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Room for Improvement: Initial Experience With Anal Cytology

Observations From the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology

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• **Context.**—Anal cytology is being used more frequently for anal cancer screening, yet many cytologists are unfamiliar with it.

Objective.—To describe the performance of anal cytology in the College of American Pathologists' Interlaboratory Comparison Program in Non-Gynecologic Cytology (CAP NGC) educational slide program during a 6-year time span, from 2006 to 2011, using participant responses (pathologist, cytotechnologist, and laboratory).

Design.—Concordance rates for the target diagnosis and general category for each slide challenge were analyzed. Four main factors were included in the analysis: (1) general category or specific responses, (2) program year from 2006 to 2011, (3) participant type (pathologist, cytotechnologist, or overall laboratory), and (4) preparation type (liquid-based or conventional).

Results.—Participants most frequently correctly classified negative for intraepithelial lesion or malignancy, low-

grade squamous intraepithelial lesion, and herpes simplex virus infection, with concordance rates of 78.8%, 85%, and 80.2%, respectively. Performance on challenges with target diagnoses of high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma, and ameba was poor, with concordance rates of 57.1%, 56.2%, and 41.5%, respectively. Significant improvement during the 6 years was seen in the concordance rates of participants' responses for low-grade squamous intraepithelial lesion challenges but not for HSIL. There was no significant difference in performance by slide preparation type.

Conclusions.—The poor performance on anal cytology in the CAP NGC program, especially with regard to correct identification of HSIL and squamous cell carcinoma, indicates that there is a need for continued education about anal cytology.

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Anal cytology is used in the evaluation of human papillomavirus-associated precancers and cancers of the anal canal, particularly in high-risk populations—primarily men who have sex with men and human immunodeficiency virus (HIV)-infected individuals—in a manner analogous to the Papanicolaou test for cervical

cancer screening.¹ Although there are currently no national guidelines for anal cancer screening in the United States, both the Centers for Disease Control and Prevention and the American Cancer Society acknowledge that some experts recommend the use of anal cytology for screening in HIV-infected men who have sex with men and others at increased risk.^{2,3} Triage algorithms use the results of anal cytology to inform initial clinical management.⁴ However, many cytologists are not familiar with the evaluation of cytologic samples from this body site.

Anal cytology was included in the 2001 Bethesda System conference and atlas of cervicovaginal cytology because of the morphologic similarity between human papillomavirus-associated squamous lesions in these sites.⁵ In the decade since the conference, more cytologists have been seeing these specimens in their practice, particularly as knowledge of increasing anal cancer rates and awareness of the potential of anal cancer screening in at-risk individuals have become more widespread. Anal cytology was first introduced into the educational slide sets of the College of American Pathologists' (CAP) Interlaboratory Comparison Program in Non-Gynecologic Cytology (NGC) in 2005. Because clinical experience with anal cytology is relatively recent and is concentrated in a few laboratories, few

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Table 1. Concordance Rates for the General Category and Target Diagnosis by Program Year

Program Year	No. of Responses	Concordance Rate, %	P Value
General category			.17
2006	1972	58.4	
2007	4722	71.2	
2008	5673	96.1	
2009	6455	95.6	
2010	9208	95.7	
2011	9640	94.9	
Target diagnosis			.32
2006	1950	68.5	
2007	4685	71.5	
2008	5634	72.0	
2009	6355	71.7	
2010	9130	68.4	
2011	9571	69.4	

cytologists have experience with it. Familiarity with this specimen type may lead to enhanced diagnostic accuracy. Here, we report the performance of anal cytology during its initial years in the CAP NGC program.

MATERIALS AND METHODS

The CAP NGC program is an educational glass-slide program mailed quarterly. In 2011, approximately 2000 laboratories participated in the program and approximately 1400 kits had anal cytology slides included in the set. Conventional smears, ThinPrep (Hologic Inc, Boxborough, Massachusetts) preparations, and Sure-Path (BD, Franklin Lakes, New Jersey) preparations of anal cytology are included in the slide sets; however, the vast majority of anal cytology challenges in the program are ThinPrep preparations. Prior to acceptance of a slide into the CAP NGC program, 2 members of the Cytopathology Resource Committee must review it and agree on the slide's target diagnosis.

Anal cytology is categorized using the same interpretive categories as the 2001 Bethesda System for cervical cytology, modified for this specimen type.⁵ For the purposes of the CAP NGC program, anal cytology with interpretations of low-grade squamous intraepithelial lesion (SIL) or higher is categorized under the general rubric of "suspicious or positive for malignancy." Benign interpretations, including negative for SIL or malignancy, reactive changes, and organisms are classified in the general category of "negative for malignancy." Concordance with the general category is classified as a participant response of positive or suspicious for a "Positive" case, or a participant response of negative for a "Negative" slide challenge. Concordance with the target diagnosis indicates the participant's specific response matched the target diagnosis for the slide challenge. Specific target diagnoses include negative for intraepithelial lesion or malignancy, low-grade SIL (LSIL), high-grade SIL (HSIL), squamous cell carcinoma (SCC), and organisms such as *Ameba*, *Candida*, and herpes virus. Questionable participant responses (for example, pituitary adenoma) that are not included on the NGC site-specific diagnostic list for anal cytology have been classified as "Invalid." Concordance with the target diagnosis indicates the participant's specific response matched the target diagnosis for the slide.

Using participant responses (pathologist, cytotechnologist, and laboratory) from 2006 to 2011, this study describes the performance of anal cytology in the CAP NGC educational slide program during a 6-year time span. Data from 2005 were excluded because of issues with the CAP NGC kit instructions. Individual laboratories vary in how the "laboratory response" is generated; it is not proscribed by the educational slide program. Some laboratories use the majority response of the participating pathologists and cytotechnologists in the laboratory, others use the pathologist's response, and others use the supervisor's response.

Two nonlinear mixed models were used to analyze the concordance rates for the target diagnosis and the general category. Four main factors were included in the analysis: (1) general category or target diagnoses, (2) program year from 2006 to 2011, (3) participant type (pathologist, cytotechnologist, or overall laboratory), and (4) preparation type (liquid based or conventional). The interaction terms between these factors were also included. Both models included a repeated-measures component to model the slide factor correlation structure, which controls for the slide-specific performance.

Statistical method: a significance level of .05 was used for these analyses.

RESULTS

This analysis includes 37 670 general category responses and 37 325 specific responses based on 708 anal cytology slides that were evaluated in the CAP NGC program during a 6-year period between 2006 and 2011. Pathologists responded to 18 960 challenges, cytotechnologist responses totaled 11 306, and overall laboratory responses totaled 7404. Overall, there was a 91.0% concordance with the "Negative" general category (N = 5238) and a 90.4% concordance with the "Positive" general category (N = 32 432).

By 2008, response concordance rates for the general category improved to present levels, ranging from a low of 58.4% concordance in 2006 to 96.1% concordance in 2008 (Table 1). Between 2006 and 2011, there was no significant change in the concordance of the specific participant responses for the target diagnosis, with concordance rates for the specific responses varying between 68.4% and 72.0%.

Cytotechnologists more accurately categorized challenges as "Positive" or "Negative" than did individual pathologists or the overall laboratory responses ($P < .001$). However, the laboratory's specific response more often matched the target diagnosis (72.0% concordance) than did either individual pathologist (70.6% concordance) or cytotechnologist (68.6% concordance) responses ($P = .03$; Table 2).

The concordance rates for the target diagnoses varied significantly ($P < .001$; Table 3). Participants most frequently correctly classified LSIL (85.0% concordance rate), with no significant difference between pathologists, cytotechnologists, or laboratories. Most anal cytology challenges with the target diagnosis of negative for intraepithelial lesion or malignancy and herpes simplex virus infection were also correctly classified, with 78.8% and 80.2% concordance rates, respectively. In fact, the best performance of any of the slide challenges was by cytotechnologists on slide challenges with herpes simplex virus infection, at a concordance rate of 90.7%. This rate was significantly better than pathologists' specific responses for slide challenges with the target diagnosis of herpes simplex virus, which was at 72.4% concordance.

Amebic organisms were infrequently recognized, with the lowest overall concordance rate for the target diagnosis at only 41.5%; pathologists and laboratory responses matched the target diagnosis significantly more often than cytotechnologists ($P < .001$; Figure 1, A). The poorest performance of any of the slide challenges was for cytotechnologist responses on slide challenges with Ameba having a concordance rate of 26.0%. Negative for intraepithelial lesion or malignancy, as well as other infectious organisms (for example, *Trichomonas* species and fungus), made up most of the other specific responses for slide challenges with

Table 2. Concordance Rates for the General Category and Target Diagnosis for General Category and Specific Response by Reader Participant Type			
Participant Type	No. of Responses	Concordance Rate, %	P Value
General category			<.001
Pathologist	18 960	88.8	
Cytotechnologist	11 306	93.4	
Laboratory	7404	90.5	
Target diagnosis			.03
Pathologist	18 810	70.6	
Cytotechnologist	11 225	68.6	
Laboratory	7290	72.0	

the target diagnosis of Ameba. Only 5.1% of the slide challenges with Ameba were classified as LSIL or worse (data not shown).

The concordance rates for the slide challenges with target diagnoses of HSIL and SCC fared only slightly better than the Ameba challenges, at 57.1% and 56.2%, respectively. Here, there were no significant differences in performance between pathologists' and cytotechnologists' specific responses; however, the overall laboratory-specific response was significantly better ($P = .01$) than that of cytotechnologists for the target diagnosis of HSIL (Figure 1, B).

During the 6-year period of experience with anal cytology as part of the NGC program, there was significant improvement in the concordance rates for participants' specific responses to the target diagnosis of LSIL but not HSIL ($P < .001$; Table 4). There are too few data to test for improvement over time for the target diagnosis of SCC.

Although the participants' specific responses matched the target diagnosis for the HSIL and SCC slide challenges less than 60.0% of the time, participants categorized most slides as abnormal, with more than 95.0% diagnosed as LSIL or greater (Table 5). The HSIL challenges were both under-called as LSIL (24.9%) and overcalled as SCC (14.3%). The SCC challenges were frequently interpreted as HSIL (40.4%).

There was no significant difference by slide preparation type—either liquid-based or conventional—for the concordance rates for the target diagnosis or the general category (Table 6).

COMMENT

The inclusion of anal cytology in the CAP NGC educational slide program allows more cytologists the opportunity to view these specimens, even if not part of their routine practice. Although there was a significant increase in participants correctly classifying slide challenges as either "Positive" or "Negative" between 2006 and 2011, most improvement occurred within the first 2 years after anal cytology was introduced into the CAP NGC educa-

Table 3. Overall Concordance Rates for the Target Diagnosis and by Participant Type			
	No. of Responses	Concordance Rate, %	P Value
Target diagnosis			<.001
LSIL	15 781	85.0	
HSIL	16 025	57.1	
SCC	329	56.2	
NILM	3019	78.8	
Amebic organisms	1696	41.5	
HSV	475	80.2	
Target diagnosis by participant type			<.001
LSIL			Path-Cyto $P = .10$
Pathologist	7844	84.4	
Cytotechnologist	4838	85.8	
Laboratory	3099	85.3	
HSIL			Lab-Cyto $P = .01$
Pathologist	8256	58.4	
Cytotechnologist	4631	53.1	
Laboratory	3138	59.6	
SCC			Path-Cyto $P = .27$
Pathologist	175	55.4	
Cytotechnologist	93	60.2	
Laboratory	61	52.5	
NILM			Path-Cyto $P = .67$
Pathologist	1490	77.9	
Cytotechnologist	947	78.8	
Laboratory	582	81.1	
Amebic organisms			Path-Cyto $P < .001$
Pathologist	813	49.6	
Cytotechnologist	566	26.0	
Laboratory	317	48.3	
HSV			Path-Cyto $P < .001$
Pathologist	232	72.4	
Cytotechnologist	150	90.7	
Laboratory	93	82.8	

Abbreviations: Cyto, cytotechnologist; HSIL, high-grade squamous intraepithelial lesion; HSV, herpes simplex virus; Lab, laboratory; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy.

Figure 1. A, Amebic cysts on anal cytology. Note their small size relative to the intermediate squamous cell and their internal structure. B, High-grade squamous intraepithelial lesion on anal cytology. The morphologic features are similar to those on gynecologic cytology (ThinPrep, Papanicolaou stains, original magnifications $\times 600$ [A]; original magnification $\times 600$ [B]).

tional program. Temporally, this improvement occurred following clarification of the kit instructions after the introduction of these specimens into the program in 2005. Although properly considered nongynecologic specimens, which require pathologist review, anal cytology uses Bethesda System terminology in a fashion that is more akin to the Papanicolaou test for cervical cancer screening. However, issues with the CAP SCORES computer system preclude the use of the gynecologic cytology template for

anal cytology in its educational program. This led to initial confusion as to the appropriate categorization of anal SIL as negative or as suspicious/positive for malignancy. After 2005, the kit instructions were clarified to label SIL with the general categorization of “Suspicious or Positive” for malignancy.

Anal cytology with LSIL was the best performer among slide challenges, having overall concordance rates of 85.0% for the target diagnosis. This compares favorably to the 84.8% rate for validated LSIL slides in the CAP PAP program for cervicovaginal cytology.⁶ Among the squamous abnormalities, the correct diagnosis of LSIL also improved during the 6 years.

Disappointingly, with the exception of LSIL, there has been no significant improvement in correctly matching the specific response to the target diagnosis over time. Overall concordance rates hover around 70.0% during the 6-year time period reported here. Indeed, HSIL and SCC were correctly classified less than 60.0% of the time, with no significant improvement noted during the 6 years. Taken together, however, squamous abnormalities were recognized in more than 95.0% of challenges.

How does the correct classification of the degree of squamous abnormality have an impact on the clinical management of patients with abnormal anal cytology? That depends. In an ideal world with unlimited resources, all high-risk patients with atypical squamous cells or with more significant abnormalities would be triaged to high-resolution anoscopy.⁴ Although the morphologic features of human papillomavirus-associated squamous lesions in the anus are similar to those in cervix, the extent and distribution of abnormal anal cytology results in this high-risk population are quite different from abnormal cytology results seen in cervical screening populations. The rate of histologic detection of high-grade squamous lesions is much higher in patients with atypical squamous cells of undetermined significance on anal cytology than in patients with atypical squamous cells of undetermined significance on a cervical Papanicolaou test.^{7,8}

As anal cytology becomes more accepted as a routine screening test, however, accurate classification of the degree of SIL is increasingly important to expedite the evaluation of those with anal precancer because of the very limited availability of those with expertise in high-resolution anoscopy. Clearly, there is room for improvement. We need to get better at evaluating these specimens.

Identification of organisms, such as herpes simplex virus and *Candida*, in anal cytology mirrors that of gynecologic cytology. However, other organisms both pathologic and commensal can be seen on anal cytology. These include those rarely encountered in gynecologic cytology, such as *Ameba*, pinworm eggs, and other helminths. Recognition of

Table 4. Concordance Rates for Target Diagnoses of Low-Grade Squamous Intraepithelial Lesion (LSIL), High-Grade Squamous Intraepithelial Lesion (HSIL), and Squamous Cell Carcinoma (SCC) by Year

Target Diagnosis	Year						P Value
	2006	2007	2008	2009	2010	2011	
LSIL concordance rate, No. (%); n = 15 781	967 (79.8)	2094 (82.7)	2701 (85.7)	2857 (84.4)	3433 (86.3)	3729 (86.5)	<.001
HSIL concordance rate, No. (%); n = 16 025	841 (54.6)	1970 (56.8)	2097 (57.1)	2618 (59.2)	4130 (55.9)	4369 (57.7)	.21
SCC concordance rate, No. (%); n = 329	—	—	—	—	97 (52.6)	232 (57.8)	Not tested

Table 5. Percentage Concordance of Participants' Responses for Target Diagnosis for Low-Grade Squamous Intraepithelial Lesion (LSIL), High-Grade Squamous Intraepithelial Lesion (HSIL), and Squamous Cell Carcinoma (SCC)

Target Diagnosis	Participants' Response			
	LSIL, %	HSIL, %	SCC, %	Total Abnormal (LSIL+), %
LSIL	85.0	8.5	1.9	95.4
HSIL	24.9	57.1	14.3	96.3
SCC	2.1	40.4	56.2	98.7

Table 6. Concordance Rates for the General Category and Target Diagnosis by Preparation Type

Preparation Type	No. of Responses	Concordance Rate, %	P Value
General category			.06
Liquid-based	37 096	90.4	
Conventional	574	96.9	
Target diagnosis			.93
Liquid-based	36 758	70.4	
Conventional	567	59.4	

amebic trophozoites and cysts was very poor on the CAP NGC challenges. Proper recognition of Ameba on anal cytology is essential for prompt appropriate clinical correlation with patient symptoms and additional studies, as warranted.

The level of performance on anal cytology in the CAP NGC program, especially with regard to specific interpretations, indicates that there is a need for continued education about anal cytology. Increased exposure evaluating a specific specimen type should theoretically improve performance, although little improvement was noted. More experience is obviously needed. At most, one anal cytologic slide was included per educational slide set. This may not be enough exposure to this sample type for individuals to become proficient. Perhaps concentrated training with multiple anal cytology samples viewed contemporaneously would improve performance. Continued educational efforts are needed.

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