Atypical Glandular Cells of Undetermined Significance

Outcome Predictions Based on Human Papillomavirus Testing

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Key Words: Atypical glandular cells of undetermined significance; AGUS; Cervical neoplasms; Human papillomavirus; HPV

DOI: 10.1309/N7KCUP0VD59GDJEL

Abstract

Cases of atypical glandular cells (AGC) diagnosed on liquid-based preparations were culled from a 3-year period. When available, residual cellular material was analyzed for human papillomavirus (HPV) by polymerase chain reaction and correlated with cytologic and histologic (biopsy) outcome.

Of 178,994 cytologic cases, 187 (0.1045%) contained AGC compared with 8,740 (4.8828%) atypical squamous cells (ASC) for an AGC/ASC ratio of 0.021. HPV results and follow-up were available for 108 specimens from 106 patients. Depending on the endpoint (histologic/cyto-logic), the sensitivity range of HPV testing for significant cervical disease (high-grade squamous intraepithelial lesion [SIL], adenocarcinoma in situ [ACIS], invasive carcinoma) was 83% with a specificity range of 78% to 82%, a positive predictive value of 57% to 61%, and a negative predictive value of 91% to 95%. Fifteen false-positive results included concurrent ASC or low-grade SIL, ASC on follow-up cytology, and previous ACIS with a negative follow-up cone biopsy result. Noncervical glandular neoplasia (including atypical endometrial hyperplasia) was confirmed in 13 cases (1 recurrent), only 2 of which scored positive for HPV.

HPV-positive AGC has a substantially higher positive predictive value for significant disease than ASC (61% vs historic 20%) and merits consideration in the triage of patients with atypical endocervical cells not otherwise specified. However, noncervical or other HPV-negative glandular neoplasia must be considered in all patients with AGC, particularly older patients.

Since the first Bethesda conference on cervical cytologic abnormalities held in 1987, the clinical and laboratory management of cervical cytologic samples showing nondiagnostic atypical squamous cells (ASC) has evolved continually. Recent consensus conferences have introduced guidelines for the diagnosis and management of ASC, including the introduction of human papillomavirus (HPV) testing for triage purposes. The preferred approach to ASC in a practice using liquid-based cytology is to use reflex HPV testing, and triage of patients to recall for colposcopy or follow-up alone is based on a positive or a negative HPV test result, respectively. Given the high sensitivity of HPV testing, the positive predictive value of this test for a high-grade squamous intraepithelial lesion (HSIL; cervical intraepithelial neoplasia 2-3) is only 15% to 20%. However, the negative predictive value is high, resulting in few patients not recalled for colposcopy based on a false-negative HPV test result. Even in such cases, the risk of an ASC coexisting with or progressing to invasive carcinoma in the immediate follow-up period is extremely low.

A cytologic interpretation of atypical glandular cells (AGC) is much less frequent than ASC, and, as with ASC, an AGC interpretation is reproduced poorly between observers. AGC frequently is attributed to benign conditions, including polyps, reactive changes, and direct sampling of the lower uterine segment. However, AGC also may be associated with high-grade squamous precursor lesions and glandular neoplasia, including not only cervical adenocarcinoma and its precursor lesion endocervical adenocarcinoma in situ (ACIS) but also remote neoplasms such as endometrial and extraterine adenocarcinomas. Thus, despite its association with benign conditions, a cytologic interpretation of AGC is associated with a high risk of neoplasia.
Relative to ASC, management of AGC is complicated by several factors. These include the lack of sensitivity of colposcopy and endocervical curettage for excluding cervical glandular neoplasia, the increased risk of adenocarcinoma associated with AGC, and concerns that noninterventional strategies might delay diagnosis of a clinically significant lesion or result in loss of follow-up completely. Recent consensus guidelines of the American Society for Colposcopy and Cervical Pathology (ASCCP) in response to the 2001 modifications of the Bethesda System recommend colposcopy and endocervical sampling for all patients given a diagnosis of AGC. However, because significant disease, including invasive carcinoma, might exist in patients after negative colposcopy and endocervical sampling, cold-knife cone biopsy with its associated morbidity often is required to exclude endocervical neoplasia.2

Like cervical squamous cell carcinoma and its precursors, cervical adenocarcinoma and ACIS are highly associated with high-risk HPV infection. However, while HPV testing now is a preferred method for managing the diagnosis of squamous atypia in liquid-based preparations, its role has not been established with AGC.2 HPV testing in association with AGC has been examined in only 1 published study,16 with promising findings. To further assess the possible role of HPV testing in the clinical management of patients diagnosed with AGC, we compared concurrent HPV status with follow-up data for these patients.

Materials and Methods

The study was performed in a hospital-based laboratory with a high-risk population and was approved by the institutional review board at Brigham and Women’s Hospital, Boston, MA. Cervical samples (conventional smears and liquid-based [ThinPrep, Cytyc, Marlborough, MA] samples) diagnosed with AGC at the Brigham and Women’s Hospital cytology laboratory were identified during the period from January 2000 through January 2003. All cases diagnosed with AGC were included regardless of the presumed site of origin of the glandular atypia and without regard to the presence of a concurrently diagnosed squamous abnormality. HPV testing by polymerase chain reaction (PCR) was performed on residual material of liquid-based samples according to previously published methods.17-19 A positive PCR result was the identification of any HPV types. In 3 cases because of clinician requests, HPV testing was performed by Hybrid Capture (Digene, Gaithersburg, MD). HPV testing was performed in a blinded manner without knowledge of the specific Papanicolaou specimen interpretation (other than AGC) or any follow-up results. HPV findings then were correlated with biopsy and/or repeated Papanicolaou sampling follow-up obtained from computerized medical records. A positive biopsy or Papanicolaou follow-up test result was the identification of a glandular or squamous high-grade cervical precursor lesion or invasive carcinoma. Endometrial hyperplasia, endometrial adenocarcinoma, and other noncervical malignant neoplasms were treated as negative outcomes for evaluating HPV testing in isolation, since HPV testing is not intended to detect these diseases. However, in evaluating HPV testing as a component of clinical triage for all patients with AGC, these lesions were treated as positive outcomes.

Results

During the 36-month study period, 187 (0.1045%) of 178,994 cytologic cases were diagnosed with AGC. During this period, there were 8,740 cases (4.8828%) diagnosed as ASC with a laboratory ASC/squamous intraepithelial lesion ratio of 1.81. Of the 187 AGC specimens, 108 specimens from 106 patients comprised the study population in which HPV test results and clinical follow-up data were available.

Figure 1. Overall, follow-up specimens averaged 3.1 per patient (1.4 biopsy specimens and 1.7 Papanicolaou specimens), and the mean follow-up period was 12 months (range, 0 [ie, concurrent biopsy] to 35 months). The 30 patients with follow-up by cytology sampling alone had an average of 2 follow-up samples with a mean follow-up period of 16 months (range, 3-35 months). Table 1 compares the original cytologic diagnosis with the follow-up diagnosis with and without the inclusion of patients having only cytologic follow-up.

Table 1

Breakdown of study cases. *Concurrent squamous atypia includes the following cases: low-grade squamous intraepithelial lesion, 1; high-grade squamous intraepithelial lesion (HSIL), 3; atypical squamous cells (ASC) of undetermined significance, 9; ASC suggestive of HSIL, 4.
Table 2
Follow-up Diagnosis and HPV Result

<table>
<thead>
<tr>
<th>Follow-up Diagnosis</th>
<th>Total No. of Cases</th>
<th>No. (%) HPV-Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma in situ*</td>
<td>17</td>
<td>14 (82)</td>
</tr>
<tr>
<td>Cervical adenocarcinoma</td>
<td>2</td>
<td>2 (100)</td>
</tr>
<tr>
<td>High-grade squamous intraepithelial lesion</td>
<td>5</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Noncervical glandular lesions†</td>
<td>13</td>
<td>2 (15)</td>
</tr>
<tr>
<td>Negative (by biopsy or cytologic examination)</td>
<td>71</td>
<td>13 (18)</td>
</tr>
</tbody>
</table>

* Includes 3 cases of adenocarcinoma in situ with concurrent high-grade squamous intraepithelial lesion.
† Includes the following cases: endometrial adenocarcinoma, 5; endometrial hyperplasia, 4; fallopian tube adenocarcinoma, 2; ovarian adenocarcinoma, 1.
‡ Includes 1 case of endometrial hyperplasia and 1 of endometrial adenocarcinoma.
§ Concurrent HSIL and ASCIS.
∥ Includes 1 case each of fallopian tube adenocarcinoma (with concurrent cytologic diagnosis of atypical squamous cells of undetermined significance suggestive of HSIL) and endometrial adenocarcinoma.
¶ Includes 1 case each of endometrial adenocarcinoma, favor reactive; 1 ACIS in a patient after cone biopsy for ACIS diagnosed as AGC, not otherwise specified (NOS); query cone biopsy effect vs endocervical atypia; 1 HSIL with AGC and concurrent atypical repair on the Papanicolaou smear; 1 HSIL and AGC with concurrent AGC and ASC suggestive of a squamous intraepithelial lesion on Papanicolaou smear; and 1 ACIS with no history diagnosed as AEC, NOS.

Discussion

The 2001 modification of the Bethesda System and the accompanying ASCCP-sponsored consensus guidelines emphasize the importance of developing evidence-based reporting terminology and treatment guidelines.2,20 Moreover, it highlighted the need for further study in a number of areas, including the diagnosis of AGC.
The treatment guidelines recommend colposcopy with endocervical sampling as the initial treatment for all women with a diagnosis of AGC with the exception of women with atypical endometrial cells. Compared with the treatment of ASC, the recommended treatment for AGC is considerably more aggressive because AGC is substantially more indicative of high-grade neoplasia—HSIL, ACIS, or invasive cervical carcinoma—than is ASC. For example, although the risk of concurrent invasive squamous cell carcinoma is estimated at 1:1,000 for ASC, the risk of invasive cervical adenocarcinoma for a woman with AGC is 10-fold higher in some studies. In the present study, 2 (1.9%) of 108 patients with AGC had invasive cervical adenocarcinoma.

Because of the strong association of high-risk HPV types with both glandular and squamous cervical neoplasia, our study was intended to further test the possible usefulness of HPV testing in managing patients diagnosed with AGC. While HPV testing has been validated for the clinical management of women diagnosed with ASC, only limited but promising data on HPV testing with AGC exist.

Our method differed from that of Ronnett et al in a number of ways. Ronnett et al studied a community-based population in which study participants were women diagnosed with AGC on routine screening with conventional Papanicolaou smears. Study participation was predicated on agreement by these women to undergo a subsequent colposcopic examination. HPV testing was performed with Hybrid Capture 2 (Digene) on residual material rinsed from the sampling device for the conventional smear. Despite the substantial differences in design between the 2 studies, the findings are similar and also are comparable to the findings with ASC; studies have indicated sensitivity from 83% to 100% with a referral rate of 31% to 56%. In the current study, a PCR-based assay detected 83% (20/24) of clinically significant cervical lesions, which falls into the lower range of reported sensitivities for the Hybrid Capture 2. Moreover, this percentage contrasts with a sensitivity of 98% for cone biopsy–proven HSIL in a follow-up study of women with a previous biopsy diagnosis of HSIL (C.P.C., unpublished data). In our experience and that of others, PCR-based assays using the MY-09/MY-11 primers are slightly less sensitive than the Hybrid Capture 2. However, the measure of security conveyed by a negative assay result with either method remains unclear. Currently, a cytologic diagnosis of ASC, favor HSIL, mandates colposcopy irrespective of the HPV test result. Thus, the contribution of a single negative

### Table 3
Detection of All Neoplasia Using Alternative Triage Strategies Following a Finding of AGC

<table>
<thead>
<tr>
<th>Criteria for Referral</th>
<th>No. (Percentage) Referred</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive Predictive Value (%)</th>
<th>Negative Predictive Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All AGC</td>
<td>106 (100)</td>
<td>100</td>
<td>0</td>
<td>34</td>
<td>—</td>
</tr>
<tr>
<td>AGC and HPV-positive</td>
<td>34 (32)</td>
<td>58</td>
<td>81</td>
<td>62</td>
<td>79</td>
</tr>
<tr>
<td>AEC favor neoplasia, noncervical AGC, concurrent ASC-H, or concurrent HSIL</td>
<td>42 (40)</td>
<td>75</td>
<td>79</td>
<td>64</td>
<td>86</td>
</tr>
<tr>
<td>AEC favor neoplasia, noncervical AGC, concurrent ASC-H, concurrent HSIL, or HPV-positive</td>
<td>57 (54)</td>
<td>86</td>
<td>63</td>
<td>54</td>
<td>90</td>
</tr>
</tbody>
</table>

AEC, atypical endocervical cells; AGC, atypical glandular cells; ASC-H, atypical squamous cells cannot exclude HSIL; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion.

### Table 4
Detection of Cervical Neoplasia Using Human Papillomavirus Testing as the Sole Basis for Colposcopy Referral Following a Finding of Atypical Glandular Cells

<table>
<thead>
<tr>
<th>Study</th>
<th>Total No. of Cases</th>
<th>Percentage Referred</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive Predictive Value (%)</th>
<th>Negative Predictive Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ronnett et al</td>
<td>137</td>
<td>28.7</td>
<td>94.1</td>
<td>80.8</td>
<td>41.0</td>
<td>99.0</td>
</tr>
<tr>
<td>Present study</td>
<td>108</td>
<td>32</td>
<td>83</td>
<td>78-82</td>
<td>57-61</td>
<td>91-95</td>
</tr>
</tbody>
</table>
HPV DNA test result to the management of a cytologic interpretation of AEC favor neoplasia is doubtful. However, a strategy using repeated HPV testing or combining HPV testing with cytologic review might be more realistic.

It is important to note that the overall AGC rate of approximately 0.1% in the present study is considerably lower than the 0.5% rate found by Ronnett et al.16 despite our higher risk study population. This reflects the fact that there is a particular interest in AGC in our laboratory, and the diagnosis (with its serious implications) is used judiciously. Thus, while we detected clinically significant pathology in 33.3%, Ronnett et al.16 detected clinically significant pathology in only 14% of their population.

In published studies, AGC rates are extremely variable, ranging from 0.1% to more than 1% of all smears, but most commonly are in the 0.4% to 0.5% range.11 Follow-up data indicate that higher AGC rates are largely due to misdiagnoses of high-grade squamous precursor lesions and/or benign glandular atypias. The association with clinically significant pathology decreases with increasing AGC rates.11 In most clinical settings, AGC rates are likely to be higher than the rate in our laboratory and will be associated with a lower disease prevalence for glandular neoplasia. As disease prevalence decreases, the predictive value of a negative HPV result increases. Thus, although triage based on HPV testing would have resulted in missing 5 of 36 clinically significant lesions found in our study, the predictive value of a negative HPV result is likely to be more useful in clinical settings in which the AGC rates are high.

The high risk of neoplasia associated with the diagnosis of AGC, favor neoplasia demands aggressive management, making HPV testing unnecessary in this setting. It is equally important to recognize that the diagnosis of AGC encompasses endometrial glandular neoplasia in addition to cervical glandular and squamous neoplasia and, less commonly, metastatic disease from more distant sites. In our study, 13 (12.0%) of 108 cases represented noncervical neoplasia (5 cases of endometrial adenocarcinoma, 1 of which was recurrent; 4 of endometrial hyperplasia; 2 of ovarian carcinoma; and 2 of fallopian tube adenocarcinoma), while cervical neoplasia comprised 24 cases (22.2%). We included all categories of AGC in our study because of the occasional difficulty encountered in accurately classifying the site of origin of abnormal epithelial cells perceived as glandular. While none of the endometrial AGC cases demonstrated cervical neoplasia, 4 cases characterized as endocervical AGC ultimately proved to arise elsewhere in the female genital tract. These findings reinforce the need to manage endometrial AGC by endometrial sampling, particularly in older women, and to evaluate the significance of any glandular atypia in the context of the patient’s age and other clinical findings.

As mentioned, some categories of AGC would not be appropriate for HPV testing, either because they represent diagnoses suggestive of a noncervical malignant neoplasm or because the risk of cervical neoplasia is so high that aggressive management is needed. In practice, HPV testing for the diagnosis of AGC should be considered only for AEC, NOS, and AGC, NOS, in 2001 Bethesda System terminology. Our results and those of the one previous study of AGC and HPV testing16 indicate that HPV testing might be a feasible alternative to colposcopy and endocervical sampling in such patients. This approach is most feasible in younger women who are at low risk for endometrial and extraterine malignant neoplasms and, particularly, in practice settings in which the AGC rates are high (>0.3%). Because a negative HPV test result cannot entirely exclude the possibility of disease, including cervical and noncervical neoplasia, follow-up Papanicolaou testing according to the ASCCP-sponsored consensus guidelines for women with AEC, NOS, or AGC, NOS, would be prudent.22 This recommendation calls for interval Papanicolaou screening every 4 to 6 months until 4 consecutive negative smears have been obtained, at which point the patient may resume routine screening.

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Presented in part at the 90th annual meeting of the United States and Canadian Academy of Pathology, Atlanta, GA, March 5, 2001.

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References


