma cases; however, 1 case in this group of undercalls (a clear cell carcinoma) had the same pattern of hypocellularity (Fig. 1A). In addition, other cases exhibited an unremarkable pattern of overall cellularity but had few recognizable tumor cells, suggesting sampling issues. In these cases, the number and characteristics of the tumor cells were insufficient to trigger the recognition of a significant lesion.

In some cases, small groups of tightly cohesive tumor cells were observed (Fig. 1B), but these were smaller than the large groups typically observed in adenocarcinoma. In addition, it was difficult to evaluate the nuclear features of the cells in these groups because of crowding. Only case 6 (Fig. 1G) had a rare group that could have raised concern for possible adenocarcinoma; however, this was only recognized on re-review.

The second problem relates primarily to well differentiated tumors of usual endocervical type in which the cytologic characteristics of the tumor cells overlap with benign, reactive changes. Recognition of these cases also was hampered by various degrees of acute inflammation. In addition, nuclear criteria were bland, with minimally enlarged nuclei; a bland, uniform chromatin pattern; and small nucleoli. Typically, the undercalled cases in our population had a combination of few tumor cells and small tumor cell groups as well as bland cytologic features. These cases also had subtle patterns of amorphous debris and ghost erythrocytes that helped obscure

TABLE 3. Key Cytologic Traits Differentiating Adenocarcinoma Interpretations From Cases With Lesser Diagnoses

	Carcinoma, N = 23	Less Than Carcinoma, N = 15		Less Than High Grade, N = 6	
Trait	No. (%)[a]	No. (%)[a]	*P*	No. (%)[a]	*P*
Overall tumor cellularity	19 (82.6)	8 (53.3)	.052	3 (50.0)	.04
Cohesive 3D cell groups	19 (82.6)	10 (66.6)	.26	3 (50.0)	.04
Round/oval cell shape	17 (73.9)	4 (26.6)	.01	1 (16.7)	.005
Groups with frayed edges	11 (47.8)	6 (40.0)	.64	0 (0.0)	.02
Columnar shape	5 (21.7)	8 (53.3)	.04	4 (66.7)	.01
Normal appearing endocervical cells	2 (8.7)	6 (40.0)	.02	2 (33.3)	.18

Abbreviation: 3D, 3-dimensional.

[a]Values indicate the sum of the number of cases with the indicated trait showing "many" and "abundant" cells

the cellular detail in the dense cell groups. Although this latter finding can raise the index of suspicion and cause the cytologist to search for more diagnostic criteria in the slide, this pattern can be observed in benign cases and cannot be relied upon alone to identify a high-risk glandular lesion. Overall, the cytologic characteristics of these undercalled cases were insufficient to warrant a diagnosis of adenocarcinoma even on re-review.

DISCUSSION

Previous studies analyzing atypical glandular cells in Pap tests, including liquid-based Pap tests, have reported difficulties in accurate interpretation, both in detection and in accurate categorization. These difficulties involved discrimination of glandular lesions from squamous lesions[13] and from benign glandular mimics.[14–19] Our previous studies with cytologic diagnosis of cervical cancers have reported greater difficulty in recognizing adenocarcinomas compared with squamous cancers.[2,3]

The current study was undertaken in an effort to increase our ability to recognize the salient features of cervical adenocarcinoma and its precursors on cytologic screening and to identify possible reasons for undercalls. The key cytologic criteria that allowed recognition of adenocarcinoma in this study reflect published criteria, including abundant, large, atypical tumor cell groups with atypical single cells. Notably, tumor diathesis was generally present but, as we have previously reported, to a lesser extent than for squamous cancers.[2] When we reevaluated the cases that we considered to be undercalled, the difficulties generally related to hypocellularity (particularly of tumor cells, even if overall cellularity was satisfactory) and well differentiated adenocarcinomas with columnar cells and minimal atypia. Although the current cytologic criteria for adenocarcinoma[20] were recognized in most of the cases in this study, we reluctantly conclude that some cases continue to be problematic upon reevaluation using cytologic features alone.

There have been various reports describing criteria for distinguishing benign glandular mimics, including reactive inflammatory changes, microcystic glandular hyperplasia, and ciliated metaplasia from glandular neoplasia.[14,17–19] Lee and colleagues[14] described a case series in which glandular lesions were both overcalled and undercalled based on cytologic criteria alone, and a subsequent report[17] documented a high level of interobserver disagreement for cases interpreted as AGC. Schoolland et al[21] reported significant sampling and diagnostic errors in 36 smears from 24 women with cervical adenocarcinoma. Ruba et al[22] described sampling and interpretive errors for the cytologic diagnosis of AIS and concluded that diagnostic errors were associated with the presence of only a few, poorly preserved, abnormal cells and an overly conservative approach to the assessment of abnormal glandular groups/cells. In comparing the screening histories of women with cervical cancer, Pak and colleagues[23] observed that women who had adenocarcinoma had been screened more regularly and were more likely to have negative Pap test results than those who had squamous cancers. Thus, although adenocarcinoma and AIS can be diagnosed accurately in a large number of cases with classical features of malignancy, a variable number of cases can be missed because of sampling and interpretative errors when evaluated by cytology alone.

Because of the variation in histologic cell types and the variety of cytologic patterns of cervical adenocarcinoma, it occurred to us that sensitivity could be improved with an adjunct test like HPV, which is more

TABLE 4. Characteristics of Cases With Undercalled ThinPrep Papanicolaou Tests

No.	Patient	Pap Diagnosis	Histology	Grade	Size, cm	HPV Type(s)	Comment
1	Age 36 y, WF, G0	Unsatisfactory	Clear cell adenocarcinoma, stage IB1	NA	1.0	Negative	Excess blood and very low cellularity; several large cell groups present (>30 cells) in bloody rim were all monomorphic (a clue that this was not a normal cell population)
2	Age 35 y, WF, G3	NILM	Cervical adenocarcinoma, usual type, stage IB1	1	1.0	16 and18	Very scant tumor cells; small lesion; tightly cohesive cell groups with overlapping nuclei
3	Age 57 y, WF	NILM	Cervical adenocarcinoma, usual type, stage IB1	2	1.4	16	The most prominent part of this case was the light diathesis in the background; rare, large groups of glandular cells with rim of ghost RBCs
4	Age 67 y, WF	ASC with obscuring inflammation	Poorly differentiated carcinoma, concerning for carcinosarcoma	3	3.0	Negative	Only rare epithelial cells present with a rim of mostly neutrophils; the epithelial cells do not exhibit definite malignant features; some laboratories could call unsatisfactory
5	Age 54 y	ASC-US	Cervical adenocarcinoma, usual type, stage IB1	1	1.0	16	Sheets of atrophic squamous cells are distractors; some large, 3-D groups of small glandular cells with tight, overlapping nuclei; little atypia; rare apoptotic cells present
6	Age 35 y	ASC-H	Cervical adenocarcinoma, usual type, stage IB1	1	1.2	18	Many of the tumor cells have bland chromatin and multiple small nucleoli; oval cytoplasm; some with tails; although many tumor cells are enlarged, others overlap with normal size nuclei; prominent neutrophils in background; several cell groups have definite mucin globules with signet ring features; diathesis inconspicuous in this case, although a few aggregates of ghost RBCs are present
7	Age 35 y	ASC-H	Mixed cervical adenocarcinoma, usual type and small-cell neuroendocrine, stage IVB	1	Not done	16	At low power, the background looks clean; however, at higher power, aggregates of ghost RBCs can be observed; very rare, small, highly cohesive cell groups are present that easily can be overlooked; small coagulum of fibrin, PMNs and occasional small epithelial cells are unusual

Abbreviations: 3D, 3-dimensional; ASC-H, atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; G, gravidity; HPV, human papillomavirus; NA, not applicable; NILM, negative for intraepithelial lesion or malignancy; Pap, Papanicolaou test; PMNs, polymorphonuclear leukocytes; RBCs, red blood cells; WF, white female.

objective. Recent, large, international studies of cervical adenocarcinomas reported that approximately 62%[24] of cervical adenocarcinomas harbor HR HPV DNA, including 71.8%[25] of adenocarcinomas of the usual type. A pooled analysis of HPV screening trials suggested that HPV screening, versus cytology screening, can lead to a stronger relative reduction of adenocarcinomas compared with squamous cell cancers.[26]

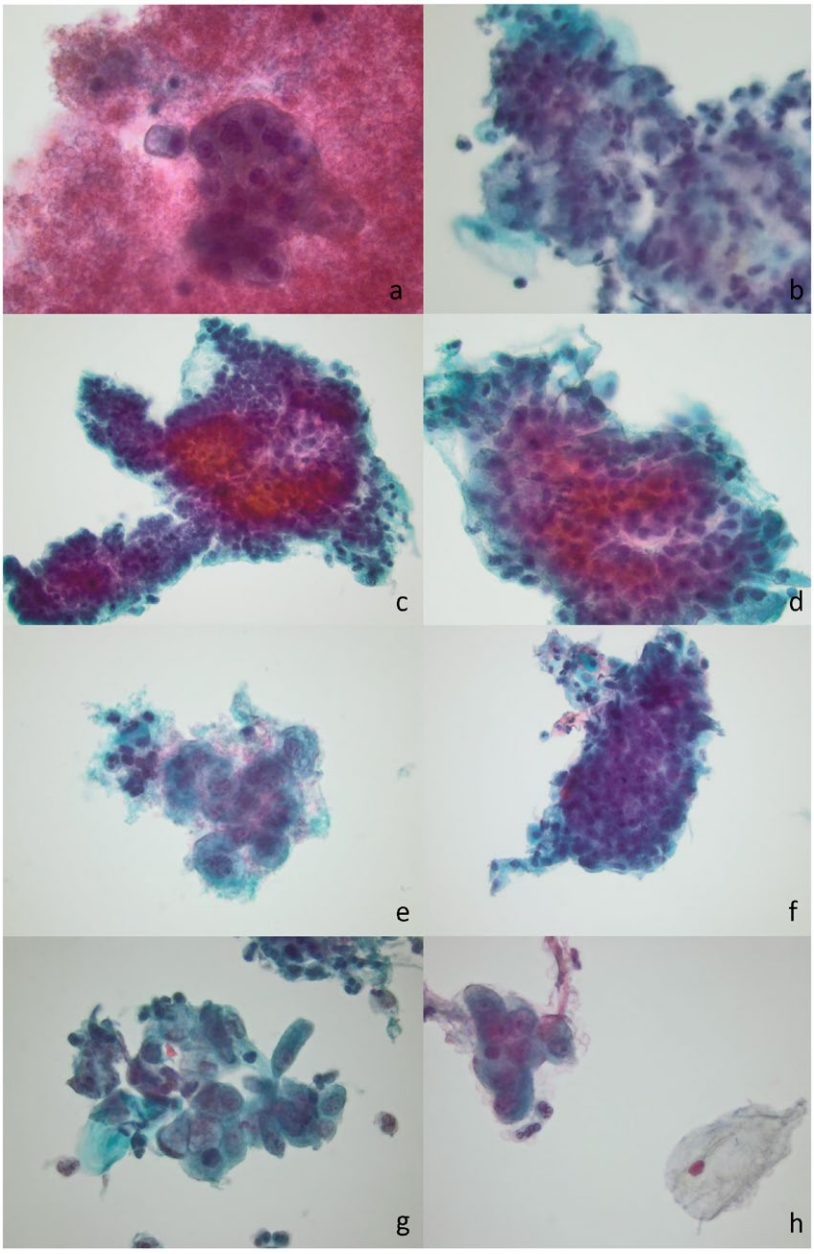

Figure 1. These are images from cases that had undercalled Papanicolaou tests (case numbers correspond to Table 4). (A) Case 1: Dense, bloody diathesis obscures a large monomorphic cell group in a hypocellular specimen (original magnification ×60). (B) Case 2: A tightly cohesive group has overlapping nuclei such that nuclear detail is obscured (original magnification ×40). (C,D) Case 3: Light diathesis is observed with a rare, large, highly cohesive group of glandular cells that have obscured cellular detail (original magnification ×20 in C, ×40 in D). (E) Case 4: A rare, small epithelial group has neutrophils in a sparsely cellular sample (original magnification ×40). (F) Case 5: This image shows a rare, large, 3-dimensional group of crowded but minimally atypical glandular cells with abundant atrophic squamous sheets (original magnification ×40). (G) Case 6: This pattern with enlarged nuclei and prominent intracytoplasmic mucin was rare in this sample (original magnification ×60). (H) Case 7: Very scant, small, cohesive cell groups with a clean background are easy to overlook (original magnification ×40).

When the Bethesda 2001 guidelines introduced HPV testing into cervical cancer screening as a reflex test, it was aimed at efficiently triaging the large number of ASC cases, but it did not include AGC.[27] Current guidelines for the follow-up of AGC call for referral to colposcopy.[28] Consequently, some cytologists may use a higher threshold for AGC compared with that for ASC. Earlier studies have indicated that AGC is associated with a high rate of high-grade lesions, particularly HSIL.[14] This may be influenced in part by conservative interpretation of AGC to prevent unnecessary colposcopy visits and loop electrosurgical excision procedures.

The results of our current study cause us to question whether the use of HPV testing for AGC should be reconsidered. Because HPV testing is widely used for ASC reflex and as a cotest for women over age 30 years, reflex HR HPV testing for AGC could increase our sensitivity for cervical glandular neoplasia with minimal cost and inconvenience. Four European countries have included AGC as an indication for triage to HPV testing in some European countries.[29] On the basis of the earlier literature[15,17,18] and our own experience, this high threshold for AGC may result in the underinterpretation of precancer and small, well differentiated lesions as reactive changes in an effort to avoid unnecessary colposcopies for reactive lesions.

Reflex HPV testing for AGC could be added to the follow-up algorithm as an initial step to distinguish HR HPV-associated cervical lesions from benign mimics and endometrial lesions. Thus, the threshold for AGC could be lowered as well if the concern for unnecessary colposcopies is removed. Additional safeguards can continue to be included for HPV-negative cases to detect endometrial lesions. Previous authors have reported the utility of HPV testing in AGC cases with favorable results.[16,30,31] Ronnett et al[16] reported that the combination of cytology interpretation and HPV testing was associated with a high rate of detection of significant glandular lesions. In our sample, 33 of 45 cytologic samples (73%) of cervical adenocarcinomas were positive for HR HPV. Thus, the detection of adenocarcinoma and precursors in a screening test can reasonably be expected to be improved by HPV testing of AGC Pap tests. Kinney and colleagues[32] have reported adenocarcinoma cases in their cotesting experience that were detected by a positive HPV test when cytology was negative. In addition, the accuracy and reproducibility of colposcopy as the gold standard for lesion detection has been questioned.[33] However, it also should be noted that a few cervical adenocarcinomas may not be detected by HR HPV testing. Indeed, 2 cases on our problematic case list were cancers of an unusual type that were not associated with HR HPV. This can be caused by false-negative HPV DNA tests or the presence of HPV-negative cancers, including special types, such as clear cell or serous cancers. The detection of these cases will continue to rely on astute cytologic evaluation and clinical parameters.

In summary, although most cervical adenocarcinomas can be detected by using classic cytologic criteria, recognition of some cases is hampered by subtle tumor diathesis, few tumor cells, and very bland cytologic features in well differentiated tumors. We propose that reflex HR HPV testing for AGC, as for ASC, can help to increase sensitivity for low-grade adenocarcinomas of the usual type, including precursor lesions, although guidelines should remain for the identification of HPV-negative cervical cancers and endometrial cancers.

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CONFLICT OF INTEREST DISCLOSURES

Nicolas Wentzensen is employed by the National Cancer Institute and has received cervical cancer screening assays in-kind or at reduced cost from Becton-Dickinson and Roche outside the submitted work. Mark Shiffman has received equipment and reagents as well as cervical human papillomavirus testing, cytology, and visual images for independent studies from Roche, Becton-Dickinson, Qiagen, and MobileODT. The remaining authors made no disclosures.

AUTHOR CONTRIBUTIONS

Rachel D. Conrad: Investigation and writing–review and editing. **Angela H. Liu:** Formal analysis and writing–review and editing. **Nicolas Wentzensen:** Conceptualization, formal analysis, and writing–review and editing. **Roy R. Zhang:** Conceptualization, and writing–review and editing. **S. Terence Dunn:** Writing–review and editing. **Sophia S. Wang:** Conceptualization and writing–review and editing. **Mark Shiffman:** Conceptualization and writing–review and editing. **Michael A. Gold:** Conceptualization and writing–review and editing. **Joan L. Walker:** Conceptualization and writing–review and editing. **Rosemary E. Zuna:** Conceptualization, investigation, writing–original draft, and writing–review and editing.

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