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Classification of Anal Squamous Intraepithelial Lesions: 2-Tiered Terminology and the Quest to Reduce the Incidence of Anal Cancer Among At-Risk Individuals

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Abstract: The incidence of anal cancer is increasing in the general population among both men and women. Its incidence is particularly high among certain risk groups such as men who have sex with men and individuals immunosuppressed because of HIV infection. In recognition of the similarity in the biology of anal cancer and human papillomavirus–associated cancer elsewhere in the genital tract, the Lower Anogenital Squamous Terminology project recommended that terminology for lesions be standardized across the anogenital tract, including the anus. Thus, a 2-tier system is recommended, with anal low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesion (HSIL) replacing older terminology. Anal cytology and histopathology use the same 2-tier terminology. Anal LSIL is not believed to be precancerous, whereas HSIL is likely the anal cancer precursor. As at other genital sites, p16 staining is recommended for lesions that are morphologically difficult to distinguish between LSIL and HSIL, and between HSIL and squamous metaplasia when necessary. Performance of anal cytology and high-resolution anoscopy–guided biopsy is performed similarly to procedures in the cervix. Identification and treatment of anal HSIL may reduce the risk of anal cancer, as at other genital tract sites, although this has not yet been formally demonstrated. Likewise, superficially invasive squamous cell carcinoma of the anus is defined similarly to superficially invasive squamous cell carcinoma elsewhere in the genital tract, but the utility of this diagnosis to guide treatment options has not yet been demonstrated.

Key Words: anal squamous intraepithelial lesion, anal cancer, human papillomavirus

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PATIENT 1

M.N. is a 45-year-old man who has sex with men (MSM) who was first seen in the University of California, San Francisco (UCSF), Anal Neoplasia Clinic in 2008 for evaluation of anal warts. He had a history of anal warts in 1980 both internally and externally. He had 2 surgeries in 1980 and 1981 and cryotherapy for recurrent perianal warts. He has not had any warts since then but also has not had anoscopy performed since the second surgery. He has a history of 75 lifetime sexual partners and practices both receptive and insertive anal intercourse. He reports a history of anal gonorrhea in 1984 and syphilis in 1988 and 1989. He believes that he has been HIV-infected for 15 years with a CD4 nadir of 220 cells/mL but has no history of any other AIDS-defining illnesses. He was begun on highly active antiretroviral therapy (HAART) in 1998. His most recent CD4 level was 800 cells/mL, and his HIV viral load was not detectable.

He was diagnosed with intra-anal high-grade squamous intraepithelial lesion (HSIL) in the right lateral octant at the squamocolumnar junction (SCJ) and at the anterior octant at the SCJ. He was treated with infrared coagulation (IRC) in December 2008. He was seen again 6 months later (July 2009) and was found to have low-grade squamous intraepithelial lesion (LSIL) on biopsy of the areas treated with IRC and on cytology. Five months later, he had HSIL on cytology and HSIL on biopsy at the treated locations 3 months after that. He underwent a second IRC in April 2010, and 4 months after the second IRC, he had persistent HSIL on biopsy at the treated locations. He underwent a third IRC in October 2010, and in February 2011, he had LSIL on cytology and biopsy of the treated locations. In August 2011, he had HSIL cytology, but no lesions were seen on high-resolution anoscopy (HRA). In November 2011, his cytology showed atypical squamous cells of undetermined significance (ASC-US), and results of his biopsies did not show abnormalities. He was seen again in February 2013, and cytology and biopsies all showed LSIL. One of these biopsies contained an adjacent area that was morphologically difficult to distinguish between HSIL and reactive squamous metaplasia. This biopsy was therefore stained with p16 (Figs. 1A, B). P16 staining of that section was negative, and that biopsy was reported as LSIL with areas of reactive squamous metaplasia. The other biopsies showed clear LSIL and were not stained with p16.

Performing Anal Cytology

Compared with cervical cytology specimens, most anal cytology specimens are relatively hypocellular and subject to air-drying artifact. Cells may also be obscured by fecal matter. Most pathologists prefer liquid-based cytology samples, although there is no evidence that these are superior to glass-slide cytology.[1] If glass-slide cytology is done, immersion in alcohol bottles is preferred to hairspray fixation, and fixation is ideally done as quickly as possible to avoid air-drying artifact. Clinicians collecting samples are instructed to use a Dacron or nylon swab that is moistened in tap water or saline. Our preferred position for the patient is left lateral decubitus, and with the patient holding his/her right buttock up to stretch the anal opening, the swab is inserted. Once through the anal sphincter, the patient is asked to stop holding his/her buttock. Clinicians should insert the swab as far as it will go while rotating the swab and applying gentle pressure to maximize cellular yield. They do this as the swab is being inserted and as it is being withdrawn, and the clinician is asked to have the swab inside the anal canal for approximately 30 seconds while collecting cells. After removal, the swab is inserted into liquid medium and vigorously rotated in the medium to maximize release of cells, or rolled onto a glass slide, and ideally immersed immediately in an

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Dr Palefsky received Merck and Co- travel support, research grant support, Hologic- speaker's honorarium, and research grant support Pharmajet: Scientific advisory board. Dr Darragh received honorarium for Webinar on anal cancer screening. She serves on the advisory board of and has stock options in OncoHealth. She is also a consultant to the National Cancer Institute for the Costa Rica Cervical Cancer Project. Currently, she has salary support for various clinical research projects for HPV-associated diseases from the National Institutes of Health and receives honoraria for lectures at ACCME-accredited continuing medical education courses in various ACCME-accredited nonprofit organizations.

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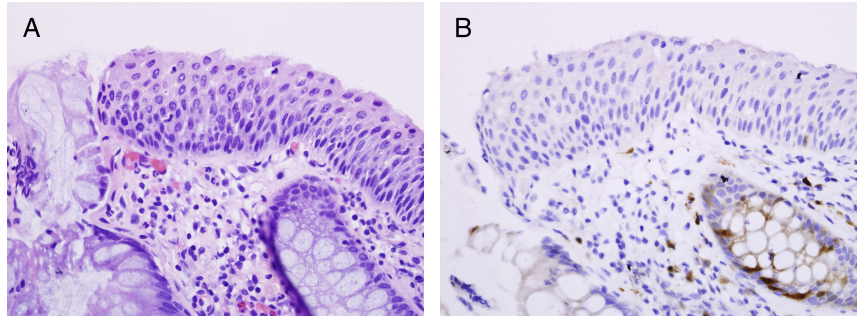

FIGURE 1. A, Biopsy of an anal lesion that had features of LSIL adjacent to the area shown in the figure. B, P16 staining was performed to distinguish between HSIL and immature squamous metaplasia. P16 staining was negative, and the biopsy was reported as squamous metaplasia.

alcohol fixation bottle. Although most anal cytology screening is performed by primary care providers, followed by referral to HRA experts if cytology is abnormal, anal cytology can also be performed using self-collection by patients.[2]

Anal Anatomy and Notation of Lesion Location

The anus is covered by squamous epithelium and extends from the SCJ to the perianal region out to a radius of 5 cm from the anal verge. The nonkeratinized epithelium of the canal becomes keratinized toward the verge and hair-bearing in the perianal region. Like the cervix, the anus contains a transformation zone where the squamous epithelium of the anus meets the columnar epithelium of the rectum. The original SCJ is at the dentate line where the distal end of the anal crypts is located. The SCJ then extends proximally to varying degrees depending on a number of poorly understood factors. The current SCJ is usually clearly visible as a discrete, sometimes thickened but flat and translucent acetowhite line spanning the entire circumference of the anal canal. The area between the original SCJ and the current SCJ constitutes the transformation zone and typically contains areas of immature squamous metaplasia.

Human papillomavirus (HPV)–related anal squamous intraepithelial lesions (ASILs) are most often found in the transformation zone but may occur anywhere in the anal canal and perianal region. There are several ways that the location of lesions can be recorded for charting purposes, including the "clock" system and the "octant system." In the clock system used at the UCSF Anal Neoplasia Clinic, the coccyx is considered to be 12 o'clock, and the anterior region is 6 o'clock. With a patient in the left lateral decubitus position, a lesion on their right would be 3 o'clock and on their left would be 9 o'clock. Lesion position may be recorded by "hour" and whether the lesion is in the anal canal or perianal region. More recently, we have been using the octant system (Fig. 2), because different medical disciplines define the 12-o'clock position differently, and this may lead to confusion in communication. In the octant system, the coccyx is always posterior regardless of the positioning of the patient. The anal canal and perianal regions are then divided into octants, in which the lesion may be posterior, anterior, left, or right, or in between, for example, left anterolateral, right anterolateral, left posterolateral, and so on. The location of the lesion may also be described with respect to its proximal or distal position, for example, at the SCJ, at the dentate line, verge, perianal, and so on. For charting purposes, lesions may also be digitally photographed and the images stored to allow for comparison between visits.

HRA Procedure

A technique known as HRA is used to visually identify anal HSIL and permit targeted biopsies for histopathologic confirmation.[3] Using a colposcope, anal HSIL is visualized using magnification, 5% acetic acid and Lugol iodine. As in the cervix, a biopsy of visible lesions is then performed for histologic confirmation. Like cervical colposcopy, optimization of HRA skills requires considerable time and experience, and the goal of the procedure is to identify the most advanced disease present. Distinguishing between anal LSIL and HSIL on HRA is facilitated using many of the same criteria recommended for selection of cervical lesions for biopsy, including atypical vessels, low Lugol uptake, acetowhitening, mosaic pattern, and flat topography.[4] In contrast to cervical colposcopy, lesion recognition on HRA may be complicated by the presence of stool and irregular topography associated with hemorrhoids, hypertrophic papillae, fissures, fistulas, sinuses, and crypts. The anal mucosa may also exhibit multiple non–HPV-associated pathologies that can mimic ASIL, including nonspecific inflammation, psoriasis, eczema, and other sexually transmitted infections. Because many lesions occur at the SCJ, HSIL may sometimes be confused for immature squamous metaplasia, and p16 staining is useful to distinguish between these 2 diagnoses.[5]

Anal Biopsy Procedure

Anal biopsies performed above the dentate line typically do not require an anesthetic since the sensory innervation of that part of the anal canal is limited. Biopsies performed distal to the dentate line and in the perianal region do require a local anesthetic. To anesthetize the area for biopsy, we typically apply topical 5% lidocaine cream (LMX-5 cream; Ferndale Healthcare, Ferndale, Mich) and allow it to absorb for approximately 5 minutes. We then inject the area with 1% lidocaine with epinephrine and buffered with 84% sodium bicarbonate to reduce stinging in a 5:1 ratio of lidocaine to bicarbonate. After injection, a small biopsy using laryngeal biopsy forceps or baby Tischler forceps can be used. Small biopsies should be done to avoid complications, which may include bleeding, infection, and fistula formation. Performed properly, the complication rate for biopsies is very low, and the procedure is very well tolerated by patients. We typically recommend avoiding heavy lifting and anal intercourse after biopsy for a period of 2 weeks. As in the cervix, the likelihood of diagnosing HSIL is increased when more biopsies are obtained. We recommend that patients being seen for the first time undergo multiple biopsies to maximize the likelihood of finding anal HSIL.

PATIENT 2

S.P. is a 53-year-old woman who was first seen in the UCSF Anal Neoplasia Clinic in June 2012, after a hemorrhoidectomy in March 2012. She was referred because pathology of the hemorrhoidal tissue revealed incidental findings of carcinoma in situ

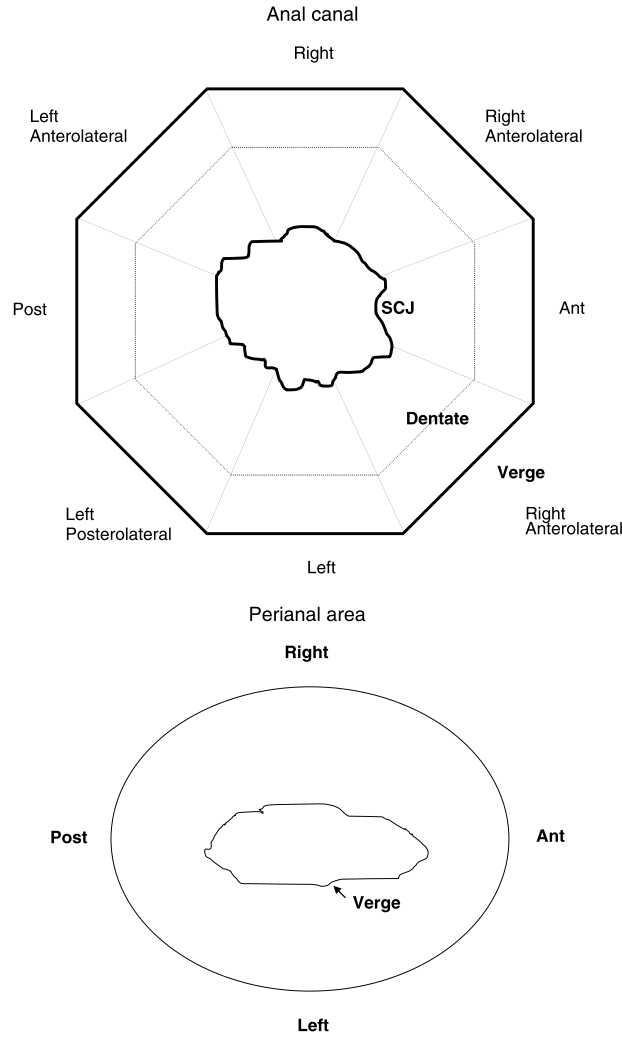

FIGURE 2. Diagram used in the UCSF Anal Neoplasia Clinic to record location of lesions seen on HRA. An octant system is used for both anal canal and perianal lesions. The coccyx is posterior, and posterior; left, right, and anterior quadrants are used. The quadrants are then divided into anterolateral and posterolateral areas.

with positive margins. The patient is married with 2 children. She had no history of anal warts, but had regular bleeding with bowel movements that led to the hemorrhoidectomy. She had no anal pain. She had an abnormal cervical cytology in 2008 with cervical HSIL treated with loop electroexcision procedure. She was followed up every 3 months for a year with normal cervical cytology at each subsequent visit. Her most recent cervical cytology in September 2011 did not show abnormalities. She has had no vaginal or vulvar disease. She has a history of 5 lifetime sexual partners, with sexual debut at age 17 years. She has no history of sexually transmitted infections and no history of anal intercourse. She has no other medical history and takes no medications other than multivitamins.

Her digital anorectal examination (DARE) was normal without masses or indurations. On HRA, discrete areas of HSIL were noted in the anterior left lateral to left anterolateral octants at the dentate line and confirmed on biopsy. In addition, she had Lugol-negative areas consistent with HSIL in the right lateral and posterior octants near the SCJ, for which a biopsy was not performed. She subsequently was referred to an anal surgeon for HRA-guided surgery because of multifocal HSIL, redundant skin tags, and prolapsing hemorrhoids and the impression that her case would be better managed in the operating room. At examination under anesthesia, extensive lesions were seen as above, extending from the SCJ to the perianal area. The lesions were ablated using electrocautery, debrided down to the submucosa, and then retreated. She recovered well after surgery, with post-surgical HRA performed 4 months later. A small possible lesion was seen posteriorly with some granulation tissue in the right anterolateral area near the SCJ. A distal fissure was noted near the verge in the anterior octant. Anal cytology was performed as described for patient 1 and was normal. Biopsy of the posterior lesion was interpreted as immature and reactive squamous metaplasia after p16 staining was performed and shown to be negative (Figs. 3A, B).

PATIENT 3

D.B. was first seen in the UCSF Anal Neoplasia Clinic in February 2013 for evaluation of ASC-US on cytology and history of warts. He was treated for warts more than 5 years ago and had not been examined since. He practices both receptive and insertive anal intercourse and has had 15 lifetime partners. His first known positive HIV test was in March 2012. He had a

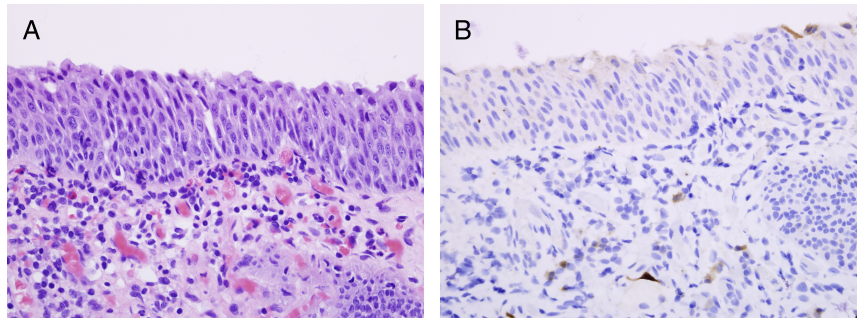

FIGURE 3. A, Biopsy of a lesion at the SCJ of a woman with multifocal HSIL found on HRA after an initial diagnosis of HSIL on routine pathology of a hemorrhoid removed at surgery. B, P16 staining was performed to distinguish between HSIL and immature squamous metaplasia. P16 staining was negative, and the biopsy was reported as immature and reactive squamous metaplasia.

CD4 nadir of 318 cells/mL and an HIV viral load greater than 100,000 copies/mL. He was immediately started on HAART.

His DARE was normal without firm, immobile masses, but several small, rubbery, soft, mobile nodules were felt. On HRA, extensive small warty nodules were noted throughout canal and distal canal and perianally. He also had areas suggestive of HSIL. Anal cytology was performed as described for patient 1 and showed LSIL. High-resolution anoscopy and biopsy were also performed as described for patient 1. A distal anal canal biopsy in the left lateral octant showed squamous mucosa with chronic inflammation and reactive changes. Biopsies in the right anterolateral octant at the SCJ showed HSIL. P16 staining was performed on a biopsy from the left anterolateral octant at the SCJ to distinguish between HSIL and squamous metaplasia. P16 staining of the biopsy was strongly and diffusely positive, and the pathologist reported the lesion as HSIL (Figs. 4A, B).

DISCUSSION AND TEACHING POINTS

Growing Incidence of Anal Cancer

Anal cancer is a squamous cell cancer that develops in the anal canal and perianal region. It is similar biologically to cervical cancer in terms of its association with oncogenic HPV, particularly HPV-16.[6] It is also very similar histopathologically to cervical cancer and is preceded by HSILs.[7] Like cervical cancer, anal cancer often develops in the transformation zone between the squamous and columnar epithelium.

Anal cancer is a relatively rare cancer in the general population and is more common among women than among men.[8] Importantly, the incidence of anal cancer has been increasing by approximately 2% per year in the general population among both men and women since the 1970s.[8] However, the risk of anal cancer is not evenly distributed throughout the population. Certain groups are known to be at increased risk of anal cancer compared with the general population. Women with a history of HSIL or cancer elsewhere in the genital tract including the cervix, vulva, and vagina are at increased risk of anal cancer, particularly women with vulvar disease. Other high-risk groups include MSMs, men and women who are immunosuppressed because of HIV or other causes, and men and women with perianal HPV-related lesions, including condyloma.[9] With a nearly 60% prevalence of anal HPV infection among HIV-uninfected MSMs,[10] it is not surprising that the incidence of anal cancer in this group of men is higher than in men in the general population, with an incidence estimated to be as high as 37 per 100,000.[11] The rate of anal HPV infection is even higher among HIV-infected MSMs, exceeding 90% in most studies, and this group has the highest incidence of anal cancer of all.[12] Combined with the increasing survival because of fewer competing causes of mortality, and absence of organized screening or prevention programs for anal cancer, the incidence of anal cancer has increased, not decreased, in the HAART era. Several HIV-anal cancer database matches showed an incidence of anal cancer of 70 per 100,000 or more.[12] More recent data from the North American AIDS Cohort Collaboration on Research and Design study show an incidence of 131 per 100,000 HIV-infected MSMs rendering the incidence of anal cancer well above the highest incidences of cervical cancer anywhere in the world.[13]

It is not clear how the incidence of anal cancer will change in the future, because there are several competing factors that will ultimately determine the incidence of anal cancer in HIV-infected men and women. One factor that may reduce the incidence of anal cancer in the future compared with current rates is changes

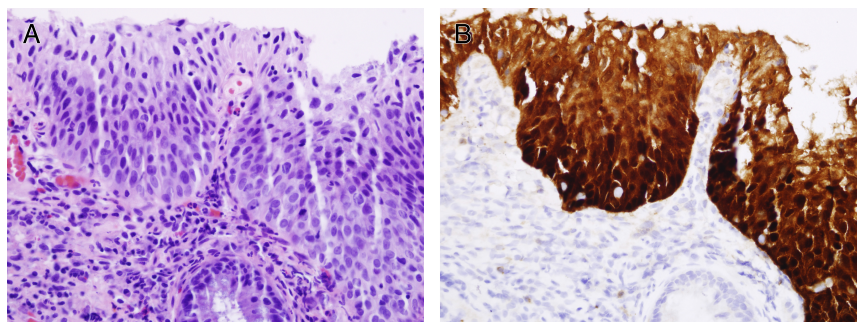

FIGURE 4. A, Biopsy from the left anterolateral octant at the SCJ from an HIV-infected man with multiple areas of LSIL and possible HSIL seen on HRA. B, P16 staining was performed to distinguish between HSIL and reactive squamous metaplasia. P16 staining was diffuse and strong, and the biopsy was reported as HSIL.

in practice regarding higher threshold CD4 levels for initiation of HAART. To the degree that immune response is needed to control HPV replication and progression from HPV infection to HSIL, initiation of HAART earlier in the course of HIV infection may result in less damage to the immune system and ultimately lead to a lower incidence of HSIL and anal cancer.[14] Unfortunately, most individuals currently infected with HIV were initiated on HAART at CD4 levels well below current guidelines, and they may not benefit from the earlier initiation of HAART. Aging of the HIV-infected population may also contribute to an increase in the burden of anal cancer in the future, as historically the incidence of anal cancer increases in the general population with increasing age over 60 years.[15] Likewise, men and women with history of solid organ transplant, such as renal transplant, and who are immunosuppressed to prevent graft rejection are at increased risk of anal cancer.[16] It is possible that improvements in approaches to immunosuppression may lead to reduction in anal cancer, but this will need to be studied.

Several studies have shown that anal HPV infection in women is as common as or more common than cervical HPV infection. HIV-infected women have an incidence of anal cancer of 30 per 100,000,[13] and their prevalence of ASIL is similar to the prevalence of cervical SIL.[17] HIV-uninfected women with concurrent cervical, vulvar, or vaginal SIL have been shown to have a prevalence of anal HSIL of 8%.[18] Anal intercourse is a risk factor for anal HPV infection, ASIL, and anal cancer in women, but it is not absolutely required. Many women with anal HPV infection report no history of anal intercourse,[19] and there are several other potential means of spread to the anal canal, including spread from fingers, toys, oral-anal contact, and from the cervix, vulva, or vagina.

Lower Anogenital Squamous Terminology Classification of Anal Lesions

Compared with the cervix, there are fewer data on the biology and natural history of ASIL and anal cancer. However, recognizing that anal and cervical cancer and their precursor lesions are histopathologically and biologically similar, the Lower Anogenital Squamous Terminology (LAST) project of the College of American Pathologists and American Society of Colposcopy and Cervical Pathology recommended adoption of uniform terminology for anal lesions and HPV-related disease elsewhere in the genital tract, including the cervix, vulva, and vagina.[5] The key principles emerging from this set of recommendations are as follows:

(1) Consistent with their shared etiologic association with HPV and similar natural history, the cervical and anal epithelium may exhibit LSIL and HSIL. As in the cervix, this 2-tier classification system replaces the older terminology of "mild, moderate, or severe dysplasia" or "anal intraepithelial neoplasia 1, 2, and 3." It eliminates the term "carcinoma in situ." Instead, only 2 distinct biological states are believed to exist, in which anal LSIL is associated with few signs of cell transformation but instead primarily reflects cytopathic changes because of high levels of HPV replication. These changes are not believed to be precancerous. Conversely, anal HSIL exhibits signs of HPV-induced transformation and is the true cancer precursor lesion. As in the cervix, moderate anal dysplasia or anal intraepithelial neoplasia 2 is not considered to be a biologically distinct or reproducible disease state, although this is based on much more limited data than are available from studies of the cervix. Uniform terminology is therefore recommended for SIL across the entire anogenital tract, including the cervix, vulva, vagina, anus, scrotum, and penis.

Anal LSIL is the most common form of ASIL and is associated with a wide variety of HPV types, both oncogenic and nononcogenic.[6] Unlike anal LSIL, a high proportion of anal HSIL contain oncogenic HPV types.[6,20] Infection with multiple HPV types is particularly common in HIV-infected individuals, but it is relatively uncommon to detect only nononcogenic HPV types in these lesions. The rate at which anal HSIL progresses to cancer is unknown and varies highly from person to person and by immune status. Among HIV-infected MSMs, estimates are that 377 per 100,000 with anal HSIL will progress to cancer per year.[20] Because the mean age at which HIV-infected individuals develop anal cancer is lower than the general population, it is likely that the time of progression from anal HSIL to invasive cancer is shorter in this group.

(2) P16 staining may be used to classify lesions as LSIL or HSIL when a lesion has features of both of these diseases and the pathologist is unable to clearly distinguish between them.[5] Lesions that show diffuse, clear p16 positivity are classified as HSIL, and those that exhibit weak or focal staining are classified as LSIL. Lesions that show clear features of LSIL or HSIL should be reported as such and not stained with p16.

(3) P16 staining may also be used to distinguish between HSIL and squamous metaplasia when the pathologist is unable to make that distinction on hematoxylin-eosin morphologic interpretation. Tissues that show strong, diffuse p16 positivity are classified as HSIL, and those that do not are classified as squamous metaplasia.

(4) P16 staining should not be used for lesions determined to be normal or LSIL on routine hematoxylin-eosin morphologic interpretation.

(5) Uniform terminology across the anogenital tract is also recommended for superficially invasive cancers. Superficially invasive squamous cell carcinomas of the anus (SISCCAAs) are defined as lesions less than 3 mm in depth below the basement membrane, 7 mm in width, and which have been completely excised. At this time, the clinical implications for distinguishing SISCCAA from more advanced invasive cancers for the purposes of staging and treatment are not yet known.

(6) As with the rest of the genital tract, both anal cytology and histology reporting would use the 2-tier system of LSIL and HSIL. The pathology report would indicate whether the sample was cytology or histology. The pathologist has the option to continue to report when the sample has features consistent with intraepithelial neoplasia grade 2 under the older terminology.

ASIL Screening

Given the biological similarity between cervical and anal cancer, and the success of cervical cancer prevention programs, anal cancer may similarly be preventable. Like cervical cancer programs, anal cancer prevention programs may take the form of primary prevention or secondary prevention. Primary prevention consists of prevention of infection with the underlying etiologic agent, HPV through prophylactic vaccination. Human papillomavirus vaccination has been shown to reduce the risk of acquisition of anal HPV infection[21,22] and development of ASIL.[22] Vaccination is primarily targeted toward males and females before sexual debut since the vaccine works to prevent initial infection with HPV.

Secondary prevention efforts are focused on those who have already been exposed to HPV and who have developed HSIL. Cervical cancer secondary prevention programs consist of screening with cervical cytology followed by referral for

colposcopy and colposcopically directed biopsy of visible cervical lesions. If cervical HSIL is shown on biopsy, the lesion is removed or ablated, reducing the risk of progression to invasive cervical cancer. Given that anal HSIL is the precursor to anal cancer, it is likely, although unproven, that removal of anal HSIL will reduce the incidence of anal cancer. As in the cervix, removal of anal HSIL requires accurate identification of the lesions, and methods to identify those with anal HSIL are largely based on those used for cervical HSIL.[3]

A similar approach to prevention of anal cancer has been advocated by several experts in the field.[23] Although it seems probable that adaptation of cervical techniques to the anal canal would be successful to reduce the risk of anal cancer, there are several challenges, particularly in HIV-infected individuals. These include large lesions, multifocal lesions, high incidence of new lesions and high recurrence rate after treatment. Well-designed clinical trials to demonstrate the efficacy of this approach have not yet been done and are urgently needed. Because of the absence of high-quality data showing that treatment of anal HSIL reduces the risk of anal cancer, screening for ASIL is not standard of care anywhere except in the state of New York, where it is recommended routinely among HIV-infected men and women. The US Public Health Service guidelines acknowledge that many expert fields recommend screening for and treating anal HSIL but require evidence of efficacy to reduce cancer before recommending routine screening of high-risk groups.[23] Recently, the American College of Colorectal Surgeons recommended anal cytology to screen for ASIL but acknowledged the low level of the quality of the supporting data.[24] Despite the lack of formal national guidelines recommending routine screening of at-risk individuals, a growing number of clinicians are being trained in HRA, and the number of facilities to identify and treat HSIL is growing. A professional society devoted to ASIL and anal cancer was recently established to focus on these diseases, known as the International Anal Neoplasia Society.

Because the risk of anal cancer is known to be concentrated largely in well-established risk groups, these groups can be targeted for ASIL and anal cancer screening. These groups include HIV-infected men and women, regardless of mode of HIV acquisition; HIV-uninfected MSMs; those with perianal HPV-related lesions; women with a history of high-grade vulvar, vaginal, and cervical SIL and cancer; and those who are immunosuppressed because of causes other than HIV (Table 1). In the interest of minimizing morbidity, consideration should be given to screening immunosuppressed men and women only after the age of 30 years, and nonimmunosuppressed men and women only after the age of 40 years, given the low incidence of anal cancer in these groups under those ages.

TABLE 1. Populations That Should Be Considered for Screening for ASILs and Anal Cancer

HIV-infected men aged >30 y
HIV-infected women aged >30 y
HIV-uninfected MSMs aged >40 y
HIV-uninfected women aged >40 y who have a history of vulvar, vaginal, or cervical HSILs or cancer
Men or women aged >40 y with perianal condyloma, squamous intraepithelial lesions, or cancer
Men or women aged >30 y who are chronically immunosuppressed because of causes other than HIV infection

Similar to cervical screening, there remains room for improvement in screening for anal HSIL. The most direct method to detect anal HSIL is to perform HRA, and the high prevalence of anal HSIL in some risk groups such as HIV-infected MSMs may theoretically justify such an approach. However, HRA requires extensive training and experience, and for optimal results, an interdisciplinary team is needed that includes an anoscopist, pathologist, surgeon, and counselor/educator. Currently, the number of clinicians performing HRA is limited, given the extensive infrastructure and training required, and there are too few well-trained clinicians performing HRA to allow it to be used as a true screening tool.

One of the more easily performed screening tools that may identify individuals who would benefit from HRA is anal cytology. Those with abnormal cytology are then referred for HRA. Like cervical cytology, the sensitivity of anal cytology is limited, and it tends to undercall the grade of lesion shown on HRA-guided biopsy.[25] Ultimately, all patients with any abnormality, including ASC-US, should be considered for HRA. However, given the high proportion of HIV-infected individuals expected to have abnormal anal cytology, the grade of cytology may also be used to triage patients and determine priority for HRA referral. The positive predictive value for anal HSIL on biopsy is highest for those with anal HSIL on cytology, followed by those with atypical squamous cells–cannot rule out high-grade lesion and LSIL and ASC-US. Cost-effectiveness studies have shown that HIV-infected MSMs should be screened annually with anal cytology if their cytology is normal, and every 2 to 3 years for HIV-uninfected MSMs.[26,27] Although there are fewer data for the other at-risk groups such as women, at UCSF we recommend similar screening intervals for these groups according to their HIV status. In addition to determining the need for referral for HRA, anal cytology can be used as a quality control tool for the anoscopist. Given the high positive predictive value of HSIL on cytology for HSIL on HRA and biopsy, if cytology is reported as HSIL but no HSIL is found on biopsy, that likely reflects a missed lesion and should prompt the clinician to repeat the HRA examination.

Human papillomavirus testing is increasingly being used in the cervix as an adjunct to cervical cytology, or as a primary screening test to identify women who should have cervical colposcopy. Given the limitations of anal cytology, some have advocated anal HPV testing to identify those who should have HRA.[28] However, given the high prevalence of oncogenic HPV in HIV-infected patients, HPV testing may be more useful for its negative predictive value. Further studies are needed to define the best use of HPV-based tests.

Diagnosis of Anal Cancer

Anal cytology and HRA-guided biopsy are primarily aimed at identifying anal HSIL, and these should therefore be considered to be primary methods of anal "precancer" screening. In contrast, DARE with palpation for hard, immobile, intra-anal, and perianal masses is a key element of anal cancer screening. Some cancers are detectable only on DARE and are not seen on HRA, whereas others may be detected on HRA but not palpated.

Like cervical cancer, survival after treatment of anal cancer correlates inversely with the stage of diagnosis. Five-year survival after treatment of stage 1 disease is approximately 70% and declines to about 20% after diagnosis of stage IV disease. In the absence of any organized, anal cancer or HSIL screening program, diagnosis of anal cancer is often made when the patient

presents with new anal pain that is not explained by any other obvious source such as hemorrhoids, fissures, or infections. Lesions such as HSIL are usually painless, and when patients develop pain, it may be a sign of progression to cancer because there may be involvement of pain nerve fibers. Patients may also present with new patterns of bleeding, discomfort upon defecation or anal intercourse, or rapid growth of a mass. With increasing recognition of SISCCAA in the setting of proactive anal HSIL screening programs, it is possible that a higher proportion of patients may be curable with wide local excision and spared the morbidity associated with the standard-of-care chemoradiation treatment regimen. Prospective studies are needed to determine if wide local excision of SISCCAA is safe and effective.

Treatment of Anal HSIL and Cancer

Once anal HSIL is identified on biopsy, every effort should be made to ablate or remove the lesion with the primary goal of reducing the risk of progression to cancer. Given that there are no data yet showing that anal HSIL treatment is effective to reduce the incidence of anal cancer, clinicians who treat anal HSIL are doing so based on the similarity between anal cancer and cervical cancer and the proven efficacy of treatment of cervical HSIL to prevent cervical cancer. Treatment for anal HSIL generally falls into 2 categories: (1) local treatment with clinician- or patient-applied creams or liquids or (2) clinician-applied ablative techniques such as electrocautery, laser or IRC, and surgery. The choice of treatments will vary with the preference of the clinician, the clinical setting, the size and number of lesions, and the location of the lesions. In general, surgery is reserved for treating those with the most extensive disease and those who require examination under anesthesia to permit biopsies large enough to definitely exclude invasive cancer or, rarely, treatment of complications of office-based procedures such as bleeding or infection. Larger perianal HSIL often requires more aggressive approaches in the setting of the operating room, such as IRC, electrocautery, laser, and surgical excision with skin flaps.

Treatment of anal cancer is based on the stage of the disease. Stages 1, 2, and 3 are treated with combined modality therapy (CMT) consisting of 5-fluorouracil and mitomycin, with radiation therapy.[29] At some centers, intensity modulated radiation therapy is used instead of 3-dimensional conformal radiation to reduce radiation-associated toxicity. Cisplatin has been assessed in place of mitomycin, but clinical trials have shown insufficient benefit to recommend it in place of mitomycin as the first-line chemotherapeutic agent in combination with 5-fluorouracil. However, cisplatin may be useful as first-line therapy for patients who are expected to be intolerant of the hematologic toxicity associated with mitomycin-based regimen.[29] HIV-infected patients can be treated with the standard CMT regimen, although careful monitoring for toxicity is required, and treatment breaks may be needed. The primary role for surgery in the treatment of anal cancer is abdominoperineal resection for patients who fail CMT.

Teaching Points From the 3 Cases

The 3 cases presented in this report illustrate several points in the diagnosis and treatment of ASIL. Patient 1 is described as an HIV-infected MSM who is typical in several ways. He comes from a risk group with the highest prevalence and incidence of anal HSIL and anal cancer. He continues to have anal HSIL despite having a good response to antiretroviral therapy and control of HIV viral load. Despite a good $CD4^+$ level, there has been no HSIL regression, implying that some of the effects of HIV on the immune system to control HPV-related lesions may have been irreversible given his low CD4 nadir. He has multiple lesions of different appearances with different levels of pathology, likely reflecting the independent course of infection with multiple different HPV types. Despite having a high current $CD4^+$ level, he also continues to have lesion recurrences after IRC, which is not uncommon among HIV-infected patients. This may reflect having multiple large lesions at the time of the first treatment, difficulty in identifying the true extent of the HSIL lesions with inadequate treatment, true lesion recurrence secondary to attenuated immune response, and, most likely, a combination of all of these factors. Infrared coagulation is an office-based procedure that is well tolerated. The goal in most cases is not to eradicate all ASIL but to eradicate all HSIL. In this case, at the visit in which the pathology is shown, all of his biopsies showed LSIL. However, one of those biopsies had an area in which it was difficult to distinguish morphologically between HSIL or squamous metaplasia. P16 was used and was negative, and the pathologist reported that biopsy as having LSIL with areas of focal squamous metaplasia. This is one of the recommended uses of p16 staining in the LAST project. Notably, the pathologist did not perform p16 staining of the biopsies that were morphologically interpreted as LSIL because there is no value in doing so. This patient required 3 IRC treatments before there was no longer any detectable HSIL, and he will need continued follow-up to monitor for recurrence of HSIL or development of new HSIL lesions at sites that were not previously shown to be lesional.

Patient 2 reflects a common presentation for anal HSIL in otherwise healthy women. Despite the fact that anal cancer is more common in women in the general population than men, it is a relatively rare cancer, and this patient did not undergo routine ASIL screening, because there is no standard of care that requires it. Instead, she came to medical attention because her hemorrhoid was sent for routine histopathology revealing the HSIL. Interestingly, although some medical centers routinely send hemorrhoids for histopathologic examination, doing so is not standard of care given the low overall yield of tissues revealing clinically relevant pathology. However, this patient had an additional risk factor that might have led some to screen her with anal cytology independent of her need for hemorrhoidal surgery, namely, a prior history of cervical HSIL. Routine ASIL screening because of prior cervical HSIL is not yet standard of care either, but there is a clear epidemiologic relationship between cervical and anal cancer.[30] The cost-effectiveness of screening women with history of cervical or vulvovaginal disease needs to be assessed. Although removal of the hemorrhoid may result in removal of the entire HSIL, as in this patient's case, the lesion may be multifocal even in otherwise healthy women and men. Identification of HSIL on a hemorrhoid should prompt HRA and biopsy to determine the extent of the disease. Treatment in her case required a combination of surgery and postsurgical HRA. From a histopathologic point of view, p16 staining was used in this case to distinguish between anal HSIL and squamous metaplasia. As in patient 1, the staining was negative, and the biopsy in question was reported as having squamous metaplasia. Also of note, a prior biopsy was reported as containing "carcinoma in situ," a term that would be replaced under the LAST terminology with anal HSIL.

Patient 3 is also an HIV-infected MSM. He was being seen for the first time in the clinic and therefore did not have any

history of ASIL. His presentation was typical in that many patients with ASIL have had prior history of warts. Warts are typically associated with HPV-6 or -11, which are not usually found in anal HSIL or anal cancer. However, a prior diagnosis of warts is a well-known risk factor for HSIL and cancer because of shared behavioral risk factors for acquisition of the oncogenic HPV types such as HPV-16 or -18, which are more likely causes of his current HSIL.[31,32] Another feature of this case that is commonly seen is that despite having HSIL on biopsy, his cytology showed LSIL. As with cervical cytology, the grade of lesion on anal cytology does not reliably indicate the true grade of disease. A high proportion of HIV-infected MSMs with LSIL or ASC-US on cytology are shown to have HSIL on biopsy; hence, the recommendation (for all at-risk groups) that any grade of cytology abnormality (ASC-US or higher) should prompt referral for HRA. In this case, one of his biopsies was stained with p16 to distinguish between HSIL and squamous metaplasia. Strong, diffuse p16 staining led the pathologist to report this biopsy as containing HSIL.

CONCLUSIONS

Anal cancer is a problem of growing importance in the general population, as well as among certain risk groups such as HIV-infected men and women. Its incidence may continue to rise as an increasing number of HIV-infected men and women reach advanced age. Diagnosis of anal cancers as early as possible may result in improved morbidity and mortality outcomes. Routine performance of simple techniques such as DARE may lead to earlier anal cancer diagnosis in some individuals. Performance of screening techniques such as anal cytology, followed by HRA-guided biopsy and ablation of anal HSIL, may reduce the risk of progression to anal cancer in some at-risk individuals. High levels of uptake of vaccination to prevent HPV infection may have a substantial impact on the incidence of anal cancer, but the impact of vaccination on anal cancer incidence will not be seen for several decades.

The LAST project recommendations standardize anal disease terminology with that of the rest of the genital tract, a logical step given the similarity of the biology of HPV infection in the anus and at other genital tract sites. The 2-tier classification of ASIL is thus the terminology of choice. Although SISCCA of the anus has a similar definition to cervical SISCCA, studies need to be done to determine the clinical relevance of anal SISSCA for guiding therapeutic choices.

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