



ORIGINAL ARTICLE

Challenges in the Pap diagnosis of endocervical adenocarcinoma in situ

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Introduction Recognition of adenocarcinoma in situ (AIS) in cervical cytology is challenging.

Materials and methods We calculated the sensitivity and accuracy of Papanicolaou (Pap) tests obtained within 1 year of a histologic diagnosis of AIS from 2007 to 2016. We also correlated it with the coexistence of squamous lesions, calculated the interobserver agreement, and compared these measures with those of endocervical adenocarcinoma (ECCA). We correlated AIS detection with high-risk human papillomavirus (hrHPV) status.

Results Of 72 patients with histologic AIS and 48 patients with ECCA, 92% and 87.5%, respectively, had abnormal Pap test results. A glandular abnormality was detected in 44.4% of the AIS and 77.1% of the ECCA cases. Complete cytohistologic concordance was reached in 8.3% of AIS and 22.9% of ECCA cases. In addition, 27.8% of AIS and 6.3% of ECCA cases were diagnosed on Pap as a high-risk squamous abnormality. Concurrent squamous lesions were present in 79.2% of patients with AIS and 29.2% of patients with ECCA. The Paps from the AIS and ECCA cases were diagnosed as pure squamous abnormalities in 47.2% and 10.4% of cases, respectively. In the AIS cases, interobserver agreement was substantial for detection of any high-risk cytologic abnormality ($\kappa = 0.67$) and fair for detection of any glandular abnormality ($\kappa = 0.34$). Among the 26 patients with AIS tested for hrHPV, 92% had positive results and 8% had negative results.

Conclusions The cytologic sensitivity for the detection of AIS remains low. It is directly related to the coexistence of squamous lesions. Cytology and hrHPV as stand-alone screening tests fail in the early detection of a small proportion of glandular lesions, although combined testing will improve their detection rates.

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Introduction

The success of Papanicolaou (Pap) cytology in the dramatic reduction of the incidence and mortality from cervical cancer has been mainly attributable to the early detection and treatment of preinvasive squamous lesions. Although cervical glandular neoplastic lesions, including adenocarcinoma in situ (AIS) and invasive endocervical adenocarcinoma (ECCA), are less common than squamous neoplasia, their proportion has increased from 5% to 25% of all cervical cancer diagnoses in the United States during the past few decades.¹⁻⁴ AIS is the precursor of invasive adenocarcinoma, and there is an interval of at least 5 years between clinically detectable AIS and invasive disease for most cases.^{5,6} Thus, the early detection and management of AIS are crucial for preventing invasive adenocarcinoma.

Patients with AIS are nearly always asymptomatic, and the lesions are generally not visible on routine pelvic examination. The diagnosis typically relies on cervical cytologic screening. Cytologically, typical AIS cells are arranged in groups and strips with rosette formation, feathering, crowding and/or pseudostratification, elongated nuclei, inconspicuous nucleoli, and increased nucleus/cytoplasm ratio.⁷ However, these characteristic features are not always present or easily recognized. In addition, these classic features refer to the precursor lesions of the usual type of adenocarcinoma, while the cytologic features of less common types (eg, minimal deviation, gastric, intestinal, signet ring) are still poorly characterized. Compared with invasive endocervical adenocarcinoma, which is detected with 45% to 76% sensitivity in cytology specimens,⁸⁻¹⁰ the reported sensitivity for the detection of AIS has only been 38% to 56%.⁸⁻¹⁴ This low detection rate can be explained, not only by sampling difficulty resulting from the location of AIS within the endocervical canal, but also by the difficulty in recognizing AIS owing to the subtle cytologic features that often overlap morphologically with neoplastic squamous and endometrial lesions and non-neoplastic endocervical and endometrial cells.

Previous studies have suggested that high-risk human papillomavirus (hrHPV) testing improves the detection of glandular lesions compared with the Pap test alone.^{15,16} The Food and Drug Administration (FDA) approved the HPV test for use as a first-line cervical cancer screening tool in women than 25 years.¹⁷ However, data on the effect of the new screening strategies have predominantly covered the detection of squamous lesions. In contrast, because of the relative rarity of glandular lesions, the effect on their detection has been less well-defined.

The aim of our study was to determine the Pap sensitivity and accuracy in the detection of AIS and compare these with those for invasive endocervical adenocarcinoma (ECCA). Our second goal was to analyze the interobserver agreement (IOA) for AIS and compare it with the IOA for the detection of ECCA and high-grade squamous

intraepithelial lesions (HSIL). The third goal was to compare the clinicopathologic characteristics of patients with AIS and ECCA to better understand the disease process and the problems with the early detection of these lesions. Our final goal was to correlate the detection of AIS with hrHPV status.

Material and methods

The campus institutional review board approved the present study. Surgical pathology cases accessioned from January 2007 to December 2016 with a diagnosis of AIS or ECCA that had had a Pap specimen collected within 1 year before the histologic diagnosis were selected from the pathology database of Parkland Health and Hospital System. All Paps were liquid-based cytology specimens (ThinPrep; HologicTM, Inc., Bedford, MA) that had been prepared according to the manufacturer's instructions. All cytologic diagnoses were made in accordance with The Bethesda System 2001.¹⁸ All hrHPV tests were performed using the FDA-approved Hybrid Capture 2 method (Qiagen, Gaithersburg, MD), which tests for the intermediate HPV and hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). HPV testing was ordered by clinicians as (1) reflex testing triggered by atypical squamous cells of undetermined significance (ASCUS) Pap test results in women aged ≥ 21 years or low-grade squamous intraepithelial lesion (LSIL) in women aged ≥ 50 years; (2) cotesting with Pap tests in women aged ≥ 30 years; and (3) cotesting, regardless of either age or Pap test results. The cytologic diagnoses, patient age, hrHPV status within 1 year before the histologic diagnosis, surgical specimen type (biopsy, loop electrosurgical excision procedure, cone excision, or hysterectomy), and corresponding histologic diagnoses were recorded.

We calculated the sensitivity of the Pap diagnoses for AIS and ECCA from 2007 to 2016. The sensitivity was calculated at four different levels: (1) overall sensitivity (a proportion of histologically proven AIS or ECCA cases that had any Pap abnormality); (2) sensitivity for the detection of AIS as any Pap glandular abnormality (a proportion of cases that had a Pap diagnosis of atypical glandular cells, not otherwise specified (AGC,NOS); AGC, favoring neoplastic (AGC,N); AIS; or adenocarcinoma [ADCA]); (3) sensitivity for the detection of AIS as a glandular malignancy on Pap (AIS or ADCA); and (4) sensitivity for a complete cytohistologic concordance (proportion of cases with cytological diagnoses exactly matching the histologic diagnoses of AIS or ADCA). The original Pap diagnosis was used to calculate the test sensitivity. The original histologic diagnosis was used as the reference standard. All available Paps ($n = 68$) with histologically proven AIS and ECCA and 56 Paps with high-grade squamous abnormality (HSIL or squamous cell carcinoma [SCCA]) were placed in random order and blindly reviewed by 3 board-certified cytopathologists (E.L., J.T., and G.R.C.). The IOA was calculated. We also

Table 1 Hysterectomy status in patients with AIS and ECCA.

Hysterectomy status	AIS (n)	ECCA (n)
Inoperable	0	10
Hysterectomy (positive for residual lesion)	16	29
Hysterectomy (negative for residual lesion)	22	2
No hysterectomy	34	7
Total	72	48

Abbreviations: AIS, adenocarcinoma in situ; ECCA, endocervical adenocarcinoma.

evaluated the presence of coexisting squamous lesions in the cases with AIS and ECCA and correlated their presence with the sensitivity for the detection of glandular lesions. All available surgical biopsy, excision, and resection slides were reviewed by 1 pathologist (E.L.) for confirmation of the histologic diagnoses. The presence of coexisting squamous lesions was determined from the histologic findings.

Statistical analysis

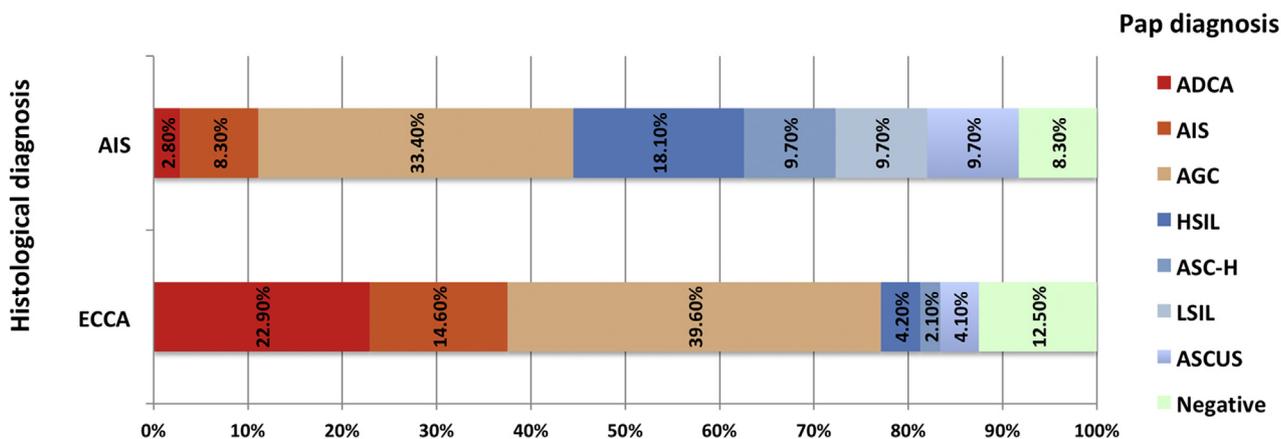
The 2-tailed unpaired *t*-test was used to compare the age differences for the AIS and ECCA patient groups. The Fisher exact test was used for comparisons between sensitivities. For AIS, the IOA was evaluated using the Fleiss kappa. When compared among the AIS, ECCA, and HSIL groups, however, we were unable to calculate the kappa because of the extreme agreement between the observers for

the diagnosis of adenocarcinoma and HSIL. The kappa statistic is influenced by the trait prevalence and rater bias, and the kappa coefficient is reduced when the agreement is extreme, a phenomenon known as the “kappa paradox.” Therefore, we used Gwet’s AC2. Gwet’s AC2 is a proven, yet less currently used, statistical tool not affected by trait prevalence or rater bias and can be used to avoid the “kappa paradox.”¹⁹⁻²¹ For the interpretation of the Fleiss kappa and Gwet’s AC2, we used a benchmark scale defined by Landis and Koch²²: <0, poor; 0 to 0.20, slight; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 to 1.00, almost perfect agreement.

Results

A total of 120 patients with a histologic diagnosis of AIS or ECCA were identified during the study period (72 cases of AIS and 48 cases of ECCA). The mean patient age was 41.1 years (range, 21-84 years). The patients with AIS were significantly younger than the patients with ECCA (37.0 ± 1.1 years versus 47.3 ± 1.8 years; *P* < 0.0001).

Of the 72 patients with AIS, 38 (52.8%) had undergone hysterectomy. Of the 48 patients with ECCA, 31 (64.6%) had undergone hysterectomy, and 10 (20.8%) had been deemed “inoperable” and had received chemotherapy with or without radiation (*P* = 0.0002). Furthermore, of the 38 patients with AIS who had undergone hysterectomy, only 16 (42.1%) had residual AIS found in the hysterectomy specimen. In contrast, of the 31 patients with ECCA who



	ADCA	AIS	AGC	HSIL	ASC-H	LSIL	ASCUS	Negative	Total
AIS	2	6	24	13	7	7	7	6	72
ECCA	11	7	19	2	1	0	2	6	48

Figure 1 Detailed Pap diagnoses of patients with histological adenocarcinoma in situ (AIS) and endocervical adenocarcinoma (ECCA). ADCA, adenocarcinoma; AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; ASCUS, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

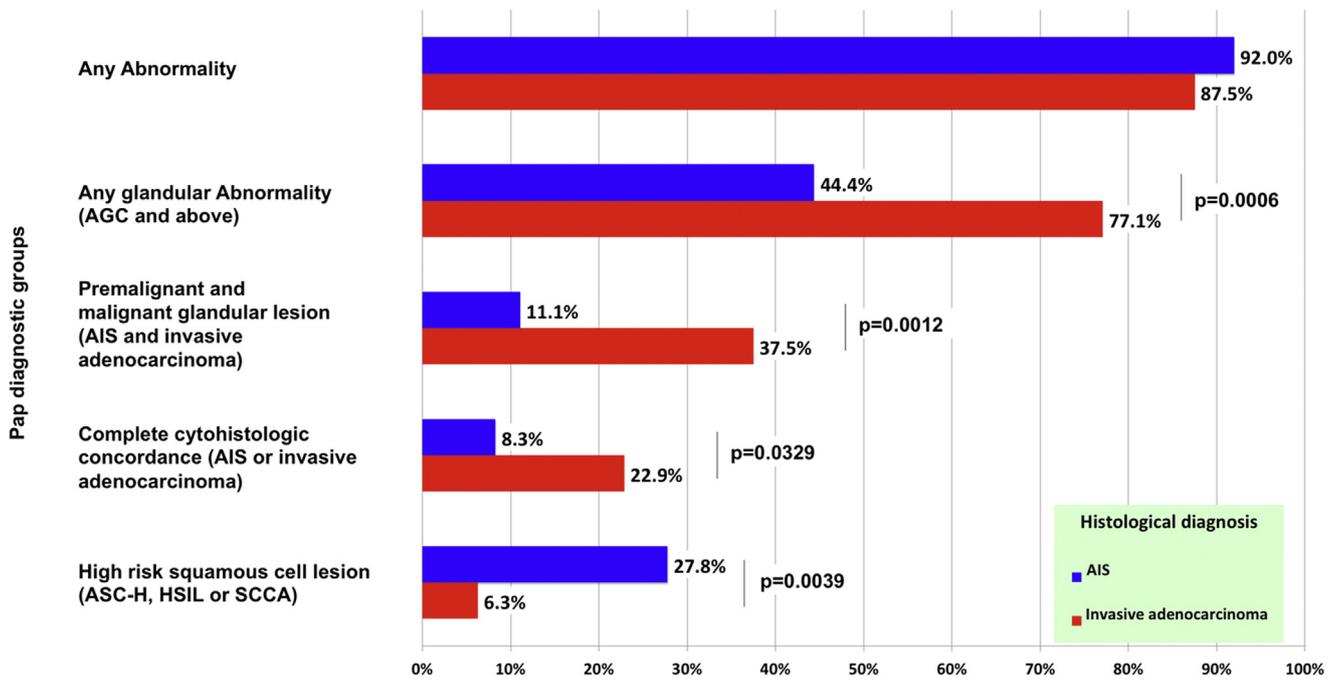


Figure 2 Comparison of Pap diagnoses between histologic adenocarcinoma in situ (AIS) and endocervical adenocarcinoma (ECCA). AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCCA, squamous cell carcinoma.

had undergone hysterectomy, 29 (93.5%) had had carcinoma found in the hysterectomy specimen ($P = 0.0001$). Details of the surgical status and diagnosis in the hysterectomy specimen are presented in [Table 1](#).

Of the 120 patients with histologic AIS or ECCA, 108 (90%) had abnormal Pap results, including 66 of 72 (92%) with AIS and 42 of 48 (87.5%) with ECCA. A glandular abnormality was detected in 32 of 72 AIS cases (44.4%) and 37 of 48 ECCA cases (77.1%; $P = 0.0006$). A definitive cytologic diagnosis of a premalignant or malignant glandular lesion (AIS or ADCA) was made in 8 AIS cases (11.1%) and 18 ECCA cases (37.5%; $P = 0.0012$). Complete cytohistologic concordance was reached for 6 AIS cases (8.3%) and 11 ECCA cases (22.9%; $P = 0.0329$). In addition, 20 of the 72 histologic AIS cases (27.8%) and 3 of the 48 ECCA cases (6.3%) were diagnosed on Pap as a high-risk squamous abnormality ($P = 0.0039$). Details of

the Pap diagnoses of ECCA and AIS are summarized in [Figs. 1 and 2](#).

The coexistence of a squamous cell lesion (histologic finding of LSIL/CIN1, HSIL/CIN2-3, or SCCA) was investigated by a review of the slides with the diagnosis of ECCA and AIS and the surgical pathology reports within 1 year of the histologic diagnosis of ECCA or AIS. Significantly more patients with AIS (57 of 72; 79.2%) had concurrent histologically confirmed squamous lesions than did patients with ECCA (14 of 48; 29.2%; $P < 0.0001$). Of all AIS cases (with and without coexisting squamous lesions), 47.2% (34 of 72) had been diagnosed on Pap as pure squamous abnormalities (atypical squamous cells of undetermined significance [ASCUS], LSIL, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion [ASC-H], HSIL, or SCCA). In contrast, only 5 of 48 ECCA cases (10.4%) had been diagnosed as pure squamous

Table 2 Coexistence of AIS and ECCA with squamous lesions.

Histologic diagnosis	Squamous lesion on histology	Pap diagnosis				Total
		Glandular	Glandular and squamous	Squamous	Negative	
ECCA	Yes	9	1	3	1	14
	No	22	5	2	5	34
	Total	31	6	5	6	48
AIS	Yes	15	7	31	4	57
	No	10	0	3	2	15
	Total	25	7	34	6	72

Abbreviations: AIS, adenocarcinoma in situ; ECCA, endocervical adenocarcinoma.

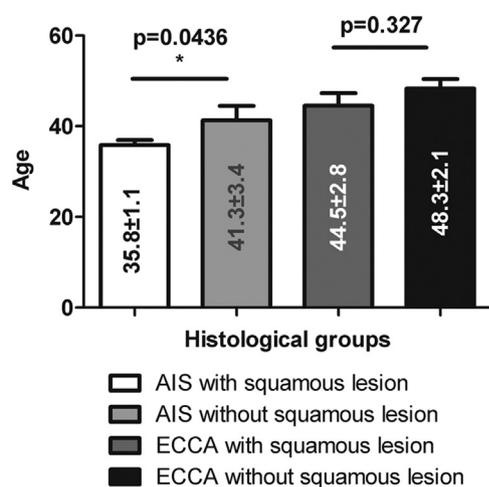


Figure 3 Comparison of patient age between adenocarcinoma in situ (AIS) and endocervical adenocarcinoma (ECCA) diagnostic groups with and without coexisting squamous lesions. *Statistically significant, $P < 0.05$.

abnormalities. Among all the cases with pure glandular neoplasia (AIS and ECCA) on histologic examination, 3 of the 15 pure AIS cases (20%) were misdiagnosed by cytologic examination as pure squamous abnormalities. In contrast, only 2 of 34 pure ECCA cases (5.9%) were misdiagnosed as pure squamous abnormalities. The details are summarized in Table 2.

Interestingly, both patients with AIS and patients with ECCA who had coexisting squamous lesions were younger than those patients with AIS or ECCA who did not have coexisting squamous lesions (35.8 ± 1.1 years versus 41.3 ± 3.4 years, $P = 0.0436$ for AIS; 44.5 ± 2.8 years versus 48.3 ± 2.1 years, $P = 0.33$ for ECCA). The difference in the patients with AIS was statistically significant (Fig. 3).

For AIS, the IOA was substantial for the detection of any high-risk cytologic abnormality (AGC/AIS/ADCA/ASC-H/HSIL/SCCA; Fleiss kappa = 0.67; $P < 0.0001$) and fair for the detection of any glandular abnormality (AGC/AIS/ADCA; Fleiss kappa = 0.34; $P < 0.001$; Table 3). The Fleiss kappa could not be calculated for ECCA and HSIL because of the extreme agreement between the observers (reduced kappa coefficient value when the agreement

between the observers is extreme, a phenomenon known as “kappa paradox”).¹⁹⁻²¹ To avoid this, Gwet’s AC2 was calculated to compare the IOA among the AIS, ECCA, and HSIL groups. For the detection of any high-risk cytologic abnormality (AGC/AIS/ADCA/ASC-H/HSIL/SCCA), the IOA was substantial for AIS (Gwet’s AC2 = 0.77; 95% confidence interval [CI], 0.61-0.94) and ECCA (Gwet’s AC2 = 0.78; 95% CI, 0.51-1.0), and almost perfect for HSIL (Gwet’s AC2 = 0.87; 95% CI, 0.78-0.96). For the detection of any glandular (AIS and ECCA) or squamous (HSIL) abnormality, the IOA was moderate for AIS (Gwet’s AC2 = 0.45; 95% CI, 0.22-0.69) and ECCA (Gwet’s AC2 = 0.60; 95% CI, 0.24-0.96), while excellent for HSIL group (Gwet’s AC2 = 0.82; 95% CI, 0.72-0.93; Table 4).

hrHPV testing was performed in 26 of 72 patients with AIS (36%) on the residual liquid-based cytology specimen. Of these 26 patients with AIS, 24 (92%) had positive hrHPV results and 2 (8%) had negative results. Of these, 1 patient had had a negative cytologic diagnosis and 2 patients had a diagnosis of ASCUS, indicating that for these patients, the decision to proceed to colposcopy and biopsy relied on the hrHPV results. We were not able to compare the hrHPV results for AIS and ECCA because of the small number of patients with ECCA tested for HPV (2 cases).

Discussion

In the present study, we compared the diagnostic sensitivity and accuracy of cervical cytology for the detection of AIS and ECCA. We also compared selected clinical parameters between AIS and ECCA. We found that the patients with AIS were significantly younger than were those with ECCA (37.0 ± 1.1 years versus 47.3 ± 1.8 years; $P < 0.0001$), indicating that progression from in situ to invasive carcinoma requires, on average, 1 decade. The sensitivity for the detection of a glandular abnormality in patients with AIS was 44.4%, significantly lower than that for ECCA (77.1%). Complete cytohistologic concordance for the diagnosis of AIS was only 8.3%, significantly lower than that for ECCA (22.9%).

The results of our investigation are in agreement with other studies that have demonstrated difficulties in the cytologic diagnosis of AIS. In a recent study by Champ et al.²³ performed on ThinPrep Paps immediately preceding the histologic diagnosis of AIS/ECCA, 88% of cases had abnormal Pap test results. For Paps performed ≤ 122 months before the biopsies, the overall sensitivity (any abnormal Pap result) was 53.7% for AIS and 45.8% for ECCA.²³ Glandular abnormalities were detected in 50.2% and 72.7% of AIS and ECCA cases respectively, comparable to our results. The true cytohistologic concordance rates were 4.4% for AIS and 12.5% for ECCA, a trend similar to our findings.²³ Several other groups of investigators have reported similar results with conventional smears. Ruba et al.¹³ reported the sensitivity for the detection of AIS diagnosed as

Table 3 Interobserver agreement (Fleiss kappa) in Pap tests of patients with AIS.

Pap diagnosis	Fleiss kappa	P value
High-risk diagnosis ^a	0.67	<0.0001
Glandular abnormality diagnosis ^b	0.34	<0.001

Abbreviations: ADCA, adenocarcinoma; AGC, atypical glandular cells; AIS, adenocarcinoma in situ; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCCA, squamous cell carcinoma.

^aAGC/AIS/ADCA/ASC-H/HSIL/SCCA.

^bAGC/AIS/ADCA.

Table 4 Interobserver agreements (Gwet's AC2) in Pap tests from patients with AIS, ECCA, and HSIL.

Pap diagnosis	Histologic group (95% CI)		
	AIS	ECCA	HSIL
High-risk diagnosis ^a	0.77 (0.63-0.93)	0.78 (0.51-1.0)	0.87 (0.78-0.96)
Glandular (for AIS and ECCA) or squamous (for HSIL) abnormality diagnosis ^b	0.45 (0.22-0.69)	0.60 (0.24-0.96)	0.82 (0.72-0.93)

Abbreviations: ADCA, adenocarcinoma; AGC, atypical glandular cells; AIS, adenocarcinoma in situ; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; CI, confidence interval; ECCA, endocervical adenocarcinoma; HSIL, high-grade squamous intraepithelial lesion; SCCA, squamous cell carcinoma.

^aAGC/AIS/ADCA/ASC-H/HSIL/SCCA.

^bAGC/AIS/ADCA.

high-grade epithelial abnormality (glandular or squamous) as 54%. Ostor et al¹⁴ found that 92% of women with AIS had presented with abnormal Pap smear results, and of these, 56% contained abnormal glandular cells. A few investigators compared Pap sensitivity for AIS and high-grade squamous lesions and demonstrated significantly greater difficulty in the diagnosis of AIS compared with HSIL. Roberts and Thurloe²⁴ reported that the ThinPrep Pap overall sensitivity (any cytologic abnormality) for AIS was 67%, AIS with coexisting HSIL was 64%, and for HSIL was 80%. In their study of the 2001 and 2002 College of American Pathologists Interlaboratory Comparison Program, Renshaw et al⁹ reported that the interpretation rate of AIS was 46.5% and for HSIL and SCCA was 73.2% and 75.1%, respectively. The sensitivity for the specific diagnosis of AIS in our study, and other similar studies, was lower than that in the College of American Pathologists PAP educational surveys.^{9,25} Zhao et al²⁵ calculated the specific AIS recognition by 50% of survey participants, and the false-negative interpretation rate for AIS was 6.9%. The reason for such a difference likely resulted from the types of reporting categories available for the survey participants (the indeterminate categories, such as AGC, widely used by pathologists in daily practice were not available for the survey participants and this might have led the participants to select a malignant or high-grade response).²⁵ In addition, 2 other factors could have potentially played a role in such a discrepancy: (1) the expectation bias (during the survey, the participants anticipated testing on a range of diagnostic categories, including glandular lesions); and (2) the method for case selection stipulates an agreement between the Pap and histologic diagnosis with independent verification of the findings by a few selection committee members. Thus, the lesions represented in surveys are more likely to demonstrate the classic cytologic features, allowing for a definitive diagnosis.

Despite the low proportion of cases with a specific cytologic diagnosis of AIS, our results have demonstrated a 92% overall sensitivity (any abnormal Pap result), comparable to that for ECCA (87.5%). Many AIS cases were diagnosed as AGC, invasive adenocarcinoma, or squamous lesions. Similar results were reported in other studies.^{26,27} Although

misinterpretation of AIS as a high-grade squamous lesion might not alter patient treatment significantly, its misdiagnosis as a negative or low-grade lesion could lead to serious undertreatment. Another reason for such misclassification is the coexistence of many AIS cases with squamous lesions (79.2% of all AIS cases). More than one half of these cases were detected as pure squamous abnormalities (47.2% of all AIS cases). Some of these Paps might have contained glandular abnormalities that were missed or misclassified as a squamous abnormality, and some might have only contained squamous abnormalities, which is usually more accessible using Pap procurement devices. The distribution of AIS cases between the 2 scenarios could not be determined with our present data. Interestingly, compared with AIS cases, significantly fewer cases with ECCA had coexisting squamous lesions on histologic examination (29.2%). This might at least be partially attributed to the early detection of AIS with coexisting squamous lesions resulting in further investigation and, consequently, preventing these cases from progressing to ECCA. Indeed, we also observed that both patients with AIS and those with ECCA with coexisting squamous lesions were approximately 5 years younger than those with AIS and ECCA with pure glandular disease, and the difference within the AIS group was statistically significant.

The reasons for the failure to diagnose AIS have been previously examined by several investigators. These have included sampling, screening, and diagnostic errors. Sampling errors appear to contribute to a significant number of false-negative cases because of an inherent difficulty in accessing glandular neoplasms. Although during the previous years, the sampling techniques have improved and newer devices have resulted in adequate sampling of the endocervical component, they often miss higher and deeper endocervical lesions. The study by Kalir et al²⁸ demonstrated that the failure of Pap testing to detect AIS and invasive adenocarcinoma was significantly more likely to occur when tumors had spared the transformation zone (54%).

The previously described potential sources of screening/interpretive error that can hamper the recognition of malignant cells include their scarcity, obscuring blood, inflammation, drying artifact, increased thickness of the

malignant cell groups, and the small size of the malignant cells, which often resemble benign endometrial cells, especially those from the lower uterine segment.^{8,11,13,26,29} Rare subtypes of AIS (eg, minimal deviation adenocarcinoma/adenoma malignum, intestinal, and gastric type) are still poorly characterized and pose an even greater challenge owing to their infrequent occurrence and, in many cases, subtle features.³⁰⁻³³ We could not assess the distribution of false-negative results by the type of error (sampling versus screening/interpretive error) because many older slides were not available for review. However, the problem with diagnostic accuracy was further demonstrated by the high interobserver variability. In our study, the IOA for the detection of AIS as a high-risk cytologic abnormality was substantial ($\kappa = 0.67$) and was only fair for the diagnosis of a glandular abnormality ($\kappa = 0.34$). In addition, although the IOA for detection of any glandular abnormality fell into the moderate agreement category in both AIS and ECCA groups, it was lower in the AIS group (Gwet's AC2 = 0.45) versus the ECCA group (Gwet's AC2 = 0.60). The IOA for both of these glandular lesions was significantly lower than the IOA for the detection of squamous abnormalities in the HSIL group (Gwet's AC2 = 0.82).

The great majority of cervical glandular lesions are caused by hrHPV. Cotesting with cytology and hrHPV is considered a potentially useful approach to increase the detection of these lesions.^{15,34} Recently, hrHPV has been approved by the FDA as a primary screening method for cervical cancer.¹⁷ We found that of the patients with AIS tested for hrHPV, 92% had positive results. In 3 of these hrHPV-positive cases, the Pap diagnosis had been ASCUS (2 cases) or negative (1 case); thus, the management decision to proceed to colposcopy and biopsy had relied on the hrHPV results. In contrast, 8% of the patients with AIS had negative hrHPV results, a small, yet significant, proportion. Our percentage of positive hrHPV results was similar to that in previously reported studies.^{10,27,35} Although our data are limited, they nevertheless indicate that hrHPV or cytology alone will not detect all cervical glandular neoplasms, whereas Pap-hrHPV cotesting might provide their optimal detection.^{27,35}

Conclusions

Our results, together with the results of other studies, have demonstrated that, although the familiarity with the cytologic features of AIS has improved during the past years, the sensitivity and accuracy of the diagnosis of AIS remains problematic. In addition, we have demonstrated that women with coexistent squamous lesions were younger than those with pure glandular lesions. One could speculate that presence of a squamous lesion might lead to the earlier detection and treatment of the disease. Testing for hrHPV or cytology alone will miss some glandular lesions, while hrHPV-Pap

cotesting will likely improve their detection. Additional studies are needed to explore other potential factors contributing to the disease progression to better understand the biology, clinical presentation, and pathologic manifestation of these lesions.

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Conflict of interest disclosures

The authors made no disclosures.

Author contributions

SN: conceptualization, methodology, visualization, formal analysis, investigation, writing—original draft. KM: funding acquisition, writing—review and editing. JT: visualization, investigation, writing—review and editing. GR: visualization and investigation. SH: writing—review and editing. WZ: writing—review and editing. EL: conceptualization, methodology, funding acquisition, visualization, formal analysis, investigation, writing—review and editing.

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