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ORIGINAL ARTICLE

The reporting rates of atypical glandular cells and their HPV testing and histologic follow-up results: a comparison between ThinPrep and SurePath preparations from a single academic institution

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Introduction The interpretation of atypical glandular cells (AGCs) remains a major challenge in gynecologic cytopathology using liquid-based cytology (LBC) (ThinPrep and SurePath). The comparison of performance of detecting glandular abnormalities using these 2 methods is lacking. We investigated the reporting rates of AGCs, *human papillomavirus* (HPV) testing, and histologic follow-up results in ThinPrep (TP) and SurePath (SP) samples.

Materials and methods In our institution, both TP and SP were utilized during the period between January 2014 and June 2017. A retrospective search was conducted to identify patients with AGCs from 58,591 LBCs (27,041 TP and 31,550 SP). Roche (Pleasanton, CA) cobas HPV testing and histologic follow-up results were collected.

Results The reporting rates of AGCs for TP (0.7%) or SP (0.2%) were within the College of American Pathologists benchmark ranges, but the reporting for TP was significantly greater than that for SP ($P < 0.0001$). The HPV-positive rates were 26.0% and 19.4% in TP-AGCs and SP-AGCs, respectively, with no statistical significance. A total of 137 (74.9%) TP-AGCs and 54 (74%) SP-AGCs had histologic follow-up. High-grade squamous intraepithelial lesions (HSIL)/squamous cell carcinoma were identified in 8.8% (12 of 137) of TP-AGCs and 13% (7 of 54) of SP-AGCs. Adenocarcinomas including endocervical and endometrial adenocarcinomas were identified in 9.5% (13 of 137) of TP-AGCs and 13% (7 of 54) of SP-AGCs. Together, 18.2% (25 of 137) of TP-AGCs and 25.9% (14 of 54) of SP-AGCs showed either HSIL or carcinoma in histologic follow-up, but with no statistical significance.

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Conclusions TP preparation detected considerably more AGCs than SP preparation. There was no statistical significant difference in HPV-positive rates or histologic follow-up outcomes between TP-detected AGCs and SP-detected AGCs.

Introduction

Cervical cancer is the fourth most frequent cancer in women and 80%-90% of cervical cancers are squamous cell carcinoma. The incidence of cervical adenocarcinoma has been increasing in the US population, however, especially among young women and black women.[1,2] The sensitivity for detecting glandular lesions is limited by sampling issues and interpretation difficulties.[3] It has been reported that 80% or more of women with adenocarcinoma in situ or invasive adenocarcinoma have an abnormality on their gynecologic cytology, ranging from "atypical" to a more definitive interpretation.[4,5] The cytologic criteria for diagnosing atypical glandular cells (AGCs) include cellular features beyond reactive changes, but short of glandular neoplasia. The incidence of AGCs ranges from 0.18% to 0.74% of gynecologic cytologic specimens.[6] Diagnosing AGCs is challenging, with relative low reproducibility, and results in a high percentage of benign conditions on histologic follow-up.[6-14] Nevertheless, a significant portion of women with AGCs do have significant lesions on histologic follow-up, including high-grade squamous intraepithelial lesion (HSIL), endocervical carcinoma, and endometrial carcinoma.[6-8]

Liquid-based cytology (LBC) including ThinPrep (Hologic, Inc, Boxborough, MA) and SurePath (BD Diagnostics, Burlington, NC) has become the standard of care in the United States for gynecologic cytology.[15] Nevertheless, the comparison of sensitivity and efficacy to detect glandular neoplasia between these 2 LBC methods is lacking. We aimed to examine AGC reporting rates, and their *human papillomavirus* (HPV) testing and histological follow-up results from SurePath and ThinPrep samples at a single institution.

Materials and methods

Patients and specimens

After obtaining approval from the institutional review board at The Ohio State University, a computer-based search was carried out on our CoPath laboratory information system to retrieve LBC Papanicolaou tests with AGC interpretation taken between January 2014 and June 2017. All specimens were processed in the pathology laboratory of The Ohio State University Wexner Medical Center. ThinPrep (TP) Papanicolaou tests were prepared according to the manufacturer's specifications from specimens in PreservCyt solution using an automated processor (ThinPrep 3000). SurePath slides (SP) were prepared according to the manufacturer's protocol from specimens in SurePath preservative fluid. Staining of slides was done on a Sakura Tissue Tek Automated Slide Stainer (Sakura Finetek USA, Torrance, CA) according to a US Food and Drug Administration (FDA)—approved manufacturer's protocol.

The interpretations of AGC were made by staff cytopathologists, based on categories defined by The Bethesda System. All cases interpreted as AGCs and coexisting squamous abnormalities were excluded.

HPV testing

HPV DNA testing was ordered by clinicians according to several ordering options as follows: reflex testing; cotesting with Papanicolaou tests in women aged 30 years and older; and cotesting regardless of either age or Papanicolaou test results. HPV DNA detection was performed using the FDA-approved Roche cobas 4800 assay (Roche Molecular Systems, Pleasanton, CA), which tests for high-risk and intermediate-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The results of HPV DNA testing were reported as either positive or negative.

Histologic follow-up

Histological follow-up results were collected from the following histologic procedures within 1 year after AGC interpretation: cervical biopsy, endocervical curettage, conization, endometrial biopsy and curettage, and hysterectomy. The most abnormal histologic diagnosis was recorded if there were 2 or more histologic procedures during the follow-up period. The histopathologic findings were categorized into the following 6 groups: 1) negative for neoplastic/dysplastic lesions, 2) cervical intraepithelial neoplasia 1 (CIN 1); 3) cervical intraepithelial neoplasia 2/3 (CIN2/3); 4) cervical adenocarcinoma in situ (AIS); 5) invasive cervical adenocarcinoma; 6) endometrial neoplastic lesions including endometrial complex hyperplasia or endometrial carcinoma.

Statistical analyses

The Pearson χ^2 test was used for statistical analysis, conducted with SAS 9.1 software (SAS Institute, Cary, NC). A P value less than 0.05 was considered statistically significant.

Table 1 HPV testing results from patients with atypical glandular cells diagnosed on ThinPrep or SurePath.

	Total		ThinPrep		SurePath		*P* value
	n	%	n	%	n	%	
Total cases	58,591		27,041		31,550		—
Total AGCs	256	0.4	183	0.7	73	0.2	<0.0001
Total AGCs with HPV	158	61.7	127	69.4	31	42.5	—
HPV-negative	119	75.3	94	74.0	25	80.6	NS
HPV-positive	39	24.7	33	26.0	6	19.4	

Abbreviations: AGCs, atypical glandular cells; HPV, *human papillomavirus*.

Results

During the period between January 1, 2014, and June 30, 2017, 58,591 LBC specimens were processed in our cytology laboratory, including 27,041 (46.2%) TP and 31,550 (53.8%) SP specimens. One hundred eighty-three AGCs (0.7%) were identified from 27,041 TP specimens and 73 AGCs (0.2%) were identified from 31,550 SP specimens. The average patient age was 43 years old (range: 22-83 years) in patients with AGCs diagnosed on TP preparations and 49 years old (range: 24-90) in patients with AGCs diagnosed on SP preparations. The reporting rates of AGCs for TP and SP were within the College of American Pathologists (CAP) benchmark ranges,[16] but the reporting rate of AGCs for TP was significantly higher than the rate for SP.

A total of 127 (69.4%) TP-AGCs and 31 (42.5%) SP-AGCs underwent an HPV test. The HPV-positive rates were 26.0% and 19.4% in TP-AGCs and SP-AGCs, respectively, with no statistical significance identified (Table 1).

A total of 137 (74.9%) TP-AGCs and 54 (74%) SP-AGCs had histologic follow-up results. Of those, 69.3% (95 of 137) of TP-AGCs and 61.1% (33 of 54) of SP-AGCs showed negative histologic findings, including benign endocervical mucosa, reactive epithelial changes, endometrial polyp, endocervical polyp, simple hyperplasia, tubal metaplasia, microglandular hyperplasia, benign endometrium, and atrophic changes.

Low-grade squamous intraepithelial lesion (LSIL) was identified in 12.4% (17 of 137) of TP-AGCs and 13.0% (7 of 54) of SP-AGCs, respectively. HSIL or squamous cell carcinoma (SqCC) was identified in 8.8% (12 of 137) of TP-AGCs and 13% (7 of 54) of SP-AGCs, respectively. Adenocarcinomas including endocervical and endometrial adenocarcinomas were identified in 9.5% (13 of 137) of TP-AGCs and 13% (7 of 54) of SP-AGCs, respectively. Together, 18.2% (25 of 137) of TP-AGCs and 25.9% (14 of 54) of SP-AGCs showed either HSIL or carcinoma in histologic follow-up, with no statistical significance between TP and SP groups (Table 2).

Among 19 patients with histologic follow-up of HSIL/SqCC, 10 had HPV testing and 8 were positive. Among 12 patients with cervical AIS/adenocarcinoma, only 1 had HPV testing and the result was positive. Among 13 patients with endometrial adenocarcinoma, 7 had HPV testing and all were negative. Among all cases with histologic neoplastic

Table 2 Histologic follow-up results from patients with atypical glandular cells diagnosed on ThinPrep or SurePath.

	ThinPrep		SurePath		Total	
	n	%	n	%	n	%
Total AGCs	183		73		256	
AGCs with histologic follow-up	137	74.9	54	74.0	191	74.6
Negative	95	69.3	33	61.1	128	67.0
LSIL	17	12.4	7	13.0	24	12.6
High-grade/worse lesion	25	18.2	14	25.9	39	20.4
HSIL/SqCC	12	8.8	7	13	19	10.0
HSIL	11	8.0	7	13.0	18	9.4
SqCC	1	0.7	0	0.0	1	0.5
Adenocarcinoma	13	9.5	7	13.0	20	10.5
AIS	4	2.9	1	1.9	5	2.6
Cervical adenocarcinoma	4	2.9	3	5.6	7	3.7
Endometrial adenocarcinoma	9	6.6	4	7.4	13	6.8

No statistically significant difference was identified in any categories between ThinPrep and SurePath.
Abbreviations: AGCs, atypical glandular cells; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SqCC, squamous cell carcinoma; AIS, adenocarcinoma in situ.

lesions, 69% (29 of 42) of TP-AGCs and 66.7% (14 of 21) of SP-AGCs showed squamous lesions (LSIL, HSIL or SqCC). In contrast, glandular lesions were much less frequent, with 31% in TP-AGCs and 33.3% in SP-AGCs, respectively.

The average age of patients with endocervical AIS or adenocarcinoma was 39.4 years (range: 33-49 years) and the average age of patients with HSIL/SqCC was 37.6 years (range: 22-63 years), significantly younger than that of patients with endometrial adenocarcinoma (average: 60.3 years old, range: 51-90 years).

Discussion

The CAP survey of cytology laboratories enrolled in its accreditation programs established the benchmarks for AGC reporting rates: 0% (5th) to 1.1% (95th) in conventional tests, 0% (5th) to 1.4% (95th) in ThinPrep tests, and 0% (5th) to 0.7% (95th) in SurePath tests, with medians of 0.1%, 0.2%, and 0.2%, respectively.[16] The reporting rates of AGC on TP (0.7%) or SP (0.2%) from our current study were within the CAP benchmark ranges, but the reporting rate of AGC on TP was significantly greater than that on SP. Furthermore, no significant difference was identified in HPV-positive rate or histologic follow-up outcomes between TP-detected AGCs and SP-detected AGCs.

A few studies have compared the performance of TP with SP. Zhao et al found both TP and SP yielded similar validity in detecting significant cervical lesions, but SP specimens yielded higher rates of satisfactory slides.[17] Rozemeijer et al found that the use of SP was associated with increased CIN2+ detection when compared with conventional smears, but the use of TP did not show a significant CIN2+ detection rate.[18] Several possible reasons have been proposed to explain the differences between SP and TP performance. First, the cell yield may be larger when using SP, where the collecting device is retained, than when using TP, where the collecting device is discarded from the vial with preservative fluid.[19,20] Second, blood, mucus, and vaginal discharge can adhere to the filter during TP processing, blocking the transfer of cells to slides.[21,22] Our results, however, demonstrated that TP preparation detected more AGCs than SP preparation. In general, TP preparation results in better preparation on cell monolayer, favoring recognition of nuclear details, than SP preparation.[23] Hyperchromatic crowded groups, a potential diagnostic pitfall of false-negative or false-positive interpretation, and cells overlapping may appear more obvious in SP than in TP. Our findings of more AGCs detected in TP specimens than in SP specimens underscore the challenges of recognizing glandular abnormalities on SP preparation.

Previous studies have demonstrated that up to 39% of patients with AGC showed malignant or premalignant diseases in histologic follow-up, including both cervical squamous lesions and glandular lesions from cervix, endometrium, or other sites.[24,25] In the current study, 20.4% of patients with AGCs demonstrated malignant/premalignant lesions (HSIL or worse), supporting current recommendations for the management of AGC cytology including colposcopy and endocervical sampling for all patients with AGCs, and additional endometrial sampling for women aged 35 years and older, or high-risk women under the age of 35 years.[26]

Compared with conventional smears, LBC usually shows smaller cell clusters and sheets and more dyscohesion architecturally. Cytologically, enhanced nuclear features and smaller cell size are more prominent.[15] Studies demonstrated that the ThinPrep Papanicolaou test improved the sensitivity of detecting glandular lesions/adenocarcinoma.[27-29] Nonetheless, the interpretation of atypical glandular cells for the early detection of female genital tract glandular neoplasia remains a major challenge in gynecologic cytopathology.[6,30,31] Indeed, more than half of AGCs in our study turned out to represent underlying benign conditions, consistent with previous studies.[6,30,32,33] Furthermore, among the remaining histologically dysplastic cases, 69% (29 of 42) of TP-AGCs and 66.7% (14 of 21) of SP-AGCs were squamous neoplasia. HSIL cells can involve endocervical gland and present clusters mimicking AGCs. HSIL cells usually show flattening at the periphery of the clusters and loss of cell polarity within the clusters. Isolated dysplastic squamous cells present in the background are suggestive of HSIL.[34] In our study, 8.8% of TP-AGCs and 13% of SP-AGCs were proven to be HSIL, including 1 squamous cell carcinoma, in histologic follow-up. Therefore, it is important for the cytopathologists to recognize the diagnostic pitfalls in the diagnosis of AGC on LBC tests.

HPV testing in glandular lesions was reported to range from 40% to 56% and was suggested to triage AGCs.[35,36] In our study, HPV-positive rates were 26% and 19.4% in AGCs on TP and SP, respectively. It is noted that, in our study, 65% of true glandular lesions were endometrial adenocarcinoma and 35% were endocervical adenocarcinoma. Because patients with endometrial adenocarcinoma are usually negative for HPV, the high percentage of patients with endometrial adenocarcinoma may explain the low HPV-positive rates in our cohort. Potential limitations exist in current study, including relatively small sample size and limited availability of HPV test results.

In conclusion, patients with AGCs detected by ThinPrep showed similar HPV-positive rates or histologic follow-up outcomes to patients with AGC detected on SurePath, but ThinPrep preparation detected significant more AGCs than SurePath preparation.

Disclosure

All authors have no potential conflict of interest.

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