Screening Women for Anal Cancers: Guidance for Health Care Professionals

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Anal cancer is rare in the general population but is steadily increasing in incidence over the past decade especially in women. Identification and screening of women with high risk facilitates detection of anal precancer and early-stage cancer, improves survival, and potentially uses less invasive therapies compared with the conventional chemoradiation treatments used for advanced cancers. No recently published guidelines currently describe details about screening women for anal squamous cell cancer (ASCC). The available evidence supports the existence of groups of women with higher prevalence of ASCC (e.g., women with human immunodeficiency virus, immune suppression, or previous lower-genital high-grade lesion or cancer) who would likely benefit from screening with some combination of anal cytology and human papillomavirus testing. Additional research is needed to establish the cost-effectiveness and the influence of screening on ASCC mortality rates.

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ANAL SQUAMOUS CELL CANCER

Anal cancer has a low overall incidence in the general population (about 1.9 per 100,000 and 0.2% lifetime risk), and it accounts for 2.5% of all gastrointestinal malignancies in the United States (1). This is more common in women with 2.2 new cases per 100,000 women and 1.6 new cases in men (2).Most cases are anal squamous cell cancers (ASCCs), and about 91% are caused by human papillomavirus (HPV) infection. With the increasing global burden of HPV-related cancers in the past decade, countries in the Western hemisphere have seen incidence rates of ASCC increasing by up to 2.2% annually (3,4). In 2020, it is estimated that there will be 8,590 new cases (5,900 in women and 2,650 in men) and 1,350 deaths (540 in men and 810 in women) with ASCC accounting for 0.5% of all cancer deaths in the United States (1). The estimated mortality rate has been increasing by an average of 3.1% annually from 2007 through 2016, and in 2020, it is 0.2 for men and 0.3 for women per 100,000 persons (2,5). Five-year survival rates for patients with ASCC from 2010 through 2016 averaged 68.7%. For patients with stage 1 or localized cancer, the 5-year survival rate was 81.9%, whereas it was 33.9% for patients with distant cancer, underscoring the importance of early diagnosis (2). Unfortunately, ASCC is often not detected at an early stage, with only 48% being diagnosed at localized stage, because initial symptoms resemble those of benign rectal and perianal conditions (e.g., hemorrhoids and anal fissures) (2,6).

With recommendations regarding ASCC screening, the discussion focuses on men who are having sex with men (MSM). But, ASCC is more common in women than men in general and the rates are increasing. Female sex increases the risk as ASCC is predominantly an HPV-related cancer. HPV is a multicentric infection, and because of the anatomical proximity of the anogenital areas in women, vaginal acquisition of HPV can cause concomitant anal HPV infection. This is one of the cancers where vigilance in high-risk groups of women is recommended, and we hope to provide helpful clinical guidance to health care professionals (HCPs).

HPV, the most common sexually transmitted infection in the United States, is considered necessary for the development of ASCC, and HPV DNA is identified in 90% of ASCC cases (7). HPV-16 and HPV-18 cause most ASCCs; HPV-16, the most persistent, is present in about 70% of high-grade lesions (8). Although most immunocompetent individuals can clear HPV infection or bring the viral load to an undetectable level, persistent high-risk HPV (hrHPV) infections lead to positive HPV tests and can lead to cytologic changes, precancerous lesions, and cancer (Figure 1) (9–12). Clinical, histologic, virologic, and epidemiologic data support this relationship by showing that a higher viral load is more strongly associated with cytologic abnormalities than lower viral loads (9,13,14).

The anus and cervix share embryologic and anatomical characteristics and may respond similarly to malignant changes induced by persistent hrHPV infection. ASCC has not been studied as extensively as cervical cancer, but a similar event progression is hypothesized (15). Cervical cancer rates markedly declined after the introduction of cervical cytology (Papanicolaou test), which paved the way for anal cytology and HPV testing to screen for ASCC. ASCC incidence rates for various example populations are shown in Table 1.

The Centers for Disease Control and Prevention indicate that anal cytology screening may be a useful preventive measure for human immunodeficiency virus–infected MSM (16). The American Cancer Society states that some experts recommend screening all high-risk individuals such as MSM (regardless of their human immunodeficiency virus status), women with a

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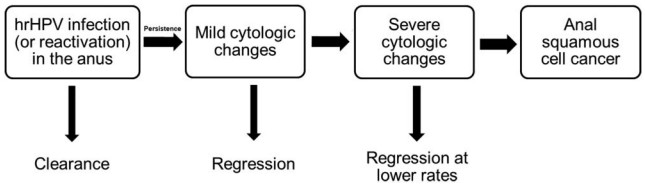

Figure 1. hrHPV infection and progression to anal squamous cell cancer. hrHPV, high-risk human papillomavirus.

history of cervical or vulvar cancer, solid-organ transplant recipients, and all human immunodeficiency virus–positive persons (17) The New York Department of Public Health Acquired Immunodeficiency Syndrome Institute and the Spanish Acquired Immunodeficiency Syndrome Study Group have provided guidelines for human immunodeficiency virus–positive patients and recommend annual anal cytology for MSM, patients with anogenital condyloma, and women with abnormal cervical or vaginal cytology (18–20).

ANAL CYTOLOGY AND HPV TESTING

High-resolution anoscopy (HRA) is considered the best screening method for very-high-risk groups (e.g., human immunodeficiency virus–positive MSM), but this procedure is complicated, painful, expensive, and has limited provider availability (21). Outside of the very-high-risk groups, HRA is a diagnostic tool and does not perform well in populations with lower risk.

By contrast, anal cytology is relatively easy to perform and cost-effective in high-risk populations (Table 2) (22,23). Persistent hrHPV infection leads to anal intraepithelial neoplasia (AIN), a precursor lesion that affects the squamocolumnar junction or the transformation zone of the anal canal (12,24,25); thus, this area is the focus of anal cytology and HPV testing.

Resource poor communities have to rely on screening high-risk patients for symptoms along with visual and digital anorectal examinations (DARE).The sensitivity and specificity of DARE are not established, but a recent study reviewing the utility of DARE as a public health screening tool, found this to be cost-effective, with minimal adverse effects and acceptable by patients (26).

How to collect a sample for anal cytology

An anal exfoliative cytology test is simple and can be taught to providers in 15 minutes (27,28). Patients should avoid anal intercourse, enemas, and douching before testing. The sample can be collected as follows:

1. Complete any indicated cervical or vaginal sample collection. Then, place the patient in either a dorsal lithotomy or lateral recumbent position.
2. With the nonexamining hand, gently part the buttocks, so that the anus is fully exposed.
3. Collect the specimen with a polyester swab moistened with water from a single-use vial (29). Do not use a cotton swab with a wooden stick because it can break and splinter (30,31). Digital rectal examination should be performed after the specimen is collected to reduce the risk of false-positive results caused by cross-contamination.
4. Insert the swab about 3–5 cm into the anus (approximate depth of the squamocolumnar junction) (Figures 1 and 2). Then, while applying outward pressure, rotate the swab 360° clockwise (5 times) and counterclockwise (5 times) for 15–20 seconds.
5. Place the swab in a vial containing liquid-based cytology media (approved by the cytopathology laboratory) and agitate vigorously for 20–30 seconds.

An adequate and satisfactory sample contains rectal columnar and anal squamous cells. Liquid-based cytology and conventional smears of the anal canal yield similar results, and either can be used for testing. Liquid-based cytology specimens more often have rectal columnar cells (indicating adequate sampling of the rectal transformation zone) and have reduced fecal and bacterial contamination and air-drying artifacts (32).

The College of American Pathologists and the American Society for Colposcopy and Cervical Pathology sponsored the Lower Anogenital Squamous Terminology project, whose consensus recommendations have been adopted by the World Health Organization and widely used in the United States. Their recommendations for reporting dysplastic anal cytology findings were simplified to a 2-tier system: (i) low-grade squamous intraepithelial lesion (LSIL) and (ii) high-grade SIL (HSIL) (31,33–35). The British Society for Clinical Cytology guidelines for reporting anal cytology, which is closely aligned with the

Table 1. Example patient populations and corresponding incidence rates of ASCC

Population	Approximate ASCC incidence per 100,000 persons
Human immunodeficiency virus–positive men who have sex with men	70–144
Women with VIN3+ (69)	35–44
Women with human immunodeficiency virus (55)	30
Women who receive a solid-organ transplant	20
Women with CIN3+ diagnosed at age <30 yr (Evans, 2003 #87)	15
Women in the general population	2

ASCC, anal squamous cell cancer; CIN3+, cervical intraepithelial neoplasia grade 3 or cancer; VIN3+, vulvar intraepithelial neoplasia grade 3 or cancer.

Table 2. Factors supporting anal cytology as an ASCC screening test

Adequate prevalence of ASCC in high-risk groups
Anal cytology influences ASCC morbidity and mortality rates
ASCC has a preclinical phase (anal intraepithelial neoplasia), during which interventions may affect outcomes
Anal cytology (with or without HPV testing) is a low-cost, low-risk test
Anal cytology (with or without HPV testing) has adequate sensitivity and specificity

Criteria for effective screening test from Obuchowski NA, et al. (88).
ASCC, anal squamous cell cancer; HPV, human papillomavirus.

Bethesda system, reports the results as AIN1, AIN2, and AIN3. AIN1 is a low-grade lesion corresponding to LSIL; AIN2 and AIN3 are high-grade lesions corresponding to HSIL (36,37). Most LSIL lesions in immunocompetent individuals spontaneously regress, but a few can progress to HSIL. Many HSIL lesions, especially p16-positive lesions, progress to ASCC. In a recent retrospective study of patients with AIN3 (and unidentified risk factors) with more than 4 years of follow-up, 8.2% had ASCC develop during a median of 2.7 years; treatment of AIN3 reduced the risk of ASCC (38).

The sensitivity (47%–90%) and specificity (16%–92%) of anal cytology tests vary, depending on the population screened, but the average sensitivity and specificity are similar to those of cervical cytology tests (20,36,39–41). Cytology tends to underestimate the grade of the disease, but high-grade cytology is often associated with high-grade histology. In a 15-year review of anal cytology screening of women seen at Mayo Clinic, sensitivity was 82% and specificity was 86%, and an 84% correlation was identified between HSIL cytology and histology (41). The sensitivity of anal cytology is affected by the disease prevalence, the surface area of anal disease, sex practices, human immunodeficiency virus positivity, and immunosuppression status.

Anal HPV testing

The US Food and Drug Administration has not approved any HPV tests for the anus, and clinical laboratories must validate their tests for this anatomical site. HPV has been identified in women who do not participate in receptive anal intercourse, but the prevalence of HPV infection varies considerably with the population being tested (42). The spectrum of HPV genotypes is similar for the cervix and anus.

hrHPV testing has high sensitivity (94.1%) resulting in a good negative predictive value (NPV) that can identify individuals with low risk of anal dysplasia. As reported by Wang et al. (43), NPV was 92% for patients with human immunodeficiency virus/acquired immunodeficiency syndrome. Conversely, the specificity and positive predictive value of anal HPV testing are poor (40,44–46). In populations with a high prevalence of anal hrHPV, the HPV test alone (without cytology) increases rates of positive screens without increasing detection of ASCC or precursor lesions (22,47,48). However, a change to a positive hrHPV status is a better predictor for high-grade anal dysplasia (49). For high-risk women, cytology plus HPV-16 genotyping better predicts AIN compared with cytology alone (40,50), but for high-risk women with a lower prevalence of anal hrHPV, negative results may help identify women who would not benefit from further screening.

In a study of high-risk Hispanic women (risk factors included human immunodeficiency virus, lower genital tract neoplasia [LGTN], and immunosuppression), when anal cytology was compared with HRA, the sensitivity of anal cytology alone to detect HSIL was 85.4% (95% confidence interval [CI], 72.2%–93.9%) and specificity was 38.8% (95% CI, 28.1%–50.3%). When anal cytology was combined with hrHPV testing to detect histologically confirmed HSIL, sensitivity increased to 100.0% (95% CI, 92.6%–100.0%) but specificity decreased to 16.3% (95% CI, 9.0%–26.2%) (51).

Before offering anal cytology to women, identify a pathologist with expertise in interpreting anal cytology (31,52–54). Women with abnormal cytology results or positive for HPV16 or 18 need to be referred for HRA, which can be performed by an HCP in gynecology, colorectal surgery, or gastroenterology (55). For patients with negative HPV and cytology findings, HRA referral is not indicated because of the low likelihood of ASCC (43,51).

IDENTIFYING AT-RISK POPULATIONS

Impaired local mucosal and systemic immunity influence the development of ASCC by increasing the risk of persistent hrHPV infection. Human immunodeficiency virus seropositivity, medical immunosuppression, history of autoimmune diseases, history of sexually transmitted infections (e.g., anogenital warts), smoking, vaginal douching, and receptive anal intercourse (56,57) can impair local and systemic immunity. Sex workers, women with

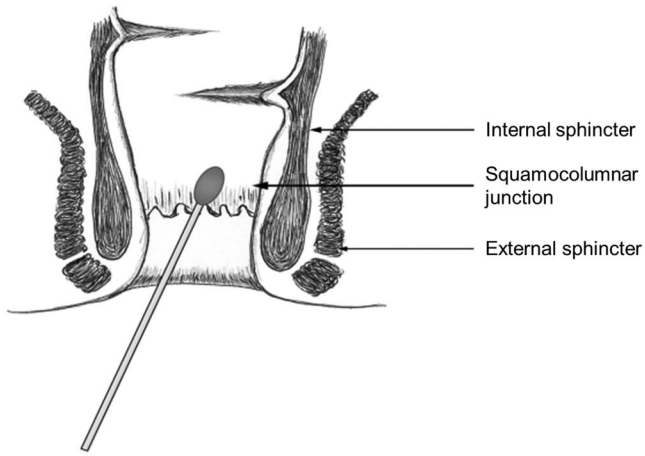

Figure 2. Anorectal site for obtaining a specimen for cytology. Used with permission of Mayo Foundation for Medical Education and Research, Courtesy of S. Vegunta.

Table 3. Sensitivity and specificity of screening modalities for anal dysplasia

Screening method	Sensitivity	Specificity	Comments
Anal cytology conventional staining	47%–90%	16%–92%	Similar to cervical cytology on average and better sensitivity and specificity in higher risk populations.
Anal HPV testing	94%	Low, by itself	HPV testing alone has good sensitivity, but poor specificity. For high-risk groups, cytology plus hrHPV better predicts AIN compared with cytology alone. (Strength in NPV)
p16 staining of anal cytology	72%	100% (tissue specimens)	High specificity in biopsy specimens. Needs more study for anal cytology thin prep/anal pap and not widely available at this time.
HPV mRNA E6/E7 testing	TBD	TBD	For cervical cancer, the mRNA E6/E7 has better specificity than hrHPV testing, more study needed for anal specimens. Testing for mRNA E6/E7 is becoming more widely available.

See text for references.
AIN, anal intraepithelial neoplasia; hrHPV, high-risk human papillomavirus; mRNA, messenger RNA; NPV, negative predictive value.

multiple sexual partners, and injection drug users have a higher incidence of anal HPV infection compared with the general population (7,58). ASCC can affect healthy individuals without human immunodeficiency virus, but the incidence rate is twice as high in patients with human immunodeficiency virus (and further increased in patients with acquired immunodeficiency syndrome) and increased by 25–50 times in MSM (Table 1) (59–61). The incidence of ASCC has been assessed in patients with autoimmune diseases such as systemic lupus erythematosus (IR, 10 [95% CI = 5–19]), ulcerative colitis (IR 6 [95% CI = 3–11]), and Crohn's disease (IR 3 [95% CI = 2–4]), and it has been reported as slightly greater than population risk (62). But, with fistulous perianal disease in Crohn's disease, there is a greater incidence of ASCC, with a high prevalence of HPV infection warranting vigilance in this disease group (63).

Women with LGTN or grade 3 intraepithelial neoplasia

The incidence of ASCC is higher in women with a history of LGTN or grade 3 intraepithelial neoplasia in the cervix, vagina, or vulva, and further increased in women who are human immunodeficiency virus–positive (25,59). Although anal HPV infection can occur in the absence of anal intercourse, a history of receptive anal intercourse increases the prevalence of abnormal cervical and anal cytology and high-grade cervical and anal lesions (particularly for individuals who are human immunodeficiency virus–positive) (64).

Women with cervical HPV infection are 8 times more likely to have anal HPV infection and anal lesions, and 50% of women with anal HPV infection have cervical HPV infection (65–67). Notably, 80% of women with concurrent anal and cervical infections show genotype concordance (68). This finding is important for women with cervical HPV-16 or HPV-18 infections because these HPV types are also drivers of high-grade anal disease (39).

In comparing risk of ASCC in women who have had LGTN, vulvar cancer confers the greatest risk (standardized incidence ratio [SIR], 17.4 [95% CI, 11.5–24.2]) compared with cancer of the cervix (SIR, 6.2 [95% CI, 4.1–8.7]) or vagina (SIR, 1.8 [95% CI, 0.2–5.3]). In addition, vulvar intraepithelial neoplasia grade 3 (VIN3), previously termed *vulvar carcinoma in situ*, is associated with a high incidence of ASCC (SIR, 22.2 [95% CI, 16.7–28.4]). The median interval to diagnosis of ASCC was 7.1 years from a diagnosis of vulvar cancer; from VIN3, 8.9 years; from cervical cancer, 11.4 years; and from cervical intraepithelial neoplasia grade 3 (CIN3), 15.7 years (69). AIN and hrHPV occur at high enough rates among human immunodeficiency virus–negative women with LGTN or VIN3 such that these women may benefit from ASCC screening.

Women living with human immunodeficiency virus infection

Cancer causes death in one-third of women with human immunodeficiency virus. ASCC, a non−acquired immunodeficiency syndrome–defining malignancy, is the fourth most common cancer in people living with human immunodeficiency virus (70). Human immunodeficiency virus disrupts the genital mucosal barrier and promotes E6 and E7 oncogene expression. Highly active antiretroviral therapy confers limited benefit in terms of reducing risk of anal HSIL and ASCC; furthermore, the prolonged survival due to highly active antiretroviral therapy increases opportunities for HPV-mediated disease (71). In the Study to Understand the Natural History of Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome in the Era of Effective Therapy (SUN study), the prevalence of anal HPV infection among human immunodeficiency virus–positive women was high (90%) (72). The Acquired Immunodeficiency Syndrome Malignancy Consortium 084 study showed a 27% prevalence of anal HSIL in human immunodeficiency virus–positive women in the United States (73).

Human immunodeficiency virus infection decreases the clearance rate of HPV, leading to high risk of anal and cervical HPV infection in human immunodeficiency virus–positive women. Lower CD4 cell counts and high viral counts are associated with HPV-related epithelial abnormalities. The Human Immunodeficiency Virus Medicine Association of the Infectious

Diseases Society of America provided guidelines for ASCC screening and management for individuals with human immunodeficiency virus (19). All human immunodeficiency virus–infected women are reasonable candidates for ASCC screening with digital rectal examinations and symptom review (pain and bleeding); patients can be referred for additional screening if initial results are positive. Data support screening with anal cytology, particularly for women with low CD4 counts or a history of CIN3+ or VIN3+. A recent retrospective analysis of a prospectively identified cohort of human immunodeficiency virus–positive men and women showed a lower incidence of ASCC with a structured screening program that included anal cytology and HRA as needed (74). High rates of positive anal HPV tests in women with human immunodeficiency virus precludes HPV testing alone from being a cost-effective screening tool for this population.

ASCC in women after organ transplantation

The increased incidence of malignancy in organ transplant recipients corresponds with the duration and type of immunosuppressive therapy. The level of immunosuppression needed can vary, depending on the type of organ transplanted and the level of human leukocyte antigen matching between the recipient and donor (75). Current immunosuppressive strategies to reduce graft rejection have improved the survival of renal transplant recipients (1-year survival rate, >90%) but at the cost of an increased risk of HPV-related cancers. Similar risks are noted for liver and heart transplant patients (76).

Older immunosuppression treatments, including cyclosporin and azathioprine, confer a 2-fold increased risk of anogenital cancers. Newer regimens include tacrolimus or cyclosporine (calcineurin inhibitors) or antimetabolites (mycophenolate or azathioprine). Corticosteroid use confers a 5-fold increased risk of AIN3.

Among transplant recipients, anal cancer occurs more commonly in women than in men (incidence rate ratio, 1.8 [95% CI, 1.3–2.7]) (75). American Society of Transplantation, Infectious Diseases Community of Practice recommends annual screening for transplant patients with a history of receptive anal intercourse or cervical dysplasia (77). The 30- to 100-fold increased incidence of anal cancer in transplant patients compared with the general population is partly due to suppression of T-cell activity, similar to that seen in patients with human immunodeficiency virus (57). In a cohort of women with kidney transplantation, hrHPV positivity increased from 25% (95% CI, 14%–37%) before transplantation to 39% (95% CI, 27%–51%) after, suggesting that immunosuppressive therapy may reactivate previous HPV infection (78). Given the good NPV of anal hrHPV tests for anal dysplasia and the lower rates of positive HPV tests in transplant patients, anal hrHPV may have a role in risk assessment.

Future direction

The reason for lack of guidelines regarding screening for ASCC could be due to the rarity of the disease in general population and perceived lack of data on the impact of treatment of preinvasive disease on the incidence of ASCC and probable lack of research funding. The Anal Cancer/HSIL Outcomes Research study will be able to provide more information regarding how progression to ASCC is altered by treatment of preinvasive disease. No consensus recommendations to screen for ASCC have been

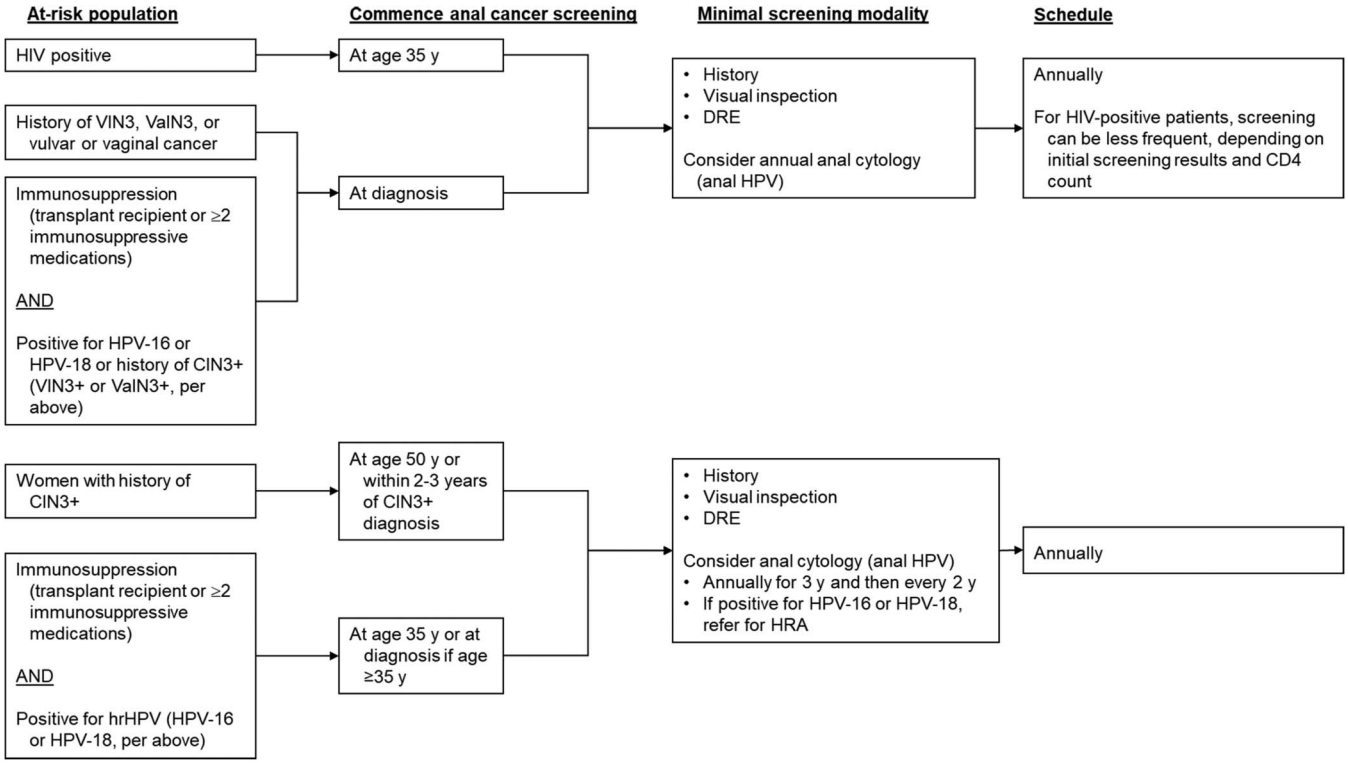

Figure 3. Screening recommendations for women with highest risk of anal squamous cell cancer. CIN3+, cervical intraepithelial lesion grade 3 or cancer; DRE, digital rectal examination; HRA, high-resolution anoscopy; hrHPV, high-risk human papillomavirus; VaIN3+, vaginal intraepithelial lesion grade 3 or cancer; VIN3+, vulvar intraepithelial lesion grade 3 or cancer.

Table 4. Recommended follow-up after ASCC screening

ASCC screening result	Recommended follow-up
hrHPV and cytology negative	Follow-up in 2–3 yr if no history of anal intraepithelial neoplasia
hrHPV-negative but cytology shows ASCUS or LSIL	Repeat testing in 1 yr
hrHPV-positive and cytology shows ASCUS or LSIL	Referral for HRA
Any HSIL or lesion greater than HSIL	Referral for HRA
hrHPV-positive (any type other than HPV-16) and normal cytology	Repeat in 1 yr If persistently hrHPV-positive, referral for HRA
Positive hrHPV-16	Referral for HRA, regardless of cytology findings

ASCC, anal squamous cell cancer; ASCUS, atypical squamous cells of undetermined significance; hrHPV, high-risk human papillomavirus; HRA, high-resolution anoscopy; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

published to date, but both the Study of the Prevention of Anal Cancer (79) and Anal Cancer/HSIL Outcomes Research (80) are currently collecting data to propose screening guidelines.

Currently, using a more sensitive test such as HPV (especially HPV 16 (81)) followed by anal cytology to improve specificity is likely a better screening strategy (10) (Table 3). Additional testing such as immunohistochemical staining for p16 and detection of HPV E6/E7 mRNA improve specificity over hrHPV DNA polymerase chain reaction tests. p16 staining and E6/E7 mRNA testing identify cellular targets that are associated with an increased risk of high-grade dysplasia and neoplasia (52).

p16 staining in anal cytology had a sensitivity of 72.3% and specificity of 100% for detecting anal dysplasia, resulting in an NPV of 92.3% and a positive predictive value of 100% (53). p16 staining can provide additional clarity for cytologic grading to determine whether the lesion is precancerous and to help avoid false-negative results (31,54).

SCREENING AND VACCINATION RECOMMENDATIONS

With optimal frequency for ASCC screening unknown, much of the societal guidance is extrapolated from cervical cancer screening recommendations. Like cervical cancer screening, reflex HPV testing or cotesting may help stratify risk and plan appropriate follow-up for patients with normal findings or atypical squamous cells of undetermined significance (16,82,83). Our recommendations for screening (high-risk populations, screening commencement, modality, and schedule) are shown in Figure 3. Recommended follow-up plans vary, depending on ASCC screening test results; these plans are summarized in Table 4.

HPV vaccines have been approved by the US Food and Drug Administration for prevention of ASCC. The 9-valent HPV vaccine covers most of the high-risk genotypes associated with ASCC, and it is recommended to all individuals aged 11–12 years for primary prevention (84). "Catch up" vaccination for HPV is recommended for individuals through age 26 years (85).Vaccination does not eliminate the need for screening. A history of HPV infection is not a contraindication for vaccination, and in fact, evidence suggests that HPV vaccines are cost-effective for secondary prevention. For patients with biopsies showing high-grade AIN, HPV vaccination reduced recurrent disease by 50% and decreased lifetime ASCC risk by 60% compared with no vaccination (83,86,87).

CONCLUSION

Given the rarity of ASCC in the general population, routine screening of healthy asymptomatic women without any risk factors or anal cancer symptoms does not provide high-value care. Screening with annual anal cytology and HPV tests is controversial because of the low specificity and moderate sensitivity of these tests in lower-risk groups. However, the sensitivity and specificity improve for higher-risk patients, and women with abnormal anal cytology or positive for HPV-16 or HPV-18 should receive a referral for HRA. Minimally invasive and cost-effective in high-risk individuals, anal cytology, and HPV tests facilitate early detection and treatment of anal dysplasia and malignancy and require minimal training for HCPs. However, many HCPs are unfamiliar with the procedure and the high-risk groups who can benefit from this screening. HCP education and training, plus appropriate triage and referral to HRA, is of paramount importance in the early diagnosis of ASCC. Promoting HPV vaccination to eligible individuals influences ASCC incidence.

CONFLICTS OF INTEREST

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70(1):7–30.
2. Seer [website]. 2020.
3. Kang YJ, Smith M, Canfell K. Anal cancer in high-income countries: Increasing burden of disease. PLoS One 2018;13(10):e0205105.

4. Van Dyne EA, Henley SJ, Saraiya M, et al. Trends in human papillomavirus-associated cancers - United States, 1999-2015. MMWR Morb Mortal Wkly Rep 2018;67(33):918–24.
5. Deshmukh AA, Suk R, Shiels MS, et al. Recent trends in squamous cell carcinoma of the anus incidence and mortality in the United States, 2001-2015. J Natl Cancer Inst 2020;112(8):829–38.
6. Singh R, Nime F, Mittelman A. Malignant epithelial tumors of the anal canal. Cancer 1981;48(2):411–5.
7. Moscicki AB, Durako SJ, Houser J, et al. Human papillomavirus infection and abnormal cytology of the anus in HIV-infected and uninfected adolescents. AIDS 2003;17(3):311–20.
8. Alberts CJ, Heard I, Canestri A, et al. Incidence and clearance of anal human papillomavirus (HPV)-16 and HPV-18 infection, and their determinants, among human immunodeficiency virus-infected men who have sex with men in France. J Infect Dis 2020;221(9):1488–93.
9. Twisk DE, van der Sande MAB, van Eeden A, et al. Detection of incident anal high-risk human papillomavirus DNA in men who have sex with men: Incidence or reactivation? J Infect Dis 2018;218(7):1018–26.
10. Lin C, Franceschi S, Clifford GM. Human papillomavirus types from infection to cancer in the anus, according to sex and HIV status: A systematic review and meta-analysis. Lancet Infect Dis 2018;18(2):198–206.
11. Damay A, Fabre J, Costes V, et al. Human papillomavirus (HPV) prevalence and type distribution, and HPV-associated cytological abnormalities in anal specimens from men infected with HIV who have sex with men. J Med Virol 2010;82(4):592–6.
12. Gosens KC, Richel O, Prins JM. Human papillomavirus as a cause of anal cancer and the role of screening. Curr Opin Infect Dis 2017;30(1):87–92.
13. Malusecka E, Chmielik E, Suwinski R, et al. Significance of HPV16 viral load testing in anal cancer. Pathol Oncol Res 2020;26(4):2191–9.
14. Maia LB, Marinho LC, Carneiro MV, et al. HPV load and anal cytological abnormalities. HIV Med 2016;17(6):467–70.
15. Palefsky JM, Rubin M. The epidemiology of anal human papillomavirus and related neoplasia. Obstet Gynecol Clin North Am 2009;36(1):187–200.
16. Ortoski RA, Kell CS. Anal cancer and screening guidelines for human papillomavirus in men. J Am Osteopath Assoc 2011;111(3 Suppl 2):S35–43.
17. https://www.cancer.org/cancer/anal-cancer/detection-diagnosis-staging/detection.html.
18. Santos J, Valencia E, Panel de Expertos de Ge S. [Consensus statement on the clinical management of non-AIDS defining malignancies. GeSIDA expert panel]. Enferm Infecc Microbiol Clin 2014;32(8):515–22. Spanish.
19. Aberg JA, Gallant JE, Ghanem KG, et al. Primary care guidelines for the management of persons infected with HIV: 2013 update by the HIV medicine association of the infectious diseases society of America. Clin Infect Dis 2014;58(1):e1–34.
20. Albuquerque A, Rios E, Schmitt F. Recommendations favoring anal cytology as a method for anal cancer screening: A systematic review. Cancers (Basel) 2019;11(12):1942.
21. Cho SD, Groves E, Lao VV. History of high-resolution anoscopy. Clin Colon Rectal Surg 2018;31(6):336–46.
22. Howard K. The cost-effectiveness of screening for anal cancer in men who have sex with men: A systematic review. Sex Health 2012;9(6):610–9.
23. Goldie SJ, Kuntz KM, Weinstein MC, et al. Cost-effectiveness of screening for anal squamous intraepithelial lesions and anal cancer in human immunodeficiency virus-negative homosexual and bisexual men. Am J Med 2000;108(8):634–41.
24. Chin-Hong PV, Vittinghoff E, Cranston RD, et al. Age-specific prevalence of anal human papillomavirus infection in HIV-negative sexually active men who have sex with men: The EXPLORE study. J Infect Dis 2004;190(12):2070–6.
25. Stier EA, Sebring MC, Mendez AE, et al. Prevalence of anal human papillomavirus infection and anal HPV-related disorders in women: A systematic review. Am J Obstet Gynecol 2015;213(3):278–309.
26. Nyitray AG, D'Souza G, Stier EA, et al. The utility of digital anal rectal examinations in a public health screening program for anal cancer. J Low Genit Tract Dis 2020;24(2):192–6.
27. Schochet E. You want to stick that where?: A guide to the anal pap smear before, during, and after. J Assoc Nurses AIDS Care 2011;22(6):502–4.
28. Bean SM, Chhieng DC. Anal-rectal cytology: A review. Diagn Cytopathol 2010;38(7):538–46.
29. Robison K, Cronin B, Bregar A, et al. Anal cytology and human papillomavirus genotyping in women with a history of lower genital tract neoplasia compared with low-risk women. Obstet Gynecol 2015;126(6):1294–300.
30. Darragh TM, Winkler B. Screening for anal neoplasia: Anal cytology - sampling, processing and reporting. Sex Health 2012;9(6):556–61.
31. Darragh TM. The LAST Project and the diagnostic bottom line. Cytopathology 2015;26(6):343–5.
32. Darragh TM, Jay N, Tupkelewicz BA, et al. Comparison of conventional cytologic smears and ThinPrep preparations from the anal canal. Acta Cytol 1997;41(4):1167–70.
33. Darragh TM, Colgan TJ, Thomas Cox J, et al. The lower anogenital squamous terminology standardization project for HPV-associated lesions: Background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical PATHOLOGY. Int J Gynecol Pathol 2013;32(1):76–115.
34. Darragh TM, Tokugawa D, Castle PE, et al. Interrater agreement of anal cytology. Cancer Cytopathol 2013;121(2):72–8.
35. Darragh TM, Winkler B, Souers RJ, et al. Room for improvement: Initial experience with anal cytology: Observations from the College of American pathologists interlaboratory comparison program in nongynecologic cytology. Arch Pathol Lab Med 2013;137(11):1550–4.
36. Nathan M, Singh N, Garrett N, et al. Performance of anal cytology in a clinical setting when measured against histology and high-resolution anoscopy findings. AIDS 2010;24(3):373–9.
37. Chiao EY, Giordano TP, Palefsky JM, et al. Screening HIV-infected individuals for anal cancer precursor lesions: A systematic review. Clin Infect Dis 2006;43(2):223–33.
38. Lee GC, Kunitake H, Milch H, et al. What is the risk of anal carcinoma in patients with anal intraepithelial neoplasia III? Dis Colon Rectum 2018;61(12):1350–6.
39. Palefsky JM, Holly EA, Hogeboom CJ, et al. Anal cytology as a screening tool for anal squamous intraepithelial lesions. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14(5):415–22.
40. Berry JM, Palefsky JM, Jay N, et al. Performance characteristics of anal cytology and human papillomavirus testing in patients with high-resolution anoscopy-guided biopsy of high-grade anal intraepithelial neoplasia. Dis Colon Rectum 2009;52(2):239–47.
41. Marnach ML, Larish AM, Kim SJ, et al. Retrospective 15-year review of anal cytology screening in women at Mayo Clinic Rochester, Minnesota. J Low Genit Tract Dis 2020;24(3):238–42.
42. Nyitray AG. The epidemiology of anal human papillomavirus infection among women and men having sex with women. Sex Health 2012;9(6):538–46.
43. Wang Y, Wang Y, Gaisa MM, et al. Negative predictive value of human papillomavirus testing: Implications for anal cancer screening in people living with HIV/AIDS. J Oncol 2020;2020:6352315.
44. Darragh TM. Anal cytology for anal cancer screening: Is it time yet?. Diagn Cytopathol 2004;30(6):371–4.
45. Salit IE, Lytwyn A, Raboud J, et al. The role of cytology (Pap tests) and human papillomavirus testing in anal cancer screening. AIDS 2010;24(9):1307–13.
46. Cachay ER, Agmas W, Mathews WC. Relative accuracy of cervical and anal cytology for detection of high grade lesions by colposcope guided biopsy: A cut-point meta-analytic comparison. PLoS One 2012;7(7):e38956.
47. Pisano L, Tiradritti L, Lorenzoni E, et al. Pap smear in the prevention of HPV-related anal cancer: Preliminary results of the study in a male population at risk. G Ital Dermatol Venereol 2016;151(6):619–27.
48. Ferraris A, Narasimhan K, Rahman I, et al. Anal pap smear in high-risk patients: A poor screening tool. South Med J 2008;101(11):1185–6.
49. Goldstone SE. High-resolution anoscopy is a crucial component of anal dysplasia screening. Dis Colon Rectum 2010;53(3):364–5.
50. Dietrich A, Hermans C, Heppt MV, et al. Human papillomavirus status, anal cytology and histopathological outcome in HIV-positive patients. J Eur Acad Dermatol Venereol 2015;29(10):2011–8.
51. Ramos-Cartagena JM, Perez CM, Guiot HM, et al. Assessment of anal cancer screening tools in detecting high-grade Anal squamous intraepithelial lesions in women. J Low Genit Tract Dis 2020;24(1):75–81.
52. Zito Marino F, Ronchi A, Stilo M, et al. Multiplex HPV RNA in situ hybridization/p16 immunohistochemistry: A novel approach to detect papillomavirus in HPV-related cancers. A novel multiplex ISH/IHC assay to detect HPV. Infect Agent Cancer 2020;15:46.
53. Roldan Urgoiti GB, Gustafson K, Klimowicz AC, et al. The prognostic value of HPV status and p16 expression in patients with carcinoma of the anal canal. PLoS One 2014;9(10):e108790.

54. Roberts JM, Ekman D. The reporting of anal cytology and histology samples: Establishing terminology and criteria. Sex Health 2012;9(6): 562–7.
55. Moscicki AB, Darragh TM, Berry-Lawhorn JM, et al. Screening for anal cancer in women. J Low Genit Tract Dis 2015;19(3 Suppl 1):S27–42.
56. Somia IKA, Teeratakulpisarn N, Jeo WS, et al. Prevalence of and risk factors for anal high-risk HPV among HIV-negative and HIV-positive MSM and transgender women in three countries at South-East Asia. Medicine (Baltimore) 2018;97(10):e9898.
57. Roka S, Rasoul-Rockenschaub S, Roka J, et al. Prevalence of anal HPV infection in solid-organ transplant patients prior to immunosuppression. Transpl Int 2004;17(7):366–9.
58. Winer RL, Lee SK, Hughes JP, et al. Genital human papillomavirus infection: Incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157(3):218–26.
59. Palefsky JM. Anal human papillomavirus infection and anal cancer in HIV-positive individuals: An emerging problem. AIDS 1994;8(3):283–95.
60. Palefsky JM, Shiboski S, Moss A. Risk factors for anal human papillomavirus infection and anal cytologic abnormalities in HIV-positive and HIV-negative homosexual men. J Acquir Immune Defic Syndr (1988) 1994;7(6):599–606.
61. Moscicki AB, Hills NK, Shiboski S, et al. Risk factors for abnormal anal cytology in young heterosexual women. Cancer Epidemiol Biomarkers Prev 1999;8(2):173–8.
62. Clifford GM, Georges D, Shiels MS, et al. A meta-analysis of anal cancer incidence by risk group: Toward a unified anal cancer risk scale. Int J Cancer 2021;148(1):38–47.
63. Ruel J, Ko HM, Roda G, et al. Anal neoplasia in inflammatory bowel disease is associated with HPV and perianal disease. Clin Transl Gastroenterol 2016;7:e148.
64. Holly EA, Ralston ML, Darragh TM, et al. Prevalence and risk factors for anal squamous intraepithelial lesions in women. J Natl Cancer Inst 2001; 93(11):843–9.
65. Sehnal B, Dusek L, Cibula D, et al. The relationship between the cervical and anal HPV infection in women with cervical intraepithelial neoplasia. J Clin Virol 2014;59(1):18–23.
66. Sehnal B, Zikan M, Nipcova M, et al. The association among cervical, anal, and oral HPV infections in high-risk and low-risk women. Eur J Obstet Gynecol Reprod Biol X 2019;4:100061.
67. Bertoli HK, Thomsen LT, Iftner T, et al. Risk of vulvar, vaginal and anal high-grade intraepithelial neoplasia and cancer according to cervical human papillomavirus (HPV) status: A population-based prospective cohort study. Gynecol Oncol 2020;157(2):456–62.
68. Hernandez BY, McDuffie K, Zhu X, et al. Anal human papillomavirus infection in women and its relationship with cervical infection. Cancer Epidemiol Biomarkers Prev 2005;14(11):2550–6.
69. Saleem AM, Paulus JK, Shapter AP, et al. Risk of anal cancer in a cohort with human papillomavirus-related gynecologic neoplasm. Obstet Gynecol 2011;117(3):643–9.
70. Welbeck M. Anal pap screening for HIV-infected men who have sex with men: Practice improvement. J Assoc Nurses AIDS Care 2016;27(1):89–97.
71. Wang CC, Silverberg MJ, Abrams DI. Non-AIDS-defining malignancies in the HIV-infected population. Curr Infect Dis Rep 2014;16(6):406.
72. Conley LJ, Bush TJ, Darragh TM, et al. Incidence and predictors of abnormal anal cytology findings among HIV-infected adults receiving contemporary antiretroviral therapy. J Infect Dis 2016;213(3):351–60.
73. Stier EA, Lensing SY, Darragh TM, et al. Prevalence of and risk factors for anal high-grade squamous intraepithelial lesions in women living with human immunodeficiency virus. Clin Infect Dis 2020;70(8):1701–7.
74. Revollo B, Videla S, Llibre JM, et al. Routine screening of anal cytology in persons with human immunodeficiency virus and the impact on invasive anal cancer: A prospective cohort study. Clin Infect Dis 2020;71(2):390–9.
75. Madeleine MM, Finch JL, Lynch CF, et al. HPV-related cancers after solid organ transplantation in the United States. Am J Transpl 2013;13(12): 3202–9.
76. Meeuwis KA, van Rossum MM, Hoitsma AJ, et al. (Pre)malignancies of the female anogenital tract in renal transplant recipients. Transplantation 2011;91(1):8–10.
77. Esser S, Kreuter A, Oette M, et al. German-Austrian guidelines on anal dysplasia and anal cancer in HIV-positive individuals: Prevention, diagnosis, and treatment. J Dtsch Dermatol Ges 2015;13(12):1302–19.
78. Hinten F, Hilbrands LB, Meeuwis KAP, et al. Reactivation of latent HPV infections after renal transplantation. Am J Transpl 2017;17(6):1563–73.
79. Machalek DA, Poynten M, Jin F, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: A systematic review and meta-analysis. Lancet Oncol 2012;13(5): 487–500.
80. Trails OC 2018. December, 30 2020.
81. Eleuterio J Jr, Cavalcante LR, Goncalves AKS, et al. Prevalence of high-risk HPV and atypia in liquid-based cytology of cervical and intra-anal specimens from kidney-transplanted women. Diagn Cytopathol 2019; 47(8):783–7.
82. Albuquerque A. Cytology in anal cancer screening: Practical review for clinicians. Acta Cytol 2020;64(4):281–7.
83. Walts AE, Thomas P, Bose S. Anal cytology: Is there a role for reflex HPV DNA testing?. Diagn Cytopathol 2005;33(3):152–6.
84. Stier EA, Chigurupati NL, Fung L. Prophylactic HPV vaccination and anal cancer. Hum Vaccin Immunother 2016;12(6):1348–51.
85. https://www.cdc.gov/vaccines/vpd/hpv/hcp/recommendations.html) (2020). Accessed December, 30 2020.
86. Swedish KA, Factor SH, Goldstone SE. Prevention of recurrent high-grade anal neoplasia with quadrivalent human papillomavirus vaccination of men who have sex with men: A nonconcurrent cohort study. Clin Infect Dis 2012;54(7):891–8.
87. Deshmukh AA, Chhatwal J, Chiao EY, et al. Long-term Outcomes of adding HPV vaccine to the anal intraepithelial neoplasia treatment regimen in HIV-positive men who have sex with men. Clin Infect Dis 2015;61(10):1527–35.
88. Obuchowski NA, Graham RJ, Baker ME, et al. Ten criteria for effective screening: Their application to multislice CT screening for pulmonary and colorectal cancers. AJR Am J Roentgenol 2001;176:1357–62.