ProExC Triage of Atypical Glandular Cells on Liquid-Based Cervical Cytology Specimens

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■ **Abstract**

Objective. Morphologic distinction between atypical glandular cells not otherwise specified (AGC-NOS) and AGC–favor neoplasia (AGC-FN) can be difficult. Distinction between these entities is important as the American Society for Colposcopy and Cervical Pathology 2006 consensus guidelines state that management of AGC-NOS differs from that of AGC-FN. The objective of this study was to determine the potential role of ProExC immunocytochemical triage of AGC-NOS.

Materials and Methods. Cytopathology records from a pathology practice were reviewed from January 2006 to December 2009 to identify AGC-NOS liquid-based Pap smears with subsequent biopsy correlation. Archival slides were examined, and ProExC immunocytochemistry was performed. The AGC groups were assessed for nuclear staining, and results were correlated with subsequent biopsy findings.

Results. Twenty-eight AGC-NOS cases with biopsy correlation were identified: 13 with subsequent high-grade neoplastic or malignant (positive) diagnoses and 15 with benign diagnoses. Of 13 AGC-NOS cases with positive diagnosis, 10 were ProExC-positive and 3 were ProExC-negative (metastatic tumors from distant sites). Of 15 AGC cases with benign follow-up, 13 were ProExC-negative and 2 were ProExC-positive (sensitivity, 77%; specificity, 87%). For patients with cervical intraepithelial neoplasia or carcinoma originating from the female genital tract, 100% (10/10) were ProExC-positive (sensitivity, 100%; specificity, 87%).

Conclusions. Results suggest that ProExC-positive AGC-NOS may be classified as AGC-FN. Although positive immunocytochemical staining for ProExC requires management similar to AGC-FN, negative staining does not rule out malignancy such as metastatic tumor. Management for ProExC-negative AGC-NOS cases should proceed according to the current guidelines for AGC-NOS. ■

Key Words: ProExC, atypical glandular cells, AGC, ASCCP guidelines

Although the diagnosis of atypical glandular cells is relatively uncommon compared with squamous cervical intraepithelial neoplasia (CIN), the interpretation of atypical glandular cells (AGC) is associated with a higher rate of neoplasia and malignancy [1]. According to the Bethesda System 2001 [2], AGC should be classified as endometrial or endocervical when possible and further designated as AGC "not otherwise specified" (AGC-NOS) or AGC "favor neoplastic" (AGC-FN). The delineation is important because follow-up data demonstrate that up to 96% of AGC-FN cases subsequently develop high-grade CIN compared with up to 41% of AGC-NOS [3]. Given the increased risk of AGC-FN, the American Society for Colposcopy and Cervical Pathology's (ASCCP) 2006 consensus guidelines for the management of women with abnormal cervical screening tests recommend women with AGC-FN to proceed to a diagnostic excisional procedure even if no invasive disease is detected via colposcopy with endometrial and endocervical sampling [4]. The AGC-NOS patients with

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negative initial workup including colposcopy with endometrial and endocervical sampling may be followed by a combination of repeat cytology and human papillomavirus testing without a diagnostic excisional procedure. Discrimination between these 2 entities is therefore important given the difference in management according to the guidelines; however, cytologic findings associated with AGC can be subtle, and accurate classification of AGC is poorly reproducible among cytopathologists [5]. Recently, ProExC (BD Diagnostics—TriPath Imaging, Inc, Burlington, NC) immunocytochemistry has been demonstrated to aid in identification of high-grade squamous CIN and glandular lesions [6–10]. Specifically, ProExC detects the overexpression of minichromosome maintenance 2 and topoisomerase 2α that may result from the activation by human papillomavirus E6 and E7 oncoproteins. Overexpression is demonstrated by a moderate to intense nuclear stain in morphologically abnormal cells [11]. The objective of this study was to determine whether ProExC immunocytochemical triage of AGC-NOS can identify patients requiring a diagnostic excisional procedure.

MATERIALS AND METHODS

A retrospective search of the electronic cytopathology records of a large pathology private practice (approximately 45,000 liquid-based cervical cytology specimens per year) was conducted to identify AGC-NOS liquid-based cervical slides with subsequent tissue biopsy or excisional specimens from January 2006 to December 2009. The study protocol was performed on fully de-identified data deemed by Mountain States Health Alliance Institutional Research Administration to be exempt from regulation.

Each liquid-based thin-layer specimen consisted of an abrasive cervical cytologic sample collected in SurePath preservative (BD Diagnostics—TriPath Imaging, Inc) that was then prepared using the semiautomated PrepStain system (BD Diagnostics—TriPath Imaging, Inc). Slides were evaluated by a cytotechnologist and cytopathologist and were classified as AGC-NOS according to the current Bethesda System. On identification of the AGC-NOS cases via electronic record search, the archival cytology slide was retrieved and reexamined by a cytopathologist to ensure appropriate classification of AGC-NOS. Residual fluid from the SurePath vials of the AGC-NOS cases was not available for preparation of additional slides for immunocytochemical staining; therefore, original archival slides were uncoverslipped for immunocytochemical staining. Removal of coverslips was accomplished via a previously described technique [12] in which archival slides were placed in xylene for up to 5 days until coverslips detached from the slides without manual manipulation. Slides and corresponding coverslips were examined before and after the uncoverslipping procedure to ensure the AGC-NOS cell groups were not inadvertently detached during the process. Antigen retrieval and immunocytochemical antibody staining were then performed on a Ventana Benchmark XT (Ventana Medical Systems, Tucson, AZ) according to ProExC package insert recommendations [11]. Positive and negative tissue controls consisting of endocervical adenocarcinoma in situ (AIS) biopsies were run concurrently with the study cases.

At the completion of ProExC immunocytochemical staining, each slide was reevaluated by a cytotechnologist and cytopathologist and scored based on the recommendations provided in the ProExC package insert [11]. Specifically, each slide was examined to ensure that AGC-NOS cells were still identifiable and to determine the presence or absence of moderate to intense dark brown nuclear staining in the morphologically abnormal glandular cells. Previously published literature has documented sporadic ProExC positivity in normal endocervical glandular cells [7–10]; however, in these cases, normal endocervical cells could still be distinguished from morphologically atypical endocervical cells. Furthermore, the positive staining of normal endocervical cells is generally sporadic but, in rare cases, has been documented in up to 25% to 50% of endocervical cells present [8]. Given the potential for sporadic positivity in endocervical cells, AGC cell groups in this study were considered positive only if greater than 50% of morphologically atypical glandular cells in any given AGC cell group demonstrated moderate to intense nuclear staining (Figure 1). Because of the possible positivity in normal endocervical cells, positive AGC cells that occurred singly were disregarded, and results were based only on AGC cell groups containing multiple morphologically abnormal cells. A summary of the criteria for determining ProExC positivity in this study is listed in Table 1. After evaluating the presence or absence of ProExC nuclear staining, the results were recorded and correlated with the findings in subsequent biopsies and/or excisional specimens.

RESULTS

Between January 2006 and December 2009, a total of 28 cases of AGC-NOS with subsequent endocervical biopsy and/or excisional procedures were identified. Of

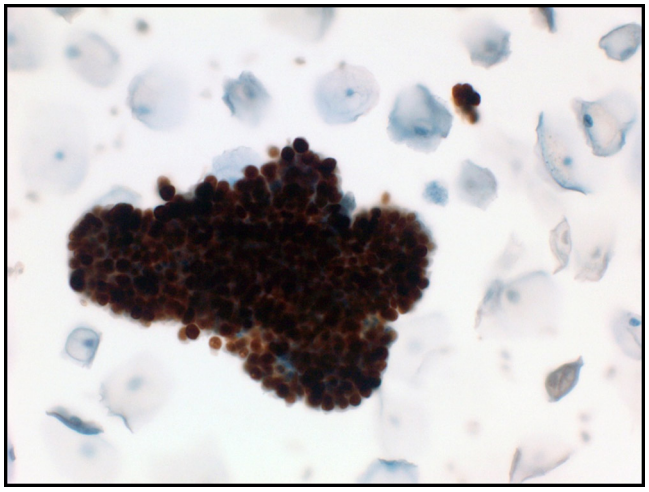

Figure 1. Atypical Glandular Cell group with strong positive ProExC staining. Subsequent biopsy demonstrated Adenocarcinoma in-situ.

these 28 AGC-NOS patients, 13 (age ranged from 24 to 72 y; mean, 43 y) were subsequently diagnosed via biopsy and/or excisional procedures with high-grade neoplastic lesions or malignancy: 6 with AIS, 1 with high-grade CIN (CIN 3), 1 with invasive poorly differentiated cervical squamous cells carcinoma, 2 with endometrial adenocarcinoma, and 3 with metastatic tumors from distant sites (Table 2). The remaining 15 of 28 AGC-NOS patients (age ranged from 19 to 55 y; mean, 39 y) had subsequent benign diagnoses on follow-up biopsies and/or excisional procedures.

Of the 13 AGC-NOS cases with subsequent neoplastic/malignant diagnoses (Table 2), 10 cases demonstrated positive ProExC nuclear staining of AGC cell groups (all 10 cases with squamous/glandular neoplasia or malignancy originating from the cervix, endocervix, or uterus), whereas 3 AGC cases demonstrated negative nuclear staining (all 3 cases of metastatic malignancy from distant sites). Of the 15 AGC-NOS cases with benign follow-up results, 13 were negative and 2 were positive for ProExC nuclear staining of AGC cell groups.

Table 1. Criteria for Positive ProExC Nuclear Staining

All 3 criteria satisfied = ProExC-positive
Less than 3 criteria satisfied = ProExC-negative (or indeterminate)

1. Morphologically abnormal cells consistent with AGC must be present on slide.
2. At least moderate to intense nuclear staining must be present in AGC cell nuclei.
3. At least 50% of nuclei in AGC cell group must demonstrate nuclear staining.

Table 2. Summary of ProExC Nuclear Staining in AGC-NOS Cases With Subsequent Neoplastic/Malignant Diagnoses (*n* = 13)

Diagnosis	ProExC status	
AIS[a]	Positive	
AIS	Positive	
AIS	Positive	
AIS	Positive	
AIS	Positive	
AIS	Positive	
Endometrial adenocarcinoma	Positive	
Endometrial adenocarcinoma	Positive	
CIN 3[b]	Positive	
Invasive cervical squamous cell carcinoma	Positive	
		n = 10
Metastatic breast carcinoma	Negative	
Metastatic colon carcinoma	Negative	
Metastatic poorly differentiated carcinoma (favor pancreatic)	Negative	
		n = 3

[a]Adenocarcinoma in situ.
[b]High-grade CIN.
AGC-NOS, AGC not otherwise specified; AGC, atypical glandular cells; AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia.

Overall sensitivity and specificity of ProExC for high-grade neoplasia or malignancy in AGC-NOS were 77% and 87%, respectively. If considering only AGC-NOS patients with neoplasia or malignancy arising from the female genital tract (excluding the 3 cases of metastases from distant sites), 10 of 10 were ProExC-positive, and therefore, sensitivity and specificity were 100% and 87%, respectively.

DISCUSSION

Of the 28 cases of AGC-NOS in the study, 13 had subsequent high-grade neoplastic or malignant diagnoses, and ProExC immunocytochemistry demonstrated positivity in 10 of the 13 cases. The finding of ProExC positivity in AGC from cases representing exfoliated cells of neoplasia or malignancy originating in the female genital tract is consistent with findings of other studies that demonstrate the efficacy of ProExC in the identification of cervical squamous and glandular neoplasia. Although less is documented regarding ProExC staining in endometrial adenocarcinoma, 2 cases of endometrial adenocarcinoma were positive for ProExC in this study.

The 3 false-negative cases in this group were all subsequently diagnosed as metastatic malignancies from distant sites as follows: (1) high-grade breast carcinoma, (2) colon adenocarcinoma, and (3) poorly differentiated carcinoma (possibly a pancreatic primary). In each case, the clinical history of known malignancy distant from the female genital tract was documented. Given the

documentation of aggressive malignant neoplasms in these patients, it is questionable as to whether management for these patients would follow ASCCP guidelines for AGC-NOS as in the general screening population. Although minichromosome maintenance 2 has been demonstrated to be overexpressed in a number of noncervical primary malignancies including breast and colon adenocarcinomas [13], there are few studies of the efficacy of ProExC for primary or metastatic tumors from sites other than cervix/endocervix. If ProExC staining is limited to patients in a general screening population (without known preexisting high-grade malignancy distant from the female genital tract) then the resulting sensitivity and specificity in this study are 100% and 87%, respectively.

Of the 15 AGC-NOS cases that were subsequently diagnosed as benign on biopsy or excisional procedure, 2 cases demonstrated false-positive ProExC nuclear staining. The follow-up data available for the 13 cases of AGC-NOS with negative ProExC stain and subsequent benign diagnoses are summarized in Table 3. Complete follow-up of these AGC cases per ASCCP recommendations could not be documented because the data are limited to specimens received at a single pathology practice. It is possible that these patients received additional follow-up at other locations. About the 2 cases of false-positive staining, 1 patient (aged 39 y) subsequently had a negative cervical biopsy result followed by complete hysterectomy. Examination of the cervical biopsy demonstrated marked inflammatory changes of endocervical and ectocervical mucosa and extensive squamous metaplasia. Likewise, hysterectomy demonstrated inflammatory changes within the endocervix but no evidence of neoplasia or malignancy. Follow-up on the second false-positive case (aged 30 y) consisted of 2 biopsies 3 months apart that included sampling of ectocervical and endocervical mucosa. Both biopsies demonstrated squamous metaplasia and reactive changes. After these 2 negative biopsy results, an additional 3 negative yearly screening liquid-based Pap tests were documented. In the 2 false-positive cases in our study, review of the cytologic findings confirmed adequate morphologic criteria to warrant the diagnosis of AGC-NOS, but no explanation for the ProExC positivity could be found other than the AGC cells, possibly representing atypical squamous metaplasia or reactive endocervical cells found in the subsequent tissue sections.

Table 3. Follow-up of ProExC-Negative AGC-NOS Cases With Subsequent Benign Diagnoses (*n* = 13)

	Initial management	Subsequent management	Approximate length of follow-up, y
1	CxBx, ECC	Hysterectomy and 3 negative Pap smears	4
2	CxBx, ECC, EMBX	Hysterectomy.	4
3	ECC, cone biopsy	2 negative Pap smears	4
4	ECC, EMBX	6 negative Pap smears	3
5	CxBx, ECC	3 negative Pap smears	3
6	CxBx, ECC	6 negative Pap smears	3
7	CxBx	4 negative Pap smears	3
8	CxBx, ECC	3 negative Pap smears	3
9	ECC	5 negative Pap smears	3
10	ECC	1 negative Pap smear	2
11	CxBx, ECC, EMBX	1 negative Pap smear	2
12	ECC, EMBX	3 negative Pap smears	2
13	ECC	1 negative Pap smear	1

CxBx, cervical biopsy; ECC, endocervical curettage; EmBx, endometrial biopsy.

Discrimination of high-grade neoplastic lesions from either benign or low-grade neoplasia based on ProExC staining can be problematic. Being an indicator of the S phase induction, ProExC can demonstrate positivity in reactive endocervical cells, squamous metaplasia, and low-grade squamous intraepithelial lesions (LSILs) [8]. Given the possibility of sporadic positive staining in normal cells, in this study, cases were determined to be positive for ProExC only if AGC cell groups had greater than 50% of cells in the group that demonstrate strong nuclear staining. Although the AGC cells may occur in large cohesive cell clusters, small cell groups of two to three cells and individual AGCs present a dilemma for determining ProExC staining because of the possibility of positive sporadic staining in nonneoplastic cells. For this reason, evaluation must be limited to cell clusters with multiple morphological atypical cells identifiable and the nuclear stain must be at least moderate to intense. The determination of "intense" versus "moderate" or "weak" staining also lends subjectivity to the determination of ProExC staining.

Although no AGC case in our study demonstrated subsequent low-grade CIN on follow-up biopsy, LSILs may demonstrate ProExC positivity. Of note, although the mean age of the patients with AGC in this study was 41 years, the youngest patient was 19 years. Caution is warranted, and additional studies are required to determine the utility of ProExC staining in AGC cases of women younger than 21 years in which the prevalence of LSILs is increased.

Another limitation of this study is that ProExC immunocytochemical stains were performed on archival

cytology slides. Although the uncoverslipping method used in this study resulted in no appreciable loss of AGC cell groups, sporadic focal heavy background staining artifact was noted on 4 of the 15 AGC cases from patients with subsequent benign diagnoses. And although the cause of the artifact could not be ascertained with certainty, the possibility that residual coverslip mounting medium contributed to the focal heavy background stain exists. Fortunately, not all areas of the slides were affected, and on each slide, there were several areas unaffected by the artifact in which morphologically abnormal AGC cell groups were identified. In each of these 4 cases, the AGC groups demonstrated negative nuclear staining. Because of the rarity of archival AGC slides available for study and the inability to repeat the stain on any given slide, these cases were included for study. Additional prospective studies using residual fluid from which liquid-based specimens is used for ProExC staining would be beneficial to avoid difficulties associated with archival slides.

With an overall sensitivity and specificity of 77% and 87%, respectively, AGC-NOS ProExC-positive cases approach a similar rate of high-grade neoplasia or malignancy as that of AGC-FN. If patients with known distant malignancies are excluded then sensitivity and specificity increase to 100% and 87%, respectively, and those of patients with subsequent high-grade neoplasia or malignancy equal that of AGC-FN. Given these results, it may be suggested that ProExC immunocytochemistry be performed on AGC-NOS cytology specimens. The AGC-NOS cases that are subsequently determined to be positive for ProExC nuclear staining (AGC cell groups with >50% of cells demonstrating moderate to intense nuclear staining) could be considered AGC-FN and managed according to AGC-FN guidelines. In summary, an AGC-NOS case that is ProExC-positive by these criteria should be considered AGC-FN. Although a positive ProExC AGC-NOS should be managed as AGC-FN, a negative (or indeterminate) ProExC AGC-NOS should not be considered entirely benign. As this study demonstrates, not all malignancies demonstrate ProExC positivity; therefore, all AGC-NOS ProExC-negative cases still require management according to the current guidelines for AGC-NOS to include colposcopy with endometrial and endocervical sampling. It should also be noted that ProExC positivity is not diagnostic for high-grade neoplasia or malignancy and that false-positives may occur secondary to occasional sporadic positivity in normal endocervical cells and squamous metaplasia.

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