Basic Science, Epidemiology, and Screening for Anal Intraepithelial Neoplasia and Its Relationship to Anal Squamous Cell Cancer

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Abstract

Despite the progress made in the reduction of squamous cell carcinoma of the cervix, the incidence of anal squamous cell carcinoma (ASCC) has been increasing since 1992. While it remains an uncommon disease, the prevalence is climbing steadily. Among human immunodeficiency virus (HIV)-infected adults, especially men who have sex with men, ASCC is one of the more common non-AIDS-defining malignancies. The precursor lesion, anal intraepithelial neoplasia (AIN), is prevalent in the HIV-infected population. More than 90% of ASCCs are related to human papilloma virus (HPV), oncogenic types (HPV 16, 18). While the biology of HPV-related intraepithelial neoplasia is consistent in the anogenital area, the natural history of AIN is poorly understood and is not identical to cervical intraepithelial neoplasia (CIN). CIN is also considered an AIDS-defining malignancy, and the methods for screening and prevention of AIN are derived from the CIN literature. This article will discuss the epidemiology of ASCC and its association with HPV and the life cycle of the HPV, and the molecular changes that lead to clearance, productive infection, latency, and persistence. The immunology of HPV infection will discuss natural immunity, humoral and cellular immunity, and how the HPV virus evades and interferes with these mechanisms. We will also discuss high-risk factors for developing AIN in high-risk patient populations with relation to infections (HIV, HPV, and chlamydia infections), prolonged immunocompromised people, and sexual behavior and tobacco abuse. We will also discuss the pre- and post-HAART era and its effect on AINs and ASCC. Finally, we will discuss the importance of anal cytology and high-resolution anoscopy with and without biopsy in this high-risk population.

Keywords

- ▶ non-AIDS-defining malignancy
- ▶ anal intraepithelial neoplasia
- ▶ human papilloma virus
- ▶ anal cytology
- ▶ high-resolution anoscopy

The epidemiology of anal squamous cell carcinoma (ASCC) has changed remarkably during the second half of the 20th century. While it remains an uncommon disease, the incidence and mortality has been increasing at a steady rate over the past several decades (▶ **Fig. 1**).¹ In 2016, it is the 26th most common malignancy in the United States affecting 1.8 per 100,000 individuals. This compares to 0.8 per 100,000 affected individuals in 1975. In 2016, the disease is expected to be diagnosed in just over 8,000 individuals in the United States and is expected to cause over 1,000 fatalities.²

The rate of new cases of ASCC has been rising 2 to 3% each year over the past 10 years. Death rates have also been rising on average of 3% each year.³ Joint-point analyses, employed to identify inflection points, identify 1997 as a significant point of change, when the slope of incidence rates statistically increased.³ Since 1996 marked the introduction of highly active antiretroviral therapy regimens, this increase in ASCC possibly couples with the increased life expectancy in the HIV-positive population with HIV antiretroviral therapy.⁴ In addition, when specifically looking at the

Fig. 1 Increasing incidence of ASCC 1975–2013. ASCC, anal squamous cell carcinoma.

incidence of ASCC prior to the AIDS era, there is a noted increase during and after the introduction of more effective medications to treat HIV-positive patients. The incidence of ASCC has particularly increased among men and those between 30 and 54 years of age since the introduction of these treatments.⁵ One study found that the incidence of anal cancer increased sixfold from before the introduction of these medications to the time after, affecting 8.5 per 100,000 person years in 1992–1996, to 53 per 100,000 person years from 2005 to 2008.^{6,7} Although there is no evidence that these medications are associated with an increase in anal cancer rates, there is some evidence that using the same medication regimen for over 4 years may decrease the risk of developing malignancy.⁸

The disease remains slightly more frequently diagnosed in women and is most commonly diagnosed in the fifth and sixth decades of life⁹ (► **Fig. 2**), with a median age of diagnosis being 61 years. Yet, ASCC is known to occur at a

much earlier age, especially in the immunocompromised population. The risk factors tend to mirror those of cervical cancer, where human papilloma virus (HPV) infection, an immunocompromised status, multiple sexual partners, and cigarette smoking are the most frequent risk factors identified.^{10,11} In addition, women with a prior history of cervical cancer are also known to have a greater risk for subsequent anal cancer.¹¹

Anal cancer rates are highest in HIV-positive men, where it is the most common malignancy diagnosed.¹² HIV-infected individuals have a risk of developing anal cancer which is 20 to 30 times higher than the general population. The ratio for anal cancer in HIV-infected men is 46 per 100,000 years compared with 2 per 100,000 in HIV-negative men.¹³ Case-control studies have demonstrated that sexual risk factors are also strongly associated with anal cancer risk. When specifically looking at the HIV-infected individuals with ASCC, there is an even greater increase among men who

Fig. 2 Age at diagnosis for new cases of anal cancer.

have sex with men (MSM). In addition, it is noted that ASCC in HIV-positive patients tends to be diagnosed two decades earlier than what is traditionally reported in HIV-negative populations. Infection with HIV is associated with up to a 30-fold increased lifetime risk of ASCC, with up to a fourfold increase in 5-year mortality.^{14–16}

Taxonomy

Part of the difficulty in comparing populations with ASCC over time is a change in taxonomy. Examples are Bowen's disease or erythroplasia of Queyrat, diagnoses that have been currently abandoned. Confusion regarding the taxonomy existed due to the different interests from diverse clinical practitioners including from pathology, gynecology, dermatology, and surgery.

The Lower Anogenital Squamous Terminology (LAST) project was cosponsored by the College of American Pathologists (CAP) and the American Society for Colposcopy and Cervical Pathology (ASCCP) and was conceived to align the terminology used for HPV-associated lesions.¹⁷ The project recommends a unified terminology across all lower anogenital sites. For intraepithelial lesions, a two-tiered terminology—low or high (low-grade intraepithelial lesion [LSIL] and high-grade intraepithelial lesion [HSIL])—reflects the biology of transient, productive HPV infections and persistent precancerous lesions.

This classification couples with the Bethesda system that is also two tiered and is used for the classification of anal cytological samples. The Bethesda system is commonly used and leads to more consistent communication between the pathologist and the clinician.¹⁸ The previously employed cervical intraepithelial neoplasia (CIN) or anal intraepithelial neoplasia (AIN) 1, 2, and 3 is abandoned in favor of this simpler classification.

Basic Science

The Human Papilloma Virus Life Cycle

HIV infection coupled with oncogenic HPV infection is a strong risk factor for ASCC and its precursor high-grade anal intraepithelial neoplasia (HGIN). HPVs are host-specific epitheliotropic small (40–50 nm), double-stranded deoxyribonucleic acid (DNA) viruses (→Fig. 3). The HPV life cycle is dependent on epithelial differentiation; it replicates exclusively within the epithelial layer. HPV infects, through micro-abrasions, the basal keratinocytes. In the basement membranes are glycosaminoglycans and laminin that serve as receptors for HPV. The virion binds to the $\alpha 6 \beta 4$ integrin cell membrane proteins, which leads to conformational change in the viral capsule enabling the HPV to enter the keratinocytes (→Fig. 4). This HPV infection has four pathways: clearance, productive infection (warts), latency, and persistence. It is believed that viral persistence leads to AIN and ASCC. The viral oncogenes E6 and E7 deregulates expression, which leads to increased and disorganized proliferation of the basal epithelial cell layer. The E6 oncoprotein binds to p53 tumor suppressor protein, causing deregulation of DNA damage and apoptotic pathways. E6 oncoprotein also activates telomerase, which is an intrinsic step of cell transformation and immortalization.¹⁹ The E7 oncoprotein releases E2F transcription activators, which inactivates retinoblastoma protein (pRb) tumor suppressor function and also disrupts DNA methyltransferase, which serves to disrupt the maintenance of CpG methylation patterns following cell replication.^{20,21} There are over 100 different subtypes of HPV, differentiated by a number.²² At least 40 of these subtypes are transmitted through sexual contact and are known to infect the anogenital region or other mucosal sites of the body. These mucosal HPV types can be subcategorized into those that are either high risk or oncogenic or low risk,

Human Papillomavirus Characteristics

- Host Specific
- Strictly Epitheliotropic
- Non-enveloped
- Double-stranded DNA
- Small (40–55 nm)
- Icosahedral Capsid formed by major L1 & minor L2 Structural Protein

- Major Oncoproteins E6 & E7
- Types 120
- Alphapapillomavirus Genus
- High Risk Carcinogenicity: type 16 (85%) or 18 (7%) (these types account for 70% of all Cervical Cancers)

Fig. 3 Human papilloma virus characteristics. DNA, deoxyribonucleic acid

Fig. 4 Human papilloma virus (HPV) life cycle.

which is not commonly associated with malignancy. HPV subtypes 6 and 11 are commonly isolated in benign genital warts, although they have low oncogenic potential.²³ Local control can be achieved with a wide selection of surgical and topical techniques with little or no malignant potential.²⁴

The oncologic HPV subtypes 16, 18, 31, 35, 39, 45, 52, 56, 58, and 59 are found in dysplastic lesions and have the potential to progress to invasive ASCC. HPV 16 is the most commonly isolated,²⁵ and is responsible for up to 80 to 93% of cancers of the anus.^{26–28}

Immunology of Human Papilloma Virus Infection

The oncogenes in HPV have evasive mechanisms to interfere with the natural immune response, and also interfere with both the humoral and cellular response to viral infection.

Natural Immunity

The immune response to HPV infection has been studied in relation to CIN. Realizing that the natural history of AIN may be different in CIN because the anatomy and hormonal differences and the overall immune response of the anus and the cervix are different. There is natural innate immunity to HPV infection, which has several components in the anus including the epithelial barrier, antimicrobial peptides (β -defensins), toll-like receptors (TLRs), and several types of defensive cells including phagocytes, natural killer cells (NKC), and intraepithelial lymphocytes. Oncogenic HPV subtypes 16 and 18 have been shown to evade and delay the adaptive immune response. These oncogenes evade tumor necrosis factor- α (TNF- α)-mediated inhibition of production of the major antiviral cytokine interferon- γ (IFN- γ) in circulation of peripheral blood mononuclear cells (PBMCs) and NK cells. The inhibition of TLR9 transcription suppresses the activation of a signaling pathway that causes stimulation of

the adaptive immunity and induction of an antiviral condition. These oncogenes also downregulate interleukin-1 β and interleukin-6, which prevent production of antiviral, proinflammatory, and chemotactic cytokines. This evasion of the natural immune response begins the unrelegated proliferation and eventual malignant transformation.²⁹

Humoral Immunity

Most of the studies have been done on women with cervical disease. In women, systemic immunoglobulin G (IgG) antibodies to L1 are HPV type specific, but seroconversion is found only in 59.5% with HPV-16 infection. There are data that indicate in men that seroconversion may be site specific.³⁰ The mucosal humoral response of HPV-16 indicates immunoglobulin A (IgA) in the cervical secretions before the detection of serum IgA. The IgA response is very short lived. HIV infection impairs cervical production of HPV-16 IgA which may also play a role in malignant transformation.

Cellular Immunity

The cellular immunity response is found in established HPV infections. There are systemic HPV-specific T cell responses; the mechanism of clearing mucosal HPV infection is unclear. The T cell response is through CD4 and CD8 circulating cytotoxic to HPV-16 E6 and E7, and has been shown that in women who are HPV-16 positive there is an absence of CIN. If there is no cytotoxic response to E6, there is persistence of HPV-16 infection. If T cell response to HPV-16 E2, E6, and/or E7 is absent, there may be progression of low-grade CIN. The mucosal immune response is based on a local microenvironment and a complex interplay of regulatory and effector cells.

In HIV-induced CD4, T cell immunodeficiency is a risk factor for the detection of anal HPV, AIN, and ASCC.³¹

Anal Intraepithelial Neoplasia

Anal intraepithelial neoplasia is the precursor lesion to ASCC. AIN is analogous to and is associated with both cervical and vulvar intraepithelial neoplasia.³² As the incidence of ASCC climbs, an increase in precursor lesions such as AIN has, not surprisingly, also been noted, climbing even higher than the invasive component, with increases of up to 10 to 15% per year.^{33,34} There are several risk factors that increase the incidence of AIN-associated HPV infections. The risk factors are categorized under sexual behavior, infections, prolonged immunosuppression, and cigarette smoking (**► Table 1**).

Risk Factors

Infections

Human Papilloma Virus Infection

ASCC and its precursor lesions are associated with the HPV. Infection with HPV is a significant risk factor for the development of ASCC. Thirty-five thousand new HPV-associated malignancies occur every year. While oropharyngeal and cervical are the most prevalent, ASCC is increasing. HPV is a group of diverse DNA viral strains that affect and invade the

Table 1 Risk factors: HPV infection and anal squamous cell cancer

Sexual behavior
Number of life time sex partners
Anal receptive intercourse
Men Who Have Sex with Men
Infections
Human Papilloma Virus
Human immunodeficiency virus
24-fold increase in HIV-infected woman
32-fold increase in HIV-infected men
52-fold increase in HIV-infected MSMs
Chlamydia infection
Higher incidence of carcinogenic HPV infection
Increases persistence of HPV
Prolonged immunosuppression
Transplant patients
CD4 count
Low CD4 < 350 cells/mm ³
Cigarette smoking
History of smoking last 12 mo
Smoking for > 10 y
Increased number of packs smoked per day

Abbreviations: HIV, human immunodeficiency virus; HPV, human papilloma virus; MSM, men who have sex with men.

host epithelium. The virus enters the basal epithelium cells in areas of local trauma. At the point of insertion, HPV viral oncogene expression drives cellular proliferation producing a clonal proliferation and differentiation in the host epithelial DNA (→Fig. 4).

HPV infection is ubiquitous and is the most common sexually transmitted infection encountered in the United States, with most sexually active persons having an exposure at some point in their lifetime.³⁴ HPV is documented in almost 80 million persons in 2008, and another 14 million more are infected each year.³⁵ Despite the widespread nature of the virus, not all individuals exhibit evidence of infection and very few go on to develop malignancy. This is due in part to the fact that there are numerous subtypes of the virus and the virus is typically well cleared by the immune system of the immunocompetent individual.

Human Immunodeficiency Virus Infection

The population that has been most extensively studied regarding the incidence and development of ASCC is the HIV-positive population, and there is clear linkage to the development of malignancy. The incidence of anal cancer developing in HIV-positive patients is 40- to 80-fold higher compared with the general population.³⁶ The incidence varies considerably between populations evaluated. Specifically when looking at female patients who are HIV positive, precursor lesions are found in 3 to 26%, compared with 0 to

3% of HIV-negative women.³⁷ In one study, precursor lesions were present in up to 30% of HIV-positive men.³⁸

It is believed that HIV increases the activity and duration of HPV infections due to the ineffectiveness of clearing the HPV in immunocompromised patients, thus leading to the increased incidence of AIN and ASCC. In another study, the associated increased risk of HPV-associated cancers is elevated in HIV-infected patients and associated increase immunosuppression. This may indicate an increased incidence of anal cancer with prolonged survival of patients with HIV infections.³⁹

HIV-Positive Infection: Incidence of AIN and ASCC Post-HAART

The association of immunosuppression and highly active antiretroviral therapy (HAART) with the risk of HPV-associated cancers is important to understand. HAART does partially “restore” the immune system, but also prolongs survival of HIV-infected individuals. In a study in 2009, records of 499,230 people with diagnosed AIDS from January 1, 1980, through December 31, 2004, were linked to cancer registry data of nine U.S. states. The authors looked at all HPV-associated cancers: anal canal and anorectal, uterine, cervix, vagina, vulva, oropharynx, lingual tonsil, base of tongue, and Waldeyer's ring. They then broke the individuals into three calendar periods of AIDS diagnosis: 1980–1989 (early AIDS period), 1990–1995, and 1996–2004 (the HAART era). From the 1980–2004 period, there was an increase in proportion of women, the median age at AIDS diagnosis, African Americans, and Hispanics with AIDS. For each site of HPV-associated cancer, there was a higher risk of in situ cancers when compared with invasive cancers like ASCC. In high-risk HIV groups, there was an increased risk of both in situ and invasive cancers, but the greatest incidence was in MSMs (standard infection ratio [SIR] = 89.7%, 95% confidence interval [CI]: 76.8–104.1: for invasive cancers, SIR = 51.8, 95% CI: – 45.3 to 59.0). In the 10-year period from 5 years before to 5 years after the onset of AIDS, there was a statistically significant increased incidence of in situ squamous cell cancers of the anus and penis in men and in woman of the cervix, vagina, or vulva but not the anus. The authors looked at the HAART era (1996–2004) the relationship of CD4 T cell count at AIDS onset with associated risk of incidence of cancers. In the 28 to 60 months after AIDS onset, a low CD4 T cell count was associated with a statistically significant increased risk of invasive anal cancer among men (relative risk [RR] per 100 cell/cc mil decline in CD4 cells = 1.59, 95% CI: 1.09–2.34; *p* = 0.016). The overall incidence of HPV-associated cancers during the 4 to 60 months after AIDS onset (1980–2004 all three calendar periods), including in situ and invasive anal cancers, increased among men (both groups MSMs and other men). This study indicated that HAART has not substantially altered HPV persistence or the rates of progression or regression of premalignant anogenital lesions, which may indicate that HAART may not restore HPV-specific immunity. This study found an elevated risk of HPV-associated cancers among persons with AIDS and an increased incidence of anal cancer during the 1996–2004 HAART era.³⁹

In a similar study done in 2010, the authors looked at 4,506 HIV-infected males with 37,806 person-years (PY) of follow-up (the incidence of anal cancer rates increased fivefold from 11/100,000 PY pre-HAART to 55/10,000 PY in the HAART era, $p = 0.02$). In the 2006–2009 era, the rates increased to 128/100,000 PY. They also found that people with HIV infections for > 15 years had a 12-fold increase higher rate than those with < 5 years (348 vs. 28, $p < 0.01$). This study also demonstrated that the incidence of ASCC among HIV-infected persons has steadily risen despite the advances in antiretroviral therapy. This is probably related to a reduction in competing causes of death and the increase in life expectancy, thus lengthens the persistence of time of the HPV virus allowing accumulation of genetic mutations that are important in the ASCC development.⁴⁰

Chlamydia Infection

In a 2013 study of life style characteristics for HIV-infected MSMs who were in a routine anal cancer-screening program, the authors screened the patients for chlamydia trachomatis and Neisseria gonorrhea. The statistical analysis was a univariate analysis and a multivariate logistic regression comparing $< \text{AIN-2}$, high risk (HR)-HPV-positive versus $< \text{AIN2}$, HR-HPV-negative patients. They showed that men with a history of chlamydia trachomatis infection had a higher incidence of $< \text{AIN-2}$ and HR-HPV-positive than the chlamydia trachomatis-negative patients. The study indicated that chlamydia trachomatis in women may increase persistence of HPV of the cervix without a biologic interaction which could be the same mechanism in men with anal HPV.⁴¹

Prolonged Immunocompromised Population

Immunocompromised individuals are at even higher risk for the development of ASCC, amplifying any exposure to oncogenic HPV.⁴² Likely, the immunosuppression is associated with failure to clear the virus and then subsequent development of malignancy. The risk of anal dysplasia progression appears to correlate directly with the degree of immunosuppression.

Renal Transplant Patients

Renal graft recipients are another population group known to be at higher risk for developing ASCC. In one evaluation of 50 renal graft recipients, 24% of patients demonstrated subclinical precursor lesions.⁴³ A review of both populations demonstrates that HIV-positive patients as well as those receiving immunosuppression following transplant are at increased risk for the development of ASCC. This demonstrates that it is likely the immune suppression itself is responsible, rather than the exact etiology of the suppression.⁴³

Sexual Behavior

As ASCC is associated with HPV, which is sexually transmitted, sexual behavior is also not surprisingly associated with developing ASCC.⁴⁴ While difficult to quantify, there is

evidence that an increased number of lifetime sexual partners is associated with an increased risk.

Number of Lifetime Sex Partners

A risk factor for anal HPV is the number of lifetime sexual partners.^{44,45} In a large study of MSM, HIV-positive men were at an increased risk of HPV infection and anal precancer when compared with HIV-negative men. The study utilizing a univariate model showed a significantly increased risk associated with the number of lifetime partners.⁴⁵

Anal Receptive Intercourse

Additionally, in patients with a history of anal receptive intercourse, the rate of anal cancer is equivalent to that of cervical cancer. As oncogenic HPV is sexually transmitted and due to the necessity of epithelial abrasion for the viral particles to be implanted, it is likely that the practice of anal receptive intercourse facilitates this due to a thinner lining of the anal canal compared with the vagina. In one study, having more than 10 lifetime receptive anal intercourse episodes was associated with a higher incidence of ASCC precursor lesions.^{46,47}

Men Who Have Sex with Men

Men who have sex with men (MSM) may be at an even higher risk. One study showed that AIN was slightly more common in MSM than in men who have sex with women.⁴⁷ In one study of 346 MSM, over 70% demonstrated an anal HPV infection, with 56% being oncogenic HPV genotypes. Precursor ASCC lesions were present in 30% of these individuals. A higher proportion of precursor lesions was noted in older MSM, those with a higher number of lifetime partners, as well as those with a history of anal warts. In a meta-analysis, the incidence of oncogenic HPV was 35%, with high-grade AIN present in 30% of patients.⁴⁸

CD4 T Cell Count

When using the T cell CD4+ count as a marker for immunosuppression, for an example, a CD4+ cell count less than 200 cells/mm³ is closely associated with an increased prevalence of ASCC.⁴⁹ In addition, the amount of time that the CD4 count was diminished increased the rate even further.⁵⁰ In a study of 363 MSMs, they looked at multiple variables and confirmed from previous studies that a low CD4 count (350 cell/mm³) was associated with carcinogenic HPV infection. The other variables were lifetime number of partners, cigarette smoking, and associated chlamydia infections.⁵¹

Tobacco Use

Tobacco use is another common associated risk factor that is associated with the development of ASCC. Cigarette smokers, for instance, are eight times more likely to develop anal cancer, and the effects may be additive to the other risk factors listed earlier. In another study, there are several smoking characteristics including smoking in last 12 months, lifetime duration of smoking, and cigarettes smoked per day, which are risk factors for precancerous anal lesions among HPV-infected men.⁵²

Similarity to Cervical Cancer

ASCC is similar clinically and histologically to the disease that is found in the cervix.

Due to these numerous similarities, attempts are made to extrapolate the data and techniques associated with cervical cancer to anal cancer. Both of the malignancies occur at a junction between squamous and columnar epithelium and are in areas that are associated with rapid turnover and are susceptible to the genetic alterations associated with oncogenic HPV. In addition, the HPV types in anal cancer are identical to those of cervical cancer.⁵² Furthermore, both cervical and anal cancers demonstrate favorable outcomes if detected early. Due to the ability to detect cervical cancer precursor lesions⁵³ using Papanicolaou (Pap) stains, a similar management strategy for anal cancer is attractive.

Symptoms of Anal Human Papilloma Virus Infection

Anal HPV infection is usually asymptomatic; however, it can present with pruritus, exophytic lesions, bleeding per rectum, pain, or fecal incontinence (if anal sphincters are involved), anal fissure (anal ulcer), anal fistula, or change in bowel habits. It may be an incidental finding on histopathology for benign anorectal surgery, for example, hemorrhoid specimens. It is important to identify inguinal lymphadenopathy at presentation.

Screening for Human Papilloma Virus and Anal Intraepithelial Neoplasia

If interventions are to be successful, as in cervical cancer, two things must be accomplished. First, precursor lesions must be able to be identified via screening. Second, there must be methods to arrest the progression of the disease. The goal of anal cancer screening is to identify and treat HGAIN before it progresses to anal cancer. In a retrospective review, a group of 329 patients (89.8% men and 10.2% women, mean age of 46 ± 10 years) classified as an increased-risk (IR) group was

compared with 44 patients (72.7% men, 27.3% women, mean age: 52 ± 8 years) classified as a standard-risk (SR) group. The IR group was defined by male sex, white race, sexual orientation, past and current receptive anal intercourse, and noncompliance with condom use. Findings were significantly different when compared with the SR group. Anal cytology detected biopsy-confirmed HSIL in 23 of 118 (19.5%) in the IR group and in 2 of 7 (28.6%) in the SR group. In the IR group, 118/187 (42%) had HSIL, and the SR group had 7/15 (16.3%) HSIL.⁵⁴ However, the indications for screening do vary in another study looking at world-wide clinics in eight different countries (80 clinics with 82 providers). It was found that over a third of the clinics do not restrict access to screening, while the rest screened based on HIV status and anal cytology results. Fifty-three percent of the clinics require abnormal anal cytology (ASCUS or higher) prior to performing HRA in asymptomatic patients. All clinics offer anal cytology and HRA.⁵⁵ There does not appear to be a universal consensus for optimal screening for an anal cancer screening; this is probably due to the lack of prospective controlled trials or well-designed observational studies (**► Fig. 5**).

Digital Anal–Rectal Examination

In high-risk populations, patients who are immunocompromised have ano-receptive intercourse, or a history of HPV infections, and especially in HIV-infected MSMs a digital examination of the anus and rectum is indicated at least every year.

Anal Cytology

Cytology is the primary screening tool for detection of AIN of the anus.

In an attempt to extrapolate the success from the use of the Pap smear for cervical cancer, similar techniques have been employed to screen high-risk populations for ASCC. Originally reported in Europe in 1989,⁵⁶ the procedure soon became adopted by American practitioners, as well.⁵⁷ Anal Pap testing is likely cost-effective in screening high-risk

Screening Algorithm For High Risk Patients with HIV/HPV-Infections

Fig. 5 Screening algorithm for high-risk patients with HIV/HPV infections. ¹Individuals with HIV positive, women HIV positive (history of HPV infection, CIN, cancer of cervix, vulva, vagina), HIV-positive men, HIV-positive MSM (men who have sex with men), HIV-positive individuals who have anoreceptive intercourse. ²High-resolution anoscopy. ³Atypical cells of undetermined significance. ⁴Low-grade intraepithelial lesion. ⁵High-grade intraepithelial lesion. ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HRA, high-resolution anoscopy; HSIL, high-grade intraepithelial lesion; LSIL, low-grade intraepithelial lesion.

populations, provided that effective treatment is available.⁵⁸ Sensitivity and specificity are argued to be similar to cervical cytology.⁵⁹ Sensitivity estimates vary widely however, ranging from 69 to 98%, while specificity ranges from 32 to 59%.^{60,61} False-negative cytology results can also be very high, increased by 45% for HIV-positive patients.⁶² Perhaps more troubling is that cytology is more sensitive for low-grade AIN, but less so for high-grade or ASCC.^{63,64} Due to this lack of sensitivity and a lack of comfort in performing the procedure, it has been slow to gain universal acceptance.⁶⁵

Anal cytology is categorized according to the Bethesda Classification: normal, atypical squamous cells of undetermined significance (ASCUS), LSIL, and HSIL. Anal histology is graded according to severity; mild abnormalities are graded as LSIL (AIN I) and moderate to severe abnormalities as HSIL (AIN II or III). Those patients with ASCUS cannot rule out HSIL. Also, anal cytology can also be utilized as a quality-control measure for the anoscopist. The recommendation for patients with ASCUS, LSIL, and HSIL should be a referral for high-resolution anoscopy (HRA). The Centers for Disease Control and Prevention (CDC) recommends screening of all HIV-positive MSM annually and HIV-negative MSM every 2 years.⁶⁶ However, to date, the literature has not shown that anal cytology (Pap) testing or HRA screening has reduced the incidence or mortality of anal cancer in this patient population.⁶⁷

Diagnosis

High-Resolution Anoscopy

The early identification of anal lesions is beneficial only if there is effective therapy that can arrest the progression to malignancy. If anal Pap smear is used as an initial screening examination, then high-resolution anoscopy (HRA) typically follows, in a manner similar to how a cervical Pap smear is followed by colposcopy. HRA is usually performed in an outpatient setting, usually the practitioner's office.

HRA uses magnification with a colposcope to examine the perianal tissue and anal canal. Using acetic acid with or without Lugol's iodine to bathe the perianal tissues, acetowhite lesions that are detected are biopsied using local anesthesia and the specimens sent for pathological review. HRA has shown to demonstrate high inter-observer agreement.⁶⁸ Anal Pap smear and HRA have a wide-ranging sensitivity and specificity.^{69,70} In one study of 300 HIV-positive men with positive anal Pap smears, only 54% demonstrated high-grade precursor lesions.⁷¹ There is much debate regarding where this technique should be used and where it should fall in the colorectal surgeon's armamentarium. In addition to the questions of the utility, there is some evidence that a great deal of experience is necessary to gain proficiency. One provider found that it took approximately 200 cases before there were no missed high-grade lesions.⁷² However, we doubt that colon and rectal surgeons would require 200 cases to become proficient in HRA screening, because we utilize anal evaluation on a daily basis.

The classification of the lesions was discussed in a study using the interobserver method of verification. They found

AIN in 71% and HGAIN in 38% of cohorts. Over 70% of the lesions were classified as flat and those with acetowhiting (13%), flat leukoplakia (75%), hyperleukoplakia (9%), punctation (71%), mosaicism (4%), and atypical vascular pattern (21%) were found to have AIN. The patterns with the highest HGAIN were found in lesions with punctation (23%), flat leukoplakia (25%), and atypical vessels (23%). The authors recommended histologic evaluation of flat lesions, punctation, flat leukoplakia, and condylomatous lesions because of the high incidence of HGAIN found in them.⁶⁸

The progression of HGAIN is unknown but over the last decade its incidence in HIV-positive MSM has increased to 52%. With the increase in ASCC HRA screening of high-risk individuals, identification and eradication of HGAIN is becoming the accepted modality and should eventually become the standard of care. Utilizing HRA after application of 3% acetic acid highlights the epithelial and vascular changes characteristic of the AIN lesions in the transitional zone (squamocolumnar junction) of the anal canal. The utilization of HRA and biopsy is currently the best method of identifying these precancerous histologic lesions. Combining with HPV polymerase chain reaction (PCR) and then specific serotyping for the presence of HPV 16, or other high-risk HPV DNA types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68), it has been shown that HRA-guided biopsy is an excellent method of detecting HGAIN in this high-risk population.⁷³

Treatment

The diagnostic or screening tests mentioned previously are of benefit only if there are effective treatments to arrest the progression from precursor lesions to invasive malignancy. Numerous strategies have been attempted including non-operative medical treatment. Nonoperative treatments include expectant surveillance as well as topical imiquimod or 5-fluorouracil (5FU). In a review of the Cochrane registry, only one study comparing imiquimod to placebo was found. In that study with only 53 patients, no demonstrable improvement was found.⁷⁴

Operative therapies include wide local excision and targeted ablation with electrocautery, infrared coagulation, or cryotherapy. Regardless of the method chosen, the recurrence rates are high and surveillance is required.⁷⁵ In a retrospective review of patients with AIN, who were treated with imiquimod and either HRA with ablation or observation, the 5-year progression rate was only 6.0% for expectant management and 4.5% for HRA.⁷⁶ Clearly, there is still much to be learned regarding the treatment of these lesions.

Human Papilloma Virus Vaccine

If there is no definitive means of preventing the progression to invasive disease, perhaps it is possible to prevent it entirely. One of the most exciting areas of research into ASCC involves the HPV vaccine.

The quadrivalent HPV vaccine has been demonstrated to provide sustained protection against low-grade lesions attributable to vaccine HPV types 6, 11, 16, and 18.⁷⁷ In girls aged 15

to 19 years, who were vaccinated, there was a reduction in genital warts of over 30%.⁷⁸ Another study demonstrated that the vaccine reduces the risk of anal cancer and anal precancerous lesions in young MSM with no prior history of anal lesions.⁷⁹ A study of over 600 healthy MSM demonstrated an efficacy of 77% against AIN in those who received the vaccine.⁸⁰

The CDC recommends that MSM should receive the vaccine through age 26 years. Men and women with HIV infection or other immunocompromised populations should also receive the vaccine through age 26 years.⁸¹ There is evidence that vaccinating HIV-negative MSM could reduce the lifetime risk of anal cancer by up to 60%.⁸² Currently, there are no data on those older than 26 years.

Future Directions

In addition to ongoing work of the HPV vaccine, there are ongoing studies that attempt to answer some of the more difficult clinical questions regarding the management of patients at higher risk for ASCC. The Study of the Prevention of Anal Cancer (SPANC)⁸³ is a 3-year prospective cohort that began recruitment in 2010 with follow-up planned through 2018 that will examine the overlapping roles of digital anorectal examination, HPV testing, anal Pap tests, and HRA. The Anal Cancer HSIL Outcomes Research (ANCHOR) Study: anal cancer high-grade squamous intraepithelial neoplasia (HSIL) Outcomes Research is an ongoing 5-year prospective randomized trial with the goal of enrolling over 5,000 HIV-positive patients over the age of 35 years, diagnosed with precursor lesions on anal Pap testing and HRA.⁸⁴ The two arms of the study include a monitoring arm and a treatment arm for HSIL by ablation through infrared coagulation, with the ultimate goal of determining whether active surveillance with ablative treatment of HSIL will ultimately decrease the incidence of anal cancer.

Conclusion

The incidence of ASCC is climbing. In the Annual Report on the state of cancer in 2013, it notes that there were increases in only two of the HPV-associated cancers: oropharyngeal and anal.⁸⁵ There is a great deal of interest and research regarding early detection and possible prevention for methods to halt the increase of this disease. The use of anal Pap smears and HRA for the detection of these lesions has not demonstrated the results that have been accomplished with cervical cancer, but there is strong clinical experience that is moving toward anal cytology and HRA as screening tools for high-risk populations. It is inherent on clinicians who treat patients who are in high-risk categories to be familiar with these techniques as well as their shortcomings and to always remain vigilant in this difficult disease.

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