Management of Anal Squamous Intraepithelial Lesions

Carlos E. Pineda, M.D.[1] and Mark L. Welton, M.D.[1]

ABSTRACT

Anal squamous intraepithelial lesions include both low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) and are caused by chronic infection with the human papillomavirus (HPV). The disease is increasing in both incidence and prevalence, especially among patients with the following risk factors: homosexual men, acquired or iatrogenic immunosuppression, and presence of other HPV-related diseases. Although the natural history of the disease is unknown, there is significant evidence that untreated HSIL progresses to squamous cell carcinoma in 11% of patients and in up to 50% of patients with extensive disease and immunosuppression. Anal cytology and reflex HPV DNA testing are used to screen for disease, particularly among patients with the aforementioned risk factors. Evaluation of the patient should include physical examination and high-resolution anoscopy (HRA) to evaluate for disease above and below the dentate line. Intervention is warranted and this can be achieved in many ways. The treatment option associated with the best outcomes is ablation directed with HRA, which can be performed in the office or in the operating room with minimal morbidity. This strategy is effective in patients with both low-volume and high-volume disease and is associated with a malignant progression rate of 0.4% in patients with treated HSIL.

KEYWORDS: Anal squamous dysplasia, high-grade squamous intraepithelial lesions, high-resolution anoscopy

Objectives: On completion of this article the reader should be able to summarize the current management of anal squamous intraepithelial lesions.

Anal squamous intraepithelial lesions (ASIL) represent a spectrum of disease that spans from mild squamous dysplasia to severe squamous dysplasia, the putative precursor to invasive squamous cell carcinoma.[1] Although the management of mild dysplasia, known as condylomatous disease when grossly visible, and invasive disease are relatively straightforward, the management of severe dysplasia is not. This is mostly due to the lack of understanding of the natural history of the disease, specifically, the risk of developing invasive carcinoma when patients remain untreated and whether value exists in screening and intervention strategies. Here we describe the recent advances in diagnosis, screening, and therapeutic modalities for ASIL.

[1]Section of Colon and Rectal Surgery, Stanford University School of Medicine, Stanford, California.

Address for correspondence and reprint requests: Carlos E. Pineda, M.D., Section of Colon and Rectal Surgery, Stanford University School of Medicine, 300 Pasteur Dr., Stanford, CA 94305 (e-mail: cepineda@ stanford.edu).

Anal Cancer and Retrorectal Tumors; Guest Editor, Jan Rakinic, M.D.

Clin Colon Rectal Surg 2009;22:94–101. Copyright © 2009 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662.
DOI 10.1055/s-0029-1223840. ISSN 1531-0043.

CLASSIFICATION

ASIL is classified into two groups based on the degree of dysplasia: low-grade SIL (LSIL) and high-grade SIL (HSIL). LSILs demonstrate nuclear atypia and perinuclear cytoplasmic cavitation, with a nucleus that is larger than that of a normal intermediate squamous cell on cytology.[2] On histology, these lesions are characterized by low nuclear/cytoplasmic ratios (koilocytes), atypical cells confined to superficial layers, and mitotic activity in the lower third of the epithelium.[3]

HSILs demonstrate high nuclear/cytoplasmic ratios on cytology, with cell sizes smaller than those with LSIL.[2] On histology, there are high nuclear/cytoplasmic ratios and full-thickness atypia, which includes parabasal atypia, loss of cell polarity, and mitotic activity in the upper third of the mucosa, as well as abnormal mitotic figures.[3] Full-thickness atypia with invasion of the basement membrane defines anal squamous cell carcinoma (SCC).

A myriad of terms are found in the literature used to describe HSIL, including Bowen's disease, anal intraepithelial neoplasia II/III, and squamous cell carcinoma in situ. However, these all describe the same lesion, even when examined with histology[4] and immunohistochemistry,[5] so in the interest of standardization we only use the term HSIL.[6] This definition has been accepted by the American Joint Committee on Cancer (AJCC) and is used in the seventh edition of their staging manual.[6a]

ETIOLOGY

ASIL is a neoplastic change secondary to chronic infection with the human papillomavirus (HPV) in a susceptible host. HPV DNA has been identified within both benign and malignant lesions along the anogenital tract and is recognized as the cause of cervical dysplasia and cancer. It is found in up to 91% of patients with anal HSIL and in up to 81% of patients with anal SCC.[7] Infection occurs when a break in the anorectal mucosa allows for the virus to reach the basal and parabasal cells of the epithelium.[8] After entry of the HPV DNA into the cell nucleus, several intranuclear processes result in cellular immortalization.[9] A susceptible host is one that runs the risk of infection through the aforementioned mechanisms and then is unable to suppress the oncogenic changes due to a lack of normal cell-mediated immunity. Thus, recognized factors that increase the risk of, but are not necessary for, developing anal dysplasia and cancer include acquired or iatrogenic immunosuppression, concurrent HPV-related diseases, number of sexual partners, and history or presence of other sexually transmitted infections (STI).[10–15] HPV serotypes associated with anal LSIL include HPV types 6, 11, 16, and 39; serotypes associated with anal HSIL and cancer (high-risk serotypes) include HPV 16, 18, 58, and 45.[16]

EPIDEMIOLOGY

The true incidence and prevalence of ASIL is unknown and will most likely remain unknown. However, a recent evaluation of the Surveillance, Epidemiology and End Results (SEER) program revealed an increasing trend in the incidence of HSIL and anal cancer between 1973 and 2000 in the United States,[17] which was more pronounced in men who have sex with men (MSM) in large urban centers.[18–20] In men, the incidence increased from 0.09 per 100,000 from 1973 to 1979 to 0.45 per 100,000 from 1994 to 2000.[17] These data underestimate the true incidence because the SEER data report only carcinoma in situ, not HSIL, anal intraepithelial neoplasia (AIN) III, or Bowen's disease. Recently, ASIL has been recognized as a disease that occurs outside of large metropolitan areas. A series of HIV-positive patients in a rural region screened with anal cytology and then biopsied reported a prevalence of 19% and 15% for LSIL and HSIL, respectively.[21]

Natural History

The natural history of this disease is largely unknown. Historical accounts of progression from premalignant disease to invasive disease reported a rate of 6%.[22] However, more contemporary series have described rates of 13 to 50% in immunocompromised patients managed expectantly.[23,24] Several case reports of anal SCC arising in areas of HSIL lend support to the theory of malignant progression.[25,26]

Clinical Evaluation

ASIL presents with many nonspecific symptoms, including bleeding, pruritus, and pain. Commonly patients are asymptomatic and disease is found incidentally in surgical specimens obtained during treatment of another anorectal disease, or found through screening with anal cytology.

Kreuter et al described a clinical classification of ASILs: bowenoid, erythroplakic, leukoplakic, and verrucous.[27] However, it is practically impossible to tell grossly whether lesions are LSIL, HSIL, or invasive. In fact, up to half of pathologically diagnosed HSIL are invisible to the naked eye.[28] Therefore, in addition to inspection and digital rectal examination, we use high-resolution anoscopy and the criteria described by Jay et al to ascertain the presence of ASIL.[29]

High-Resolution Anoscopy

Because ASIL shares many of the pathogenic features of cervical SIL, a technique similar to colposcopy has been successfully transferred to the anus as high-resolution anoscopy (HRA).[30] This technique is based on the principle that with the application of acetic acid dysplastic tissue exhibits distinct changes and patterns in the anal

and rectal mucosa similar to the changes seen with cervical HSIL. The examination is performed in the office or in the operating room, by physicians or allied health personnel.[31] After generous application of 3% acetic acid to the anal canal and perianal skin, tissues that harbor ASIL turn acetowhite.[32] Acetowhitening alone is nonspecific. It sets the background upon which the clinicians identify the characteristic vascular changes of LSIL and HSIL. HSILs, in addition to being acetowhite, tend to be flat and exhibit vascular punctation and mosaicism, whereas LSILs are generally raised lesions that have warty vessels.[29] Lugol's solution may be applied in areas of diagnostic uncertainty. Areas that do not take up Lugol's are considered at high risk for harboring HSIL.[31] However, if Lugol's is used as the only staining method, its accuracy decreases to 33% for the detection of HSIL.[33] Thus, we recommend its use as an adjunct to acetic acid for lesions that are hard to evaluate. HRA-directed biopsies of lesions can be performed in the office and equipment is generally available in most gynecology practices. Identification requires training. This is offered through various organizations and societies, like the American Society for Colposcopy and Cervical Pathology (http://www.asccp.org). HRA is critical to directing ablative therapy. It is indispensable in the diagnosis and management of HSIL.

Anatomic Considerations

A clear and accurate description of the location and size of the lesions is imperative for successful therapy and follow-up in patients with ASIL, particularly when communicating across specialties. Thus, we use a classification system in which the location of the lesions are described as being intraanal, perianal, or on the skin.[8] Intraanal lesions are either only partially visible or not visible at all when gentle traction is placed on the buttocks. Perianal lesions are visualized in their entirety and are within a 5-cm radius of the anal opening with the same gentle traction. Finally, skin lesions are those that lie outside of this 5-cm radius. Furthermore, it is better to describe lesions as anterior, posterior, or lateral rather than "at 6:00" because patients are often examined in different positions.

The newly defined anal transformation zone is a region of squamous metaplasia overlying the distal rectal mucosa. The distal margin of this zone is the dentate line and it extends proximally in a dynamic fluid fashion. As in the cervix, the metaplastic tissue within the transformation zone is susceptible to HPV infection and thus should be included in the area of evaluation.[8]

ROLE OF SCREENING

The goal of screening with anal cytology is to identify patients who will benefit from an intervention to prevent malignant progression. When screening is performed, the clinician (or the patient) uses a moistened Dacron swab and takes a blind smear of the anal transition zone.[34,35] The swab is introduced into the anal canal, then using the pelvic floor as a fulcrum and employing a circular motion that generates a 360 rotation of the swab, the swab is slowly withdrawn and placed in a liquid medium. Cytologic interpretation is performed using the 2001 Bethesda classification as mentioned above.[36] Controversy lies in defining the population in which this study is cost effective and of benefit. Cytology screening has a sensitivity that ranges from 47 to 90% and a specificity that ranges from 16 to 92%.[35,37–42] The sensitivity is higher in HIV-positive patients,[35] and in detecting internal disease.[43] A note of caution though, correlation between cytology findings and HRA-directed biopsy findings is moderate,[44] with an absolute agreement of 74.7% in one series,[45] and moderate interobserver agreement for cytology specimens and biopsy specimens in another series.[46] Through multivariate analysis in a series of HIV-positive patients, risk factors for an abnormal cytology included a CD4-lymphocyte count of less than 200 cc/mm^3 at the time of cytology and anal disease on physical examination.[47] Other risk factors described in the literature include MSM, HPV infection, history of anogenital warts, and a lower CD4+ lymphocyte nadir.[21]

The diagnosis of atypical squamous cells of unknown significance (ASCUS) on cervical cytology requires "reflex" testing for HPV to increase the yield of HSIL.[48] Similar results have been found with anal ASCUS. In a retrospective review of patients with anal ASCUS, the prevalence of HSIL was higher in patients with high-risk HPV than in those with low-risk HPV ($p < 0.05$).[49] The differences in both HIV-positive and HIV-negative groups were statistically significant. Higher degrees of dysplasia have been found in patients with ASCUS and high-risk HPV types.[50] Reflex testing also increases the ability to detect HSIL in patients with condylomas.[51]

A Markov model was developed to establish the clinical and cost-effectiveness of screening for ASIL in HIV-positive and HIV-negative MSM, using disease progression data from San Francisco and Seattle.[52,53] The studies revealed that screening with Pap smears every year for HIV-positive MSM and every 2 to 3 years for HIV-negative MSM, respectively, was associated with an increased quality-adjusted life expectancy at a cost comparable with other accepted preventive measures (e.g., the use of colonoscopy to prevent colorectal cancer).

Therefore, we recommend screening HIV-positive MSM yearly and HIV-negative MSM every 2 to 3 years with cytology and reflex testing for HPV DNA during each screening session to patients with the following risk factors: MSM, immunosuppression, and

concurrent HPV infection in another site. Patients with cytologic abnormalities consistent with LSIL, HSIL, and ASCUS with high-risk HPV types should be subsequently evaluated with HRA.

TREATMENT

Treatment options for HSIL vary widely due to the unknown cancer progression rate and the availability of HRA in different regions. Options vary in terms of invasiveness of the intervention and its resulting morbidity. We classify these as nonoperative and operative.

Nonoperative Management

EXPECTANT MANAGEMENT

Expectant management is based on the premise that the malignant progression rate is unknown and thus most patients simply warrant careful observation. Patients, with a diagnosis of HSIL are observed for progression of disease by a change in symptoms or the development of a palpable mass. In one series of 98 HIV-positive patients, of which 28 had HSIL, 3 (11%) developed SCC within a period of 10, 16, and 84 months.[23] In another series of 55 patients with HSIL, 8 (15%) progressed to SCC despite local excision of abnormal lesions.[28] In this series, 9 patients became incontinent of feces and two required abdominal perineal resections. Thus, while heralded as the least invasive option, the reactionary nature of treating grossly visible disease or permitting disease progression ultimately results in higher morbidity than other more-invasive strategies. The risks associated with observation alone are underlined in another series where six immunosuppressed patients with multifocal disease identified with HRA were left untreated due to the extent of disease.[24] Of these, three (50%) developed SCC with one patient developing metastases 2 years later. Finally, in a longitudinal study of patients who were successfully treated for anal warts, 38 (19%) later developed HSIL, and of these, six (16%) went on to develop SCC, a year to 10 years after enrollment in the study.[15] Thus, given the high progression rate, we do not support this approach of watchful waiting, particularly in high-risk patients.

TOPICAL IMMUNOMODULATION

Imiquimod, a topical immunomodulator, acts on the Toll-like receptor 7 of the humoral immune system resulting in the secretion of various proinflammatory cytokines and activation of both humoral and cell-mediated immunity. It is commonly used for the treatment of anogenital warts.[54] In a series of 28 HIV-positive men with high-risk HPV types who were treated overnight with imiquimod 3 times a week for 16 weeks, 17 showed a complete clinical and pathologic response.[55] Of the patients with HSIL, 78% had a complete response, with a concomitant decrease in HPV DNA. Two with LSIL progressed to HSIL. All patients developed erythema where the cream was applied. Compliant patients were followed for an average of 30.3 months in a follow-up study.[56] This study revealed a recurrence rate of 26% at an average of 26 months, with higher recurrence rates for patients with lower CD-4 lymphocyte nadirs. Furthermore, 11 patients developed new dysplastic lesions and at the end of the follow-up period, 74% still had high-risk HPV types, though the number of HPV types was significantly lower. Thus, imiquimod may be safely recommended for treatment of patients with LSIL and HSIL with higher CD-4 lymphocyte counts, as an adjunct to HRA-guided therapies. As with all treatment protocols, close follow-up is required given the high recurrence rate.[57]

EXPERIMENTAL STRATEGIES

The therapeutic vaccine and photodynamic therapy are two treatment modalities that have shown promising results in small series, though long-term data are lacking and their use should be regarded as experimental until larger series evaluate their effectiveness.

Therapeutic Vaccine A phase I/II trial using a therapeutic vaccine consisting of a fusion of HPV 16 E7 protein and the *Mycobacterium bovis* heat shock protein 65 (SGN-00101) was performed in 15 HIV-positive patients with HSIL.[58] Heat shock proteins are loaded with antigens that elicit significant T and B cell responses against tumor antigens.[59] Patients were assessed at 48 weeks. Four of these were found to have regression to LSIL and one had a complete clinical response. None had drug-related adverse events.

Photodynamic Therapy Photodynamic therapy is used for the treatment of dysplasia in other sites (gastrointestinal and genital tracts). The photosensitizer is applied to the affected tissues and is then activated by an appropriate light wavelength that results in selective destruction. Five patients with HSIL were sensitized with aminolevulinic acid (ALA) by mouth and photodynamic therapy was performed under conscious sedation 4 hours later, followed with cytology and HRA at 5 months.[60] Patients were followed with anal cytology and HRA at 5 months. One patient (20%) had a recurrence.

Operative Management

MAPPING AND WIDE LOCAL EXCISION

This technique was initially described by Fazio et al and involves four-quadrant mapping with punch biopsies

around a clockface, random biopsies, and biopsies of suspicious lesions.[61–63] This is followed by intraoperative frozen section analysis and wide local excision of biopsy-proven disease. This is a morbid procedure that creates defects with an average size of 17.4 cm^2.[64] This sacrifices uninvolved healthy tissues to achieve widely clear margins on frozen section and typically requires flap reconstruction. Patients may even undergo three-stage procedures with creation and takedown of an intestinal stoma.[65] Despite the magnitude of the procedure, there is a 23% recurrence rate.[62]

This procedure limits sampling to the dentate line and below extending distally to the perianal region and skin, leaving disease above the dentate line untreated. HPV 16, the type most commonly associated with HSIL and cancer, has a particular affinity for the immature squamous metaplasia found above the dentate line in the transformation zone. Thus, mapping the tissue below the dentate line is missing the zone at highest risk. Mapping in the traditional approach is based on arbitrary site selection, random sampling, and sampling of grossly visible disease, thus relying on random opportunity to detect disease, potentially overtreating patients with minimal external disease, and undertreating patients with internal disease. Interobserver and intraobserver variability in the interpretation of ASIL is great, making intraoperative decisions based on this information less than ideal.[66] It is our hope that HRA-guided therapies that spare normal tissue integrity and function will replace wide-local excision as the preferred treatment of choice.

HRA-BASED THERAPIES

The advantages of using HRA to direct therapy include detection of grossly invisible disease, evaluation of the transformation zone and tissues above the dentate line, and the ability to spare destruction of normal tissue thereby reducing the risk of anal stenosis.[31]

HRA-Directed Infrared Coagulator Ablation

HRA-directed infrared coagulator ablation (IRC) is performed in the office or in the operating room with local anesthesia. Its advantages include good tolerance by patients, minimal adverse events, and the ability of nonsurgically trained clinicians to perform the procedure. In a retrospective series of 68 HIV-positive MSM treated in this fashion, recurrence rates after a first, a second, and a third treatment were 65%, 58%, and 40%, respectively.[67] Recurrence rates are lower in HIV-negative patients, with none requiring a third session.[68] Furthermore, no patient in either group progressed to SCC or developed significant complications. However, all of these patients had small and limited disease. None had circumferential or bulky disease. Technical difficulties encountered with this technique include successfully managing anorectal mucosal folds, engorgement of hemorrhoids with blood, and distortion of anatomical landmarks due to twisting of the anoscope.[69]

HRA-Directed Cautery Ablation Our group has used this modality for the past 15 years. It is our preferred mode of treatment for (1) patients with circumferential or near-circumferential disease, (2) for those who cannot be evaluated and treated in the office due to disease location (e.g., over hemorrhoids), (3) when coexisting anorectal pathology precludes evaluation (anal fissure or fistula), or (4) when patients refuse office-based therapy.[70] In our initial prospective series of 37 patients, we established the safety of the procedure. In our experience of ASIL in HIV-negative patients, all patients with LSIL were cured with treatment, whereas 45% of patients with HSIL recurred and required reintervention.[26] In our 10-year review of 246 patients with HSIL, the vast majority of patients (81%) had large-volume disease (>25% of the anal circumference affected by HSIL).

We treated patients with near-circumferential or circumferential disease in a staged fashion, with disease being purposely left behind to be treated in a second stage. The goal was to preserve as much normal tissue as possible, even if it involves leaving small areas of HSIL or LSIL behind. This second stage was performed either in the operating room or in the office, depending on the volume and location of the remaining disease.

In our series, patients that were treated with intent to cure had a recurrence rate of 57% at an average of 19 months.[70] However, most recurrences were small and successfully controlled in the office using IRC. The factor that correlated with recurrence was initial extent of disease. We controlled HSIL in 78% of patients. Three (1.2%) progressed to SCC. Two were high-risk HIV-positive MSM with known untreated HSIL lost to follow-up until 11 and 13 months when they represented with painful masses. The other patient was an HIV-negative woman with severe anal stenosis from procedures performed elsewhere, significantly limiting our ability to visualize the anus and distal rectum. Thus, our progression rate of 1.2% overall and 0.4% in patients with treated HSIL is superior to the 6% reported in the literature[22]—and vastly superior to the 11 to 50% reported for expectant management.[23,24,28] Nine patients (<4%) experienced complications that included bleeding requiring reoperation in one, anal stenosis in two (both patients had wide local excisions performed in the past), anal fissures in four patients, postoperative myocardial infarction in one, and cellulitis at the local anesthetic injection site in one patient. This approach has been successfully performed in community-based settings with similar results.[33]

In another series of 181 patients with ASIL, HSIL was present in 49%.[71] Patients received treatment with imiquimod, excision or laser ablation, or a combination of

all three. Ablation was guided with HRA, and follow-up consisted of cytology and HRA over a median period of 19.1 months. Although the study did not report recurrence rates, it reported a median time to cure of 31.5 months for the entire cohort. Factors that negatively affected the time to cure included HIV-positivity and extent (volume) of disease.

We recommend treating all patients with HRA-guided ablation because it permits a full evaluation of the anorectal anatomy, allows for targeted therapy, protects normal tissues, has minimal morbidity, allows for treatment of large-volume disease, and minimizes progression to SCC when compared with both less morbid and radical procedures.

CONCLUSIONS

ASIL represents a spectrum of disease that is rapidly increasing in incidence and prevalence, particularly among high-risk patients. Knowledge of its natural history is still evolving, though the current evidence supports that untreated disease leads to malignant progression rates much higher than previously anticipated. Thus, we recommend a management paradigm in which high-risk individuals are screened with cytology, evaluated for HPV DNA, then further examined with physical examination and HRA. Although there are many treatment modalities available to the practicing clinician, we recommend using those modalities that employ use of HRA in both the diagnostic phase and the therapeutic phase. Patients with large-volume disease can be safely and successfully treated in a staged fashion in the operating room and in the office, minimizing the risk for anal stenosis and development of cancer. Finally, long-term follow-up is necessary and can be achieved using anal cytology and HRA with HRA-targeted retreatment.

REFERENCES

1. Pineda CE, Welton ML. Controversies in the management of anal high-grade squamous intraepithelial lesions. Minerva Chir 2008;63(5):389–399
2. Apgar BS, Zoschnick L, Wright TC Jr. The 2001 Bethesda System terminology. Am Fam Physician 2003;68(10):1992–1998
3. Longacre TA, Kong CS, Welton ML. Diagnostic problems in anal pathology. Adv Anat Pathol 2008;15(5):263–278
4. Grodsky L. Bowen's disease of the anal region: squamous cell carcinoma in situ; report of three cases. Am J Surg 1954;88(5):710–714
5. Welton ML, Amerhauser A, Litle VR, Leavenworth JD, Darragh TM, Palefsky JM. Anal Bowen's disease and high-grade squamous intraepithelial lesions are histologically and immunohistochemically indistinguishable. [meeting abstract] Dis Colon Rectum 2001;44:A32
6. Fleshner PR, Chalasani S, Chang GJ, Levien DH, Hyman NH, Buie W; DStandards Practice Task Force, American Society of Colon and Rectal Surgeons. Practice parameters for anal squamous neoplasms. Dis Colon Rectum 2008;51(1):2–9

6a. Edge SB. AJCC Cancer Staging Manual. 7th edition. In press

7. Varnai AD, Bollmann M, Griefingholt H, et al. HPV in anal squamous cell carcinoma and anal intraepithelial neoplasia (AIN). Impact of HPV analysis of anal lesions on diagnosis and prognosis. Int J Colorectal Dis 2006;21(2):135–142
8. Welton ML, Sharkey FE, Kahlenberg MS. The etiology and epidemiology of anal cancer. Surg Oncol Clin N Am 2004;13(2):263–275
9. Litle VR, Leavenworth JD, Darragh TM, et al. Angiogenesis, proliferation, and apoptosis in anal high-grade squamous intraepithelial lesions. Dis Colon Rectum 2000;43(3):346–352
10. D'Souza G, Wiley DJ, Li X, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. J Acquir Immune Defic Syndr 2008;48(4):491–499
11. Adami J, Gäbel H, Lindelöf B, et al. Cancer risk following organ transplantation: a nationwide cohort study in Sweden. Br J Cancer 2003;89(7):1221–1227
12. Stier EA, Krown SE, Chi DS, Brown CL, Chiao EY, Lin O. Anal dysplasia in HIV-infected women with cervical and vulvar dysplasia. J Low Genit Tract Dis 2004;8(4):272–275
13. Frisch M. On the etiology of anal squamous carcinoma. Dan Med Bull 2002;49(3):194–209
14. Abramowitz L, Benabderrahmane D, Ravaud P, et al. Anal squamous intraepithelial lesions and condyloma in HIV-infected heterosexual men, homosexual men and women: prevalence and associated factors. AIDS 2007;21(11):1457–1465
15. Sobhani I, Walker F, Roudot-Thoraval F, et al. Anal carcinoma: incidence and effect of cumulative infections. AIDS 2004;18(11):1561–1569
16. Gohy L, Gorska I, Rouleau D, et al; HIPVIRG Study Group. Genotyping of human papillomavirus DNA in anal biopsies and anal swabs collected from HIV-seropositive men with anal dysplasia. J Acquir Immune Defic Syndr 2008; 49(1):32–39
17. Johnson LG, Madeleine MM, Newcomer LM, Schwartz SM, Daling JR. Anal cancer incidence and survival: the surveillance, epidemiology, and end results experience, 1973-2000. Cancer 2004;101(2):281–288
18. Cress RD, Holly EA. Incidence of anal cancer in California: increased incidence among men in San Francisco, 1973-1999. Prev Med 2003;36(5):555–560
19. Koblin BA, Hessol NA, Zauber AG, et al. Increased incidence of cancer among homosexual men, New York City and San Francisco, 1978-1990. Am J Epidemiol 1996;144(10):916–923
20. Frisch M, Melbye M, Møller H. Trends in incidence of anal cancer in Denmark. BMJ 1993;306(6875):419–422
21. Ciobotaru B, Leiman G, St John T, Hyman N, Ramundo M, Grace C. Prevalence and risk factors for anal cytologic abnormalities and human papillomavirus infection in a rural population of HIV-infected males. Dis Colon Rectum 2007;50(7):1011–1016
22. Marfing TE, Abel ME, Gallagher DM. Perianal Bowen's disease and associated malignancies. Results of a survey. Dis Colon Rectum 1987;30(10):782–785
23. Devaraj B, Cosman BC. Expectant management of anal squamous dysplasia in patients with HIV. Dis Colon Rectum 2006;49(1):36–40

24. Scholefield JH, Castle MT, Watson NF. Malignant transformation of high-grade anal intraepithelial neoplasia. Br J Surg 2005;92(9):1133–1136
25. Herat A, Shirato K, Damian DL, Finlayson R, Whitfeld M. Invasive squamous cell carcinoma arising in refractory perianal Bowen's disease in a HIV-positive individual. Australas J Dermatol 2006;47(2):120–123
26. Pineda CE, Berry JM, Jay N, Palefsky JM, Welton ML. High resolution anoscopy in the planned staged treatment of anal squamous intraepithelial lesions in HIV-negative patients. J Gastrointest Surg 2007;11(11):1410–1415; discussion 1415–1416; discussion 5–6
27. Kreuter A, Brockmeyer NH, Hochdorfer B, et al. Clinical spectrum and virologic characteristics of anal intraepithelial neoplasia in HIV infection. J Am Acad Dermatol 2005;52(4): 603–608
28. Watson AJ, Smith BB, Whitehead MR, Sykes PH, Frizelle FA. Malignant progression of anal intra-epithelial neoplasia. ANZ J Surg 2006;76(8):715–717
29. Jay N, Berry JM, Hogeboom CJ, Holly EA, Darragh TM, Palefsky JM. Colposcopic appearance of anal squamous intraepithelial lesions: relationship to histopathology. Dis Colon Rectum 1997;40(8):919–928
30. O'Connor JJ. The study of anorectal disease by colposcopy. Dis Colon Rectum 1977;20(7):570–572
31. Pineda CE, Berry JM, Welton ML. High resolution anoscopy and targeted treatment of high-grade squamous intraepithelial lesions. Dis Colon Rectum 2006;49(1): 126
32. Tramujas da Costa e Silva I, de Lima Ferreira LC, Santos Gimenez F, et al. High-resolution anoscopy in the diagnosis of anal cancer precursor lesions in renal graft recipients. Ann Surg Oncol 2008;15(5):1470–1475
33. Chung AP, Rosenfeld DB. Intraoperative high-resolution anoscopy: a minimally invasive approach in the treatment of patients with Bowen's disease and results in a private practice setting. Am Surg 2007;73(12):1279–1283
34. Cranston RD, Darragh TM, Holly EA, et al. Self-collected versus clinician-collected anal cytology specimens to diagnose anal intraepithelial neoplasia in HIV-positive men. J Acquir Immune Defic Syndr 2004;36(4):915–920
35. Chin-Hong PV, Berry JM, Cheng SC, et al. Comparison of patient- and clinician-collected anal cytology samples to screen for human papillomavirus-associated anal intraepithelial neoplasia in men who have sex with men. Ann Intern Med 2008;149(5):300–306
36. Solomon D, Davey D, Kurman R, et al; Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287(16):2114–2119
37. Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, Darragh TM. Anal cytology as a screening tool for anal squamous intraepithelial lesions. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14(5):415–422
38. de Ruiter A, Carter P, Katz DR, et al. A comparison between cytology and histology to detect anal intraepithelial neoplasia. Genitourin Med 1994;70(1):22–25
39. Panther LA, Wagner K, Proper J, et al. High resolution anoscopy findings for men who have sex with men: inaccuracy of anal cytology as a predictor of histologic high-grade anal intraepithelial neoplasia and the impact of HIV serostatus. Clin Infect Dis 2004;38(10):1490–1492
40. Arain S, Walts AE, Thomas P, Bose S. The anal Pap smear: cytomorphology of squamous intraepithelial lesions. Cytojournal 2005;2(1):4
41. Fox PA, Seet JE, Stebbing J, et al. The value of anal cytology and human papillomavirus typing in the detection of anal intraepithelial neoplasia: a review of cases from an anoscopy clinic. Sex Transm Infect 2005;81(2):142–146
42. Nadal SR, Calore EE, Nadal LR, Horta SH, Manzione CR. [Anal cytology for screening of pre-neoplasic lesions]. Rev Assoc Med Bras 2007;53(2):147–151
43. Garcia FU, Haber MM, Butcher J, Sharma M, Nagle D. Increased sensitivity of anal cytology in evaluation of internal compared with external lesions. Acta Cytol 2007;51(6):893–899
44. Cranston RD, Hart SD, Gornbein JA, Hirschowitz SL, Cortina G, Moe AA. The prevalence, and predictive value, of abnormal anal cytology to diagnose anal dysplasia in a population of HIV-positive men who have sex with men. Int J STD AIDS 2007;18(2):77–80
45. Mathews WC, Sitapati A, Caperna JC, Barber RE, Tugend A, Go U. Measurement characteristics of anal cytology, histopathology, and high-resolution anoscopic visual impression in an anal dysplasia screening program. J Acquir Immune Defic Syndr 2004;37(5):1610–1615
46. Lytwyn A, Salit IE, Raboud J, et al. Interobserver agreement in the interpretation of anal intraepithelial neoplasia. Cancer 2005;103(7):1447–1456
47. Scott H, Khoury J, Moore BA, Weissman S. Routine anal cytology screening for anal squamous intraepithelial lesions in an urban HIV clinic. Sex Transm Dis 2008;35(2):197–202
48. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D; 2006 ASCCP-Sponsored Consensus Conference. 2006 consensus guidelines for the management of women with abnormal cervical screening tests. J Low Genit Tract Dis 2007;11(4):201–222
49. Goldstone SE, Kawalek AZ, Goldstone RN, Goldstone AB. Hybrid Capture II detection of oncogenic human papillomavirus: a useful tool when evaluating men who have sex with men with atypical squamous cells of undetermined significance on anal cytology. Dis Colon Rectum 2008;51(7): 1130–1136
50. Walts AE, Thomas P, Bose S. Anal cytology: is there a role for reflex HPV DNA testing? Diagn Cytopathol 2005;33(3):152–156
51. Papaconstantinou HT, Lee AJ, Simmang CL, et al. Screening methods for high-grade dysplasia in patients with anal condyloma. J Surg Res 2005;127(1):8–13
52. Goldie SJ, Kuntz KM, Weinstein MC, Freedberg KA, Welton ML, Palefsky JM. The clinical effectiveness and cost-effectiveness of screening for anal squamous intraepithelial lesions in homosexual and bisexual HIV-positive men. JAMA 1999;281(19):1822–1829
53. Goldie SJ, Kuntz KM, Weinstein MC, Freedberg KA, Palefsky JM. 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):634–641
54. van Poelgeest MI, van Seters M, van Beurden M, et al. Detection of human papillomavirus (HPV) 16-specific CD4+ T-cell immunity in patients with persistent HPV16-induced vulvar intraepithelial neoplasia in relation to clinical impact of imiquimod treatment. Clin Cancer Res 2005;11(14): 5273–5280

55. Wieland U, Brockmeyer NH, Weissenborn SJ, et al. Imiquimod treatment of anal intraepithelial neoplasia in HIV-positive men. Arch Dermatol 2006;142(11):1438–1444
56. Kreuter A, Potthoff A, Brockmeyer NH, et al; German Competence Network HIV/AIDS. Imiquimod leads to a decrease of human papillomavirus DNA and to a sustained clearance of anal intraepithelial neoplasia in HIV-infected men. J Invest Dermatol 2008;128(8):2078–2083
57. Sanclemente G, Herrera S, Tyring SK, et al. Human papillomavirus (HPV) viral load and HPV type in the clinical outcome of HIV-positive patients treated with imiquimod for anogenital warts and anal intraepithelial neoplasia. J Eur Acad Dermatol Venereol 2007;21(8):1054–1060
58. Palefsky JM, Berry JM, Jay N, et al. A trial of SGN-00101 (HspE7) to treat high-grade anal intraepithelial neoplasia in HIV-positive individuals. AIDS 2006;20(8):1151–1155
59. Einstein MH, Kadish AS, Burk RD, et al. Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III. Gynecol Oncol 2007;106(3):453–460
60. Webber J, Fromm D. Photodynamic therapy for carcinoma in situ of the anus. Arch Surg 2004;139(3):259–261
61. Strauss RJ, Fazio VW. Bowen's disease of the anal and perianal area. A report and analysis of twelve cases. Am J Surg 1979;137(2):231–234
62. Marchesa P, Fazio VW, Oliart S, Goldblum JR, Lavery IC. Perianal Bowen's disease: a clinicopathologic study of 47 patients. Dis Colon Rectum 1997;40(11):1286–1293
63. Beck DE, Fazio VW, Jagelman DG, Lavery IC. Perianal Bowen's disease. Dis Colon Rectum 1988;31(6):419–422
64. Margenthaler JA, Dietz DW, Mutch MG, Birnbaum EH, Kodner IJ, Fleshman JW. Outcomes, risk of other malignancies, and need for formal mapping procedures in patients with perianal Bowen's disease. Dis Colon Rectum 2004;47(10):1655–1660; discussion 1660–1661; discussion 60–1
65. Brown SR, Skinner P, Tidy J, Smith JH, Sharp F, Hosie KB. Outcome after surgical resection for high-grade anal intraepithelial neoplasia (Bowen's disease). Br J Surg 1999;86(8):1063–1066
66. Colquhoun P, Nogueras JJ, Dipasquale B, Petras R, Wexner SD, Woodhouse S. Interobserver and intraobserver bias exists in the interpretation of anal dysplasia. Dis Colon Rectum 2003;46(10):1332–1336; discussion 1336–1338; discussion 6–8
67. Goldstone SE, Kawalek AZ, Huyett JW. Infrared coagulator: a useful tool for treating anal squamous intraepithelial lesions. Dis Colon Rectum 2005;48(5):1042–1054
68. Goldstone SE, Hundert JS, Huyett JW. Infrared coagulator ablation of high-grade anal squamous intraepithelial lesions in HIV-negative males who have sex with males. Dis Colon Rectum 2007;50(5):565–575
69. Cranston RD, Hirschowitz SL, Cortina G, Moe AA. A retrospective clinical study of the treatment of high-grade anal dysplasia by infrared coagulation in a population of HIV-positive men who have sex with men. Int J Std AIDS 2008;19(2):118–120
70. Pineda CE, Berry JM, Jay N, Palefsky JM, Welton ML. High-resolution anoscopy targeted surgical destruction of anal high-grade squamous intraepithelial lesions: a ten-year experience. Dis Colon Rectum 2008;51(6):829–835; discussion 835–837; discussion 35–7
71. Nathan M, Hickey N, Mayuranathan L, Vowler SL, Singh N. Treatment of anal human papillomavirus-associated disease: a long term outcome study. Int J Std AIDS 2008;19(7):445–449