Anal Cytology

Institutional Statistics, Correlation With Histology, and Development of Multidisciplinary Screening Program With Review of the Current Literature

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• Context.—The incidence of anal cancer in the United States is on the rise in high-risk populations. The anal Papanicolaou test (APT) is advocated as a screening tool, in addition to digital rectal examination and high-resolution anoscopy.

Objective.—To review our experience and the current literature to create, in cooperation with clinicians, a standardized screening and treatment algorithm given our large volume of APTs.

Data Sources.—All APTs collected between January 2013 and June 2015 were reviewed and correlated with follow-up/concurrent biopsy diagnoses, and clinical and social history. In total, 1417 APTs were performed on 1185 patients and APT results were as follows: 17.4% (247 of 1417) unsatisfactory; 27.9% (395 of 1417) negative; 19.5% (276 of 1417) atypical squamous cells of undetermined significance (ASC-US); 24.1% (342 of 1417) low-grade squamous intraepithelial lesion (LSIL); 3.6% (51 of 1417) atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (HSIL) (ASC-H); and 7.5% (106 of 1417) HSIL. In total 376 cases (26.5%) had concurrent/follow-up biopsy. Review of all unsatisfactory cases with squamous intraepithelial lesion (SIL) on biopsy showed LSIL in 19.2% (5 of 26). Anal Papanicolaou test with cytologic abnormality (ASC-US+) had an 83.8% (315 of 376) rate of biopsy-proven disease, and sensitivity was higher (92%) for high-grade anal intraepithelial neoplasia or worse (AIN2+). Overall detection of AIN2+ using ASC-US+ showed specificity of 26%, negative predictive value of 92%, and positive predictive value of 26%.

Conclusions.—Analytical cytology has a high abnormal rate (54.7%) and sensitivity but poor correlation with histologic grade. High unsatisfactory rate indicates need for improvement in sampling with 68.4% of cases having SIL on biopsy. Multidisciplinary effort led to improvements in sampling, cytologic interpretation, and development of a standardized management algorithm.

the relationship between HIV status and anal cytology performance is inconsistent across studies.\textsuperscript{6}

Before the HIV epidemic the incidence of anal cancer was estimated to be as high as 37/100 000 MSM.\textsuperscript{7} The potential impact of anal cancer screening among this population can be best illustrated in comparison with cervical cancer epidemiology. The incidence of cervical cancer before the advent of screening with the Papanicolaou test was approximately 40 to 50 cases per 100 000 and dropped to 8 to 10/100 000 in screened populations.\textsuperscript{8} Historically, it is the MSM population that has been the major risk group targeted for anal cytology screening. Unlike cervical cancer, there are currently no standardized screening or management guidelines that are endorsed by professional societies for anal cancer. Examination of the literature and the near tripling of the incidence of anal cancers in HIV-positive MSM reiterates the need to focus on the best strategies to prevent anal cancer.

Anal squamous cell carcinoma shares several similarities with cervical squamous cell carcinoma. Both have a causal association with HR-HPV, a presumed origin at a mucosal transformation zone, and precursor lesions and precancer (high-grade squamous intraepithelial lesion [HSIL]) that can be detected by exfoliative cytology. However, uncertainty still remains regarding the natural history of HPV in the anus and the role of anal cytology and high-resolution anoscopy (HRA) as screening tests for anal cancer.

Two major studies, Anal Cancer HSIL Outcomes Research (ANCHOR) and the Study of the Prevention of Anal Cancer (SPANC), are currently in progress to evaluate the potential value of anal screening, early detection, and destruction of AIN for the prevention of invasive squamous cell carcinoma. SPANC is a prospective study of the epidemiology of low-risk and high-risk anal HPV infection and related cytologic and histologic abnormalities in HIV-negative and HIV-positive homosexual men aged 35 years and older. The study aims to recruit 600 men from community-based settings in Sydney, Australia. The participants will have 6 study visits during 3 years, and at the first 5 visits undergo a digital anorectal examination, anal Papanicolaou test (APT), and HPV genotyping, followed by HRA and directed biopsy of any visible abnormalities.\textsuperscript{5} ANCHOR is a multicenter phase III clinical trial sponsored by the United States National Cancer Institute’s Office of HIV and AIDS and Malignancy. It will enroll 5058 participants, both men and women aged 35 and older who have HIV infection and biopsy-proven HSIL at baseline. Eligible participants will have no past history of anal cancer, or treatment or removal of HSIL and be randomly assigned to treatment or active monitoring at baseline. Participants will be followed up every 6 months for HSIL outcomes for at least 5 years. Throughout the study, the incidence of invasive cancer in both arms will be monitored, and biospecimens and associated participant data will be collected for correlative scientific studies.

Our current study data originating from a quality assurance audit performed in 2015, much like the larger ANCHOR study, endeavor to examine the current relationship between HR-HPV, AIN, and the development of squamous cell carcinoma. The practice setting at Northwestern Memorial Hospital, Chicago, Illinois, is composed of academic and nonacademic community providers with a substantial volume of APTs. Thus, we sought to establish a strong multidisciplinary group for anal cancer prevention and management of anal dysplasia in the Chicago metro area by reviewing results of anal cytology, anoscopy, anal biopsy, and early results of HR-HPV reflex testing for atypical squamous cells of undetermined significance (ASC-US). We also performed an extensive review of the current literature to ensure our methods and practices are in line with other high-volume institutions.

\section*{MATERIALS AND METHODS}

This single-center, retrospective study was approved by the Northwestern University Feinberg School of Medicine (Chicago, Illinois) Institutional Review Board. All APT cytology specimens collected between January 1, 2013, and June 30, 2015, were included in the study. Cytology specimens were primarily collected by primary care practitioners and advanced practitioners in a high-risk clinic and by colorectal surgeons who manage the patients. Most providers used a blind swab of the anal canal with either a bristled cytobrush or Dacron-tipped swab, and placed it directly into a ThinPrep jar (Cytyc, Marlborough, Massachusetts). However, a few providers used a cotton swab. Some of the providers instructed patients to use a Fleet enema (C.B. Fleet Company, Inc, Lynchburg, Virginia) before the procedure to optimize visualization of the anorectal junction; however, this was not standard amongst all.

In our cytopathology laboratory, the specimens are prepared via ThinPrep methodology (Cytyc) and interpreted by using the Bethesda system terminology as unsatisfactory; benign; ASC-US; low-grade squamous intraepithelial lesion (LSIL); atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H); HSIL; and squamous cell carcinoma (SCC).\textsuperscript{9} Human papillomavirus testing is performed in-house in our molecular laboratories, using the Roche cobas 4800 (Roche Diagnostics, Indianapolis, Indiana), which provides partial genotyping information for HPV 16, HPV 18, and 12 “other” high-risk types. Our medical center uses conventional anoscopy, performed by 12 colorectal surgeons and their physician assistants. Of note, HRA is not yet used by our institution. Anal histology results were collected for individuals who underwent conventional anoscopy and subsequent biopsy, performed by colorectal surgeons. Anal biopsies were categorized as benign, LSIL (AIN1), HSIL (AIN2 and AIN3), and SCC, and all equivocal cases were immunostained with p16 per Lower Anogenital Squamous Terminology Standardization Project criteria.\textsuperscript{10} Available demographic information and relevant clinical history were retrieved from the electronic medical record and included age, sex, sexual orientation, HIV status, and presence of any other pertinent comorbidities (ie, history of malignancy, organ transplant, autoimmune disease). Available HR-HPV data were collected but routine reflex HR-HPV testing for ASC-US was not implemented until September 2015. HR-HPV testing was performed according to the manufacturer’s instructions and after internal validation, on anal cytology specimens, using the cobas 4800 HR-HPV test (Roche Diagnostics). The cobas 4800 assay uses probes for 14 types of oncogenic HPV for detection of HPV 16, HPV 18 and 12 “other” types (31, 33, 35, 39, 45, 51, 52, 58, 59, 66, and 68). Categorical variables were compared by using the chi-square test or Fisher exact test, where appropriate. A P value < .05 was considered statistically significant.

\section*{RESULTS}

A total of 1417 APTs prepared by ThinPrep methodology from 1185 individuals were included in the study. Patients’ ages ranged from 19 to 83 years, 1066 of 1185 (90%) were male and 119 (10%) were female, and approximately 806 (68%) patients were HIV positive, based on available clinical data. Of the 68% HIV-positive patients, 790 (98%) were male and 16 (2%) female. Of 1066 male patients, 362 (34%) were MSM, and 170 of 362 MSM (47%) were HIV positive. Other significant clinical comorbidities included hepatitis C virus or hepatitis B virus in 27 of the 1185 patients (2.3%).
and in 21 patients (1.8%), some form of autoimmune condition leading to immune compromise, such as systemic lupus erythematosus, inflammatory bowel disease, or history of malignancy.

Cytology findings were abnormal, defined as ASC-US or worse (ASC-US+), in 775 of the 1417 APTs (54.7%). The overall breakdown by Bethesda category was as follows (n = 1417): HSIL, 106 (7.5%); ASC-H, 51 (3.6%); LSIL, 342 (24.1%); ASC-US, 276 (19.5%); negative, 395 (27.9%); and unsatisfactory, 247 (17.4%). Of note, the presence or absence of transition zone epithelium was not documented in this study; however, the literature has conflicting reports on whether or not the presence of transition zone epithelium improves sensitivity in detection of HSIL.8 Biopsy correlation was available in 376 of 1417 cases (26.5%) and the results are displayed in Table 1.

Of the 376 APTs with biopsy correlation, 315 tests (83.8%) with an interpretation of ASC-US or higher had biopsy-proven disease (AIN1 or worse). When further broken down, 84 of 130 APTs (64.5%) interpreted as LSIL had AIN1 on biopsy and 31 of 60 (50.8%) interpreted as HSIL had a diagnosis of AIN2 or higher. Overall test performance for the detection of AIN2+ using any abnormal cytology (ASC-US+) was sensitivity 92%, specificity 26%, negative predictive value 26%, and positive predictive value 92%. We compare our data with those of 2 other major academic institutions with a high volume of APTs and anal biopsy as part of anal cancer screening programs in Table 2. While our ASC-US rate of 20% was comparable, our unsatisfactory rate of 17.4% was significantly higher.

Thus, review of all unsatisfactory cases with LSIL or higher on biopsy follow-up was undertaken. There were 26 cases in total and most cases (21 cases, 80.8%) remained truly unsatisfactory owing to a number of factors including hypocellularity (11 cases, 52.3%), inadequate nucleated squamous cells (8 cases, 38.1%), or excess obscuring organic material or lubricant (2 cases, 9.5%). We also noted that APT providers who used cotton swabs had a much higher inadequacy rate. However, 5 cases (19.2%) on review were found to have atypical cells that warranted at least a diagnosis of ASC-US. Overall, of the 38 total unsatisfactory cytology cases with follow-up biopsy, 26 (68.4%) had squamous intraepithelial lesion (SIL) on follow-up biopsy (15 [57.9%] AIN1 and 3 [10.5%] AIN2/3). Cytologic features noted in unsatisfactory cases, which on review were found to have diagnostic cells, included nuclear enlargement, hyperchromasia and pyknosis, koilocyte-like cells, and a feature noted in ASC-US cases that correlated with LSIL on biopsy and included “cell-in-cell pattern” (Figure 1, A through D).

While ancillary HR-HPV testing was performed sporadically throughout the study period per clinician request, it was formally integrated as part of the anal cancer screening algorithm by our recommendation to the multidisciplinary team toward the end of our initial study window starting from September 2015 onward. All anal cytology cases with a diagnosis of ASC-US were systematically followed up by reflex HPV testing so the data that will be discussed reflecting testing are from September 2015 to July 2016. A total of 119 cases in this time frame were tested, and 117 had adequate material to yield diagnostic results; these results are displayed in Table 3. Although most cases were HPV negative (57 of 117, 48.7%), it was noted that of the HPV-positive cases, most cases (41 of 60, 68.3%) tested for other high-risk types, and an additional 10 cases tested positive for 1 of the high-risk types in combination with “other” HR-HPV type for an overall total of 51 of 60 (85%), similar to numbers seen in the literature.11

### DISCUSSION

Anal cytology was included in the second edition of the Bethesda Atlas in 2001 and we have been using the Bethesda terminology to evaluate APTs since 2002. Our annual anal cytology volume ranges from 550 to 600 cases. Overall anal cytology at our institution had a high abnormal rate (775 of 1417, 54.7%) and sensitivity (92%) but low positive predictive value and specificity (both 26%) for the detection of HSIL+. This is consistent with data in large-scale review articles and meta-analyses, which have shown sensitivity of anal cytology ranging from 47% to 90% and specificity ranging from 16% to 92%.12,13

Early data from the SPANC showed that among homosexual men mainly recruited from community-based settings in Sydney, 59% had abnormal cytology and 35% had histologic HSIL. The overall sensitivity of liquid-based anal cytology in the detection of histologic HSIL was just

### Table 1. Cytologic-Histologic Correlation for Anal Papanicolaou Tests (January 2013 to June 2015)

<table>
<thead>
<tr>
<th>Bx dx/Pap</th>
<th>Unsat, % (n = 38)</th>
<th>NILM, % (n = 73)</th>
<th>ASC-US, % (n = 60)</th>
<th>LSIL, % (n = 130)</th>
<th>ASC-H, % (n = 14)</th>
<th>HSIL, % (n = 61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>31.6</td>
<td>34.2</td>
<td>25</td>
<td>11.5</td>
<td>35.7</td>
<td>13</td>
</tr>
<tr>
<td>LSIL</td>
<td>57.9</td>
<td>57.3</td>
<td>63</td>
<td>64.5</td>
<td>50.0</td>
<td>36</td>
</tr>
<tr>
<td>HSIL</td>
<td>10.5</td>
<td>6.8</td>
<td>10</td>
<td>21.5</td>
<td>14.3</td>
<td>46</td>
</tr>
<tr>
<td>SCC</td>
<td>0</td>
<td>1.4</td>
<td>1.7</td>
<td>1.5</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviations: AIN, atypical squamous cells; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; Pap, Papanicolaou smear diagnosis; SCC, squamous cell carcinoma; Unsat, unsatisfactory.

### Table 2. Comparison of Anal Cytology Statistics by Bethesda Category Between 3 High-Volume Academic Institutions

<table>
<thead>
<tr>
<th>Bethesda Category</th>
<th>NMH, %</th>
<th>UCSF, %</th>
<th>MGH, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>17</td>
<td>&lt;5</td>
<td>~8</td>
</tr>
<tr>
<td>Negative (NILM)</td>
<td>28</td>
<td>30</td>
<td>48.6</td>
</tr>
<tr>
<td>ASC-US</td>
<td>20</td>
<td>20</td>
<td>25.7</td>
</tr>
<tr>
<td>LSIL</td>
<td>24</td>
<td>30</td>
<td>~12</td>
</tr>
<tr>
<td>ASC-H</td>
<td>3.5</td>
<td>4</td>
<td>~2</td>
</tr>
<tr>
<td>HSIL+</td>
<td>7.5</td>
<td>10–15</td>
<td>~3</td>
</tr>
</tbody>
</table>

Abbreviations: AIN, atypical squamous cells; AIN+, high-grade squamous intraepithelial lesion (**+** indicates HSIL or squamous cell carcinoma); HSIL+, high-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; NMH, Northwestern Memorial Hospital, Chicago, Illinois; UCSF, University of California San Francisco.
above 80%, and the specificity was 53%. The sensitivity and specificity did not differ between HIV-positive and HIV-negative men, although the positive predictive value was significantly higher (53.9% versus 40.3%) and the negative predictive value was lower (79.2% versus 89.9%) in the men who were HIV positive. The specificity of anal cytology increased with age. The sensitivity increased significantly in those with more extensive HSILs, and in those from whom more biopsies were obtained and for whom cytology showed metaplastic cells. This review of ongoing studies and of our own institutional anal cytology and biopsy statistics and correlation provided us a roadmap to begin efforts to improve our sensitivity and specificity and revealed a number of possible corrective actions that were subsequently instituted for our clinical providers, cytotechnologists, and pathologists.

Our first challenge was to address our unacceptably high unsatisfactory rate. When reviewing the literature, while other major high-volume institutions had unsatisfactory rates in the range of 5% to 8%, our unsatisfactory rate for the study period was 17% (240 cases). This is significant because other studies like that of Zaccarini and Khurana in 2015 have shown that a significant number of unsatisfactory cases, particularly in high-risk groups, are found to have AIN on follow-up biopsy. In that particular study, 47% of patients with unsatisfactory anal cytology had AIN on follow-up biopsy (39% AIN1 and 18% AIN2/3), and we had similarly high numbers with a total of 26 patients (68.4%) with AIN (15 [57.9%] AIN1 and 3 [10.5%] AIN2/3) on follow-up, with the remaining minority having benign diagnoses. This prompted review of all of the

| Table 3. Results of NMH ASC-US Reflex HR-HPV Testing (September 2015–July 2016) |
|-------------------------------|-----------------|
| **No. (%) of Cases (n = 119)** | **Result**      |
| 57 (47.8)                      | Negative        |
| 8 (6.7)                        | HPV 16          |
| 8 (6.7)                        | HPV 16 + others |
| 41 (34.4)                      | HPV non-16/18 only |
| 2 (1.6)                        | QNS – test not performed |
| 1 (0.8)                        | HPV 18          |
| 2 (1.6)                        | HPV 18 + others |

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; NMH, Northwestern Memorial Hospital, Chicago, Illinois; QNS, quantity insufficient.
unsatisfactory cases with a diagnosis of SIL on follow-up biopsy to determine the true incidence of unsatisfactory cases versus cases where lesional cells may have been missed, owing to reviewer error or extracellular obscuring material. While most cases (21, 80.8%) were truly unsatisfactory, certain key cytologic features in hypocellular or partially obscured cases were noted and when used could help decrease the number of unsatisfactory cases and more importantly, cases where reflex HR-HPV testing or biopsy should be pursued. The relatively high incidence of cases with excess extracellular material or anucleated squames on review also prompted a discussion with clinicians and physician assistants, emphasizing the importance of the transition zone as opposed to the distalmost aspect of the anorectal canal and exfoliating only anucleated squames, using the proper type of cytobrushes as opposed to cotton swabs), not using excess (if any) lubricant, and also promoting cleaning/preparation of the area to prevent extra cellular material from obscuring the sample. We collected and distributed methods for anal cytology collection to our providers with high unsatisfactory rates and provided individual adequacy statistics to our clinical providers. Since then the quality of anal cytology samples has improved, and while not enough time has elapsed to reach statistical significance, a positive trend and decrease in inadequate samples has been noted.

Unlike our unsatisfactory rates, our ASC-US rate of 19.5% (276 cases) was on par with the published literature, with other large institutions like University of California San Francisco (UCSF, San Francisco) and Massachusetts General Hospital (Boston) reporting rates of 20% and 25%, respectively. Also in keeping with published literature, most patients who tested positive with HR-HPV were associated with the non-16/18 “other” types. Overall the ASC-US category comprised 36% (279 cases) of abnormal results, which would overwhelm our current anoscopic capacity. Early publications from SPANC also expressed concern about the resources required to follow-up the 59% of abnormal cytology results seen in the study’s community-based population. To better manage the patients with ASC-US with our limited anoscopy resources, we addressed this issue with a 2-fold approach: first, to better recognize and define cytologic features that can be more definitively classified as LSIL or reactive/degenerative; and second, to implement reflex HR-HPV testing for all ASC-US cases and only triage HR-HPV+ patients with ASC-US results to anoscopy/biopsy, and have HR-HPV-negative patients return for cytology in 1 year. This approach, however, was not meant to discount the importance of the ASC-US category, which has been shown in several studies to have a higher positive predictive value for high-grade disease in the anus among at-risk populations than in the cervix among the general population. Among the 119 patients for whom reflex HR-HPV testing was performed after an ASC-US interpretation, 60 (50.4%) were positive. Of these 60 patients, 51 (85%) tested for 1 of the 2 high-risk types (16 or 18) in conjunction with 1 of the “other” non-16/18 high-risk types and 41 (68%) tested with non-16/non-18 other types only.

Analysis of our data showed a higher rate of HSIL on cytology as opposed to biopsy, which is different from previously published studies that show higher rates of high-grade dysplasia on histology compared to cytology. A total of 80 cases (21%) were diagnosed as HSIL+ on cytology; however, on biopsy follow-up only 61 cases (16%) had a histologic diagnosis of HSIL. One of the possible explanations for this discordance includes lack of consistent referral to anoscopy. Until recently, a significant number of patients who received an abnormal cytologic diagnosis were not systematically triaged for further biopsy. Another possible reason is undersampling of lesional tissue on biopsy due to the fact that our surgeons use conventional anoscopy as opposed to HR-HPV. As a result, it is evidence that HR-HPV is technically more difficult than colposcopy. There are convoluted mucosal folds of the anal canal, and performance characteristics of HR-HPV also vary with the training and experience of the anoscopist. For these reasons, ASC-US results to anoscopy/biopsy, and have HR-HPV–

CONCLUSIONS
In this study we successfully worked with our clinical providers to carry out a large-scale quality assurance analysis of our anal cancer screening by analyzing anal
cytology results with histologic correlation, identifying methods to improve our adequacy rate, implementing reflex HR-HPV testing for ASC-US to effectively use limited anoscopy resources, and developing a systematic approach to patient follow-up. A limitation of our study is the fact that reflex HR-HPV testing for ASC-US cases began after our initial study window; however, we felt it was important to include this early data to show the relationship between ASC-US cytology cases and the incidence of non-16/non-18 other HR-HPV positivity. Clearly, there remains much work to be done but we feel our initial findings help contribute to the growing literature regarding utility and best practice guidelines for anal cancer screening by cytology. The incidence of HPV-associated AIN and anal squamous cell carcinoma in at-risk populations is on the rise, and whether screening with anal cytology will help prevent anal cancer will be answered by studies such as ANCHOR and SPANC in the next several years as will the impact of HPV vaccination in prevention of anal cancer.

Figure 2. Northwestern Memorial Hospital Clinical Algorithm. Abbreviations: APT, anal Papanicolaou test; ASC-US, atypical squamous cells of undetermined significance; bx, biopsy; EUA, examination under anesthesia; HIV, human immunodeficiency virus; HR-HPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; Pap, Papanicolaou; SIL, squamous intraepithelial lesion.

References