Diagnostic Problems in Anal Pathology:
The Role of the Pathologist in Screening and Treatment of Anal Squamous Intraepithelial Lesions (Anal SIL)

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Bullet Points

- The incidence of anal squamous cell carcinoma and its precursor lesions is increasing in the United States and Europe
- New terminology has been proposed to describe cutaneous and anal canal tumors.
- Specific lesions are discussed including anal condyloma acuminatum, Verrucous Carcinoma (Giant Condyloma of Buschke and Lowenstein), flat dysplasia, and invasive carcinoma.
- Although the cervical model of pathogenesis can be applied to anal squamous cell carcinoma, screening programs for anal dysplasia pose unique problems.

Introduction

The incidence of anal squamous cell carcinoma and its precursor lesions is increasing in the United States and Europe and pathologists are seeing increasing numbers of intra-anal and perianal biopsies that require an assessment for the presence of dysplasia, the degree of dysplasia, and in some instances, the estimated risk for progression to an invasive carcinoma. As in cervical squamous cell carcinoma, the relationship between HPV infection and anal squamous cell carcinoma, regardless of its histological appearance, is well established. Although the progression rate has yet to be fully defined, progression through a precursor anal squamous intraepithelial lesion is likely. The poor interobserver and intraobserver agreement in the classification of anal intraepithelial neoplasia has prompted renewed interest in a more uniform and reproducible diagnostic terminology in the clinical, histologic and surgical assessment of these lesions. This presentation will focus on recently proposed standardized terminology, specific risk lesions, current screening strategies and the role of the pathologist in initial diagnosis and evaluation of anal resection specimen(s). Key differential diagnostic problems are addressed and the role of HPV typing, anal cytology, and recently identified biomarkers in precancer lesions is also discussed.

Terminology

Discussions regarding anal dysplasia and cancer require clear terminology with respect to anatomic location and distribution of disease. The indiscriminate use of the term “anal carcinoma” to reflect cutaneous and anal canal tumors alike and the confusion engendered by the terms anal margin (versus anal verge) and anal canal, has led to a proposed new terminology, which is provided in Table 1. Using this terminology, lesions are either intra-anal, perianal or
cutaneous (skin), based on whether or not they can be visualized in entirety when gentle traction is placed on the buttocks and if completely visualized, whether they lie within or beyond 5 cm of the anal opening. This is the preferred terminology proposed by a consensus conference composed of gynecologists, dermatologists, internists, radiation oncologists, medical oncologists, and surgeons and is the terminology used at our center, but in truth, any disease classification that 1) clearly and unambiguously distinguishes skin lesions from intra-anal canal lesions and 2) is universally understood by all health care workers in this field would be equally acceptable. As will be evident in the following discussion, much of the confusion regarding epidemiology, risk of progression, HPV subtype, and treatment of anal condyloma, anal dysplasia, and anal carcinoma is largely attributable to blurring of these anatomic sites.

A second source of confusion in the diagnosis and management of anal cancer is the terminology used to describe the putative precancer lesions. Although most pathologists use the classic anal intraepithelial neoplasia (AIN) terminology (AIN I, II, or III), AIN is subject to the same poor interobserver reproducibility suffered by the 3-tiered CIN terminology applied to uterine cervical lesions. With the introduction of the 2-tiered (low-grade squamous intraepithelial lesion and high-grade squamous intraepithelial lesion) Bethesda system for cervical cytology specimens, we, as have many pathologists now also report out cervical biopsies as either low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL), with the corresponding CIN grade in parenthesis for those clinicians who are still accustomed to a 3-tiered nomenclature. Due to the before mentioned reproducibility issues and the additional terms that are sometimes also used in anal dysplasia (Bowen’s disease, mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma in situ), we have also adopted the 2-tiered LSIL/HSIL terminology for anal lesions (Table 2) with the corresponding AIN terminology in parenthesis. Classifying the lesions into 2 categories affords direct correlation with anal Pap smear diagnoses, reduces intraobserver and interobserver variability in diagnosis, and facilitates a more uniform set of treatment options amongst medical disciplines. However, the parallels between cervical and anal lesions are incomplete. While condyloma is considered to fall under the umbrella of LSIL in the uterine cervix, the term “condyloma” in anal lesions often means different things to dermatologists, surgeons, and pathologists. Indeed, there is ongoing controversy as to whether or not condyloma represents a premalignant lesion when it occurs in the anal region (see Anal Condyloma Acuminatum below).

**Who’s At Risk?**

Risk factors for anal dysplasia (LSIL and HSIL) and squamous carcinoma include anoreceptive intercourse, history of genital tract dysplasia (LSIL and HSIL) or carcinoma, persistent high-risk HPV genotype infection, cigarette smoking, HIV seropositivity, and immunosuppression (Table 3). The prevalence of anal squamous cell cancer has increased in HIV positive patients since the initiation of highly active antiretroviral therapy, presumably because it allows patients to live long enough for progression to malignancy to occur. In line with the experience with genital HPV infection and dysplasia, HSIL has been more strongly associated with progression to carcinoma than LSIL, but the data demonstrating this is somewhat limited compared to that which has been gathered for cervical lesions. In practice, the frequent association of intra-anal LSIL with HSIL (either concurrently or on follow-up examinations) in
HIV-positive at risk patients with high-risk HPV infection often triages them into a surveillance program regardless of the degree of dysplasia in some centers (Figure 1).

Although most epidemiologic work in this area has focused on the HIV seropositive group of patients with HPV infection, there is accumulating evidence that other forms of immunosuppression may also place patients at increased risk. In addition to HIV seropositive patients, the two most widely recognized risk groups are renal transplant patients and cardiac allograft patients. The prevalence of anal intraepithelial neoplasia in renal transplant patients is approximately 20% and the relative risk for anal squamous cell carcinoma is 10; meanwhile, the prevalence of anal HPV infection in established renal transplant patients is 47%, while the prevalence of anal HPV infection in new transplant patients is 23%.

Other potential risk groups are patients with chronic disease or patients receiving chronic immunosuppressive therapy, most notably patients with longstanding ulcerative colitis. Squamous metaplasia, when present, is not uncommonly found in the distal rectal mucosa of patients with ulcerative colitis. The presence of squamous epithelium in the rectal mucosa is presumed to develop from reserve cells in damaged mucosa, but the proposed fluidity of the anal transformation zone (= the squamocolumnar junctional tissue, typically extending 2 to 4 cm from the dentate line) in some patients may permit viral replication in a similar manner to that which occurs in the immature squamous metaplastic epithelium in the transformation zone of the uterine cervix. The presence of high-risk HPV in rectal squamous cell metaplasia, dysplasia, and carcinoma in some patients with ulcerative colitis, suggests that heightened surveillance may be indicated in patients with ulcerative colitis and squamous metaplasia on colorectal biopsy, especially if there is a known history of prior high-risk HPV infection or HPV-related disease.

The specific surveillance programs utilized to monitor high risk patients vary from one center to another, but typically include periodic anorectal Pap smear screening, preferably with ThinPrep specimens, and high resolution anoscopy and perianal mapping for all HSIL. In our center, patients are treated by high-resolution anoscopy and targeted ablation using an infrared coagulator. Punch biopsy mapping followed by wide local excision, the more traditional approach to intra-anal dysplasia, is reserved for more intractable or extensive disease. In some centers, Hybrid Capture 2 (Digene, Gaithersburg, MD) HPV testing is performed in tandem with anal cytology to detect high-risk genotypes, although the use of this technology is not as well established as in cervical specimens. One proposed screening strategy that utilizes both methods is depicted in Figure 1.

**Anal Condyloma Acuminatum (Anal Wart)**

Anal condyloma acuminatum, when used in the usual sense, refers to a soft, polypoid, and cauliflower-shaped pedunculated excrescence, consisting of acanthotic papillomatous squamous mucosa with a variably thickened stratum corneum and superficial parakeratosis. The key histologic feature is koilocytosis, in which there is perinuclear clearing and a characteristic irregular nuclear membrane; nuclear atypia and binucleation may also be present. Dyskeratotic cells and mitotic figures are not uncommon, but there is an orderly progression of maturation of
the squamous epithelium. When such lesions occur over the vulva and perineum (skin), the koilocytosis may be subtle.

A significant proportion of perianal and cutaneous condylomata, particularly those on the perineum, harbor low risk HPV types 6 or 11. For this reason, anal condylomas are often interpreted as relatively innocuous, low risk lesions which may recur but have little, if any, risk of progression to carcinoma. Although this interpretation is probably true in most instances, given the loose criteria used in diagnosing many cutaneous and perianal condylomas, the occurrence of carcinoma in situ and invasive squamous cell carcinoma has been well documented in association with at least some intra-anal condylomas. In addition, some perianal and perhaps more intra-anal condylomas harbor high risk HPV types (or a mixture of low risk and high risk types, particularly HPV type 16) and it is likely that it is these types of condylomas that exhibit transition to epithelial dysplasia, carcinoma in situ, and invasive carcinoma. Because of the variable risk attached to these two types of condylomas, the possible role for HPV typing in triage of patients with anal disease has been discussed. However, whether the presence of specific subtypes will allow more accurate and cost effective prediction of those lesions that are likely to behave aggressively has not been adequately tested.

In the meantime, in absence of HPV typing, we diagnose all perianal and intra-anal condyloma with no evidence of high grade dysplasia as “condyloma acuminatum (low-grade squamous intraepithelial lesion)” (Anal Condyloma: Is It A Significant Risk Lesion?) An alternative approach is to diagnose the lesion as condyloma acuminatum with a note in the report that there is no evidence of high grade dysplasia. Either approach is similar to that used in the uterine cervix and provides no commitment to the notion that anal condyloma is or is not a premalignant lesion.

Anal Condyloma: Is It A Significant Risk Lesion?

This is posed as a question, because there is as yet no clear answer with respect to perianal and anal canal lesions. The literature surrounding this issue is confusing due to a variety of methodologic problems that include nonuniformity in patient mix, absence of a consensus-driven gross and microscopic definition of condyloma, absence of an approved “gold standard” assay to identify and type HPV, and incomplete screening and/or clinical follow up. As indicated above, most anal skin condylomata harbor low risk HPV and appear to follow a benign clinical course. As a result, criteria used to diagnose a condyloma in this location are often based on subtle and very subjective cytoarchitectural features. However, foci of high grade dysplasia, in situ carcinoma, and occasionally, microscopic invasive carcinoma may be seen in what are otherwise clinically considered to be benign intra-anal or perianal condylomata. Such foci tend to increase in incidence with increasing size of the lesion, but may be seen in up to 15% of condylomata <5 cm in size. These latter lesions typically feature more robust cytoarchitectural features of human papilloma virus infection and have been found to harbor high-risk HPV. The observations that condylomas without dysplasia are associated with low-risk HPV, while condylomas with dysplasia are associated with high-risk HPV has led to the assumption that the pathologist can reproducibly distinguish condylomas that harbor low-risk HPV from those that harbor high-risk HPV on the basis of histology alone. This untested (and likely erroneous)
assumption has cast the pathologist in the role of deciding who gets closer surveillance and who doesn’t.

Therefore, until there is more information, all condylomata, especially those arising in immunocompromised patients or patients otherwise considered to be at risk (Table 3) should be carefully evaluated and the presence or absence of HSIL (or worse) should be so noted (Figure 1).

Histologically, a high grade squamous intraepithelial lesion (HSIL) is recognized on the basis of loss of orderly surface maturation, an increased nuclear-to-cytoplasm ratio, and increased numbers of mitotic figures, often in the upper one-third of the epithelium. If a two-tiered system is used in diagnosis, there is practically speaking no need to separate moderate dysplasia from severe dysplasia or carcinoma in situ. However, in instances where we suspect the clinician may be uncomfortable with the new terminology and the distinction between moderate dysplasia (AIN II) and carcinoma in situ (AIN III) is relatively straightforward, we will also include an AIN designation in parentheses. Tangential sectioning or a small biopsy may lead to overdiagnosis of HSIL and so difficult cases may require additional level sectioning or re-biopsy, if the entire lesion was not removed. As in the cervix, dysplasia should not be assessed in areas of thermal artifact. Piecemeal removal of condylomas may preclude pathologic evaluation of resection margins and this should be so stated in the report.

### Verrucous Carcinoma (Giant Condyloma of Buschke and Lowenstein)

We regard giant condyloma of Buschke and Lowenstein and verrucous carcinoma as the same entity; the latter term is preferred. As in other sites, the diagnosis of verrucous carcinoma can be quite difficult, particularly in a limited biopsy specimen. Clinically, the lesion presents as a verrucous, slow growing and recurring large condyloma that is generally resistant or poorly responsive to topical therapy – if identified early in the course of disease, the lesion may be cured by complete surgical excision. If discovered late, it may be extremely difficult to eradicate due to fistula and sinus involvement. In the perianal region, it may extensively involve perianal skin, perirectal tissue, and the ischiorectal fossa. When fully developed, verrucous carcinoma is a large, fungating lesion that is clinically indistinguishable from an invasive carcinoma. However, since the lesion tends to burrow along a broad front, a microscopic infiltrative pattern of tissue invasion cannot be identified, despite repeated biopsies. Verrucous carcinoma may lack the classic microscopic features of HPV infection, despite the documented presence of the virus, and bona fide dysplasia may not be present or may be muted and present only in the basal layers. In a limited biopsy, the pathologist may only see hyperkeratotic benign epithelium; although a definitive diagnosis cannot be made on such a sampling, correlation with the clinical presentation is required in order to suggest the appropriate differential diagnosis. Verrucous carcinoma is distinguished from ordinary condyloma by a thicker stratum corneum, more marked papillary proliferation, and the tendency to displace deep tissues, as well as large size. In general, the larger (>10 cm), more long-standing lesions are more likely to behave in a locally destructive fashion. Despite locally destructive behavior, metastasis does not occur.
Little information is available concerning HPV type(s) in anorectal verrucous carcinoma, but HPV types 6 and 11 have been disproportionately reported in the few cases of giant condylomas that have been studied. Why this lesion would be associated with a low-risk HPV viral type is unclear, but an alteration involving control genes for early transcription of HPV 6 has been reported in some cases.

**Flat Lesions**

In some respects, flat lesions are more difficult lesions to assess. These lesions are often invisible to the naked eye and require high resolution anoscopy with biopsy confirmation; they also have a greater frequency of involvement by high risk HPV types than condylomata. The distinction between anal LSIL and HSIL is based on the same criteria (with the same associated inaccuracies) used to distinguish cervical LSIL and HSIL.

Recent efforts to improve diagnosis have drawn on experiences with cervical lesions. As in cervical dysplasia, the pathway that leads to HPV-mediated transformation requires integration of the virus into the host genome, which typically only occurs with the high-risk types. Integration leads to inactivation of p53 and retinoblastoma tumor suppressor proteins through increased expression of the E6 and E7 oncoproteins. E7 overexpression leads to overexpression of p16ink4a, a tumor suppressor protein that has been shown to be overexpressed in cervical dysplasia and carcinoma, and also a subset of vulvar carcinomas. Although it has been claimed that p16ink4a overexpression distinguishes LSIL from HSIL, in our experience with cervical and anal canal lesions, p16ink4a overexpression is also seen in LSIL associated with high-risk HPV types. A similar result has also been observed with p16ink4a expression in anal cytology specimens. Therefore, in our opinion, p16ink4a is most useful for distinguishing hyperplastic and atypical metaplastic squamous epithelial processes from dysplastic processes and we continue to rely on standard histologic criteria to separate LSIL from HSIL. Used in this manner, the sensitivity and specificity of diffuse, strong p16ink4a expression in the detection of dysplastic cervical lesions is 82% and 100%, respectively. HPV in situ offers increased specificity, but the platforms that are currently commercially available are less sensitive in evaluation of atypical squamous lesions.

ProEx C (Becton Dickinson), another recently introduced immunohistochemical marker that targets topoisomerase II-alpha and minichromosome maintenance protein-2, two genes known to be overexpressed by high-risk HPV, may offer additional corroboration for the presence of dysplastic lesions harboring high-risk HPV when used in conjunction with p16ink4a.

**Invasive Squamous Cell Carcinoma**

The spectrum of invasive squamous cell carcinoma in the anorectum ranges from well differentiated, keratinizing tumors to poorly differentiated tumors that may require immunohistochemical markers to confirm epithelial differentiation on the one hand, and exclude melanocytic, hematolymphoid, and neuroendocrine differentiation on the other. In the older literature, the poorly differentiated carcinomas have been referred to as cloacogenic, basaloid, or...
transitional carcinomas, but such terminology should be avoided since 1) there are no diagnostic, prognostic, or therapeutic reasons to assign separate histogenetic groups to these tumors and 2) this terminology only creates confusion. The cloacogenic variety of squamous carcinoma tends to occur in the upper anal canal while the more traditional, keratinizing squamous carcinoma tends to occur in the lower anal canal, but even this distinction is arbitrary and lesions with overlapping features are not uncommon. Intra-anal invasive squamous carcinomas may exhibit an adenoid cystic pattern or contain small, mucinous microcysts (so-called mucoepidermoid or microcystic squamous carcinomas); it has been suggested that the latter variant may have a poor prognosis, but the number of reported cases is limited.

In contrast to the lack of predictive value of histologic type, the degree of differentiation should be reported, since there is some evidence to support increased risk of lymph node metastases, recurrence and death with the more poorly differentiated carcinomas.

A separate, but not uncommon diagnostic problem, is the assessment of early or microscopic invasion in a large or recurrent condyloma that has been removed either piecemeal or embedded in a poorly oriented manner. Since condylomas frequently involve anal ducts and exhibit a deep, undulating epithelial-stromal interface, often blurred by a chronic inflammatory cell infiltrate, the distinction between an irregular interface and tissue invasion may be difficult. We reserve the diagnosis of invasion for only those tumors that demonstrate unequivocal infiltration into underlying tissue, i.e., jagged or irregular downward extensions, individual cells or discohesive, small cell nests surrounded by inflamed and edematous stroma. Cases that exhibit highly suspicious, but not definitive foci of invasion are designated by the modifier “suspicious for invasion” or “focal invasion cannot be excluded”. This is particularly important for lesions that have been incompletely excised, since the possibility of an invasive lesion may alter subsequent treatment. Level sections of the problematic areas should always be performed, since in some cases, this may resolve the uncertainty.

Assessment of Anal Resection Specimens

Anal mucosal resection specimens should be processed in a similar manner to skin excision specimens for malignancy. Ideally, the surgeon either submits the specimen pinned to styrofoam or corkboard with notated margins or designates the orientation for the pathologist during an intraoperative consultation so that the specimen can be pinned prior to fixation. The deep (and peripheral) margins should also be inked to assist in orientation after processing. The specimen is then serially sectioned along the longitudinal (or main) axis at 2 to 3 mm intervals and the parallel serial sections are sequentially submitted for processing. The axis of sectioning may be modified depending on the specimen and the area(s) of particular concern. Margins should be read out as negative, positive (with the site specified – i.e., focally at lateral or deep margin, etc), or indefinite (or indeterminate) if lesional tissue is present in the closest en face margin (i.e., the first or last parallel section) or in an area of thermal artifact. When assessing margins, the pathologist should distinguish HSIL from invasive carcinoma, since a positive HSIL margin will often be managed differently than a positive invasive margin.
Serendipitous Condyloma/LSIL/HSIL in Hemorrhoidectomy Specimens

We routinely exam hemorrhoidectomy specimens. On occasion, unsuspected lesions such as LSIL/HSIL, melanoma, lymphoma, and even invasive squamous cell carcinoma are detected. In most instances, the lesions were completely unsuspected clinically and were removed simply because they did not respond to traditional less invasive therapies. While the presence of HSIL and microinvasive carcinoma, if completely excised, may warrant no additional therapy in immunocompetent patients, the presence of incompletely excised or more aggressive lesions should trigger further evaluation, especially in any patient who is immunocompromised or otherwise at risk for progression.

Differential Diagnosis

Squamous Hyperplasia

The clinical and histologic diagnosis of perianal condyloma is often based on architectural features that are not specific to HPV infection and therefore a large variety of skin lesions can mimic a condyloma acuminatum and in some instances, verrucous carcinoma in the anogenital region. Most of these mimics are well recognized by dermatologists and dermatopathologists, but they can pose potential pitfalls to the unwary anorectal surgeon or general surgical pathologist. Pseudoepitheliomatous hyperplasia secondary to granular cell tumor is widely recognized, but other entities, such as verruciform xanthoma, nodular amyloid, and lymphangiectasis can impart a prominent verruciform clinical appearance that can be misinterpreted as a condyloma on limited biopsy. In some instances, the clinical appearance is so convincing for a verrucous carcinoma or giant condyloma that we have received wide, complete excisions in our laboratory without a prior diagnostic biopsy. When a prior biopsy is performed, it may be inconclusive, i.e., showing some but not all the features of condyloma; rather than base a diagnosis of condyloma on architecture alone, the possibility of a reactive papillomatous or hyperplastic process should be mentioned in such cases, particularly if the clinical presentation is unusual.

Vulvovaginal and perianal fibroepithelial stromal polyps can be confused with condylomata acuminata due to the fronded architecture and occasional hyperplastic squamous epithelium. Rapid growth during pregnancy has been reported. Fibroepithelial polyps can usually be recognized by the presence of stellate and multinucleate stromal cells, but occasional cases occur in which the histologic features are incompletely expressed and misdiagnosis can occur. The use of proliferation markers (Ki-67/mib1) and various surrogate markers for high risk HPV – p16INK4a and recently, ProExC - may be useful in such cases (see Flat Lesions, above).

Reactive Atypia in Squamous Metaplasia

This distinction is usually not responsible for as many diagnostic problems as in the female genital tract, but may on occasion pose difficulties in areas of ulceration or marked inflammation. In many cases, it may not make a significant difference in patient management; in those in which a management decision hinges on the presence or absence of dysplasia (i.e., mapping studies for a planned excisional procedure), we use p16INK4a to confirm the presence
of dysplasia. We do not use Ki-67 for this purpose, since reactive and/or proliferative epithelium frequently demonstrates increased Ki-67 expression.

**Anogenital Paget’s Disease**

Anogenital extramammary Paget’s disease can be associated with a variety of hyperplastic epithelial changes, including simple hyperplasia, fibroepithelioma-like hyperplasia, and papillomatous hyperplasia. The unusual appearance of the Paget cell nuclei in the fibroepithelioma-like and papillomatous hyperplastic variants – dark, shrunken and pyknotic with perinuclear vacuolar clearing – may be mistaken for koilocytic atypia. In some cases, the hyperplastic process forms downward, irregular extensions simulating an invasive well differentiated squamous cell carcinoma. In other cases, the Paget cells are so florid that they mimic florid HSIL (Bowen’s disease). To make matters even more difficult, Paget’s disease and condyloma/HSIL/invasive squamous cell carcinoma may, on occasion, coexist – but when they do so they are usually sharply demarcated and not extensively intermingled. As long as the possibility of Paget’s disease is considered, this differential diagnostic problem is usually easily resolved. In all cases, CK7 will highlight the Paget cells, but not the hyperplastic, koilocytic, or malignant squamous epithelial cells.

**Basal Cell Carcinoma (skin)**

This differential diagnosis is only relevant to skin or perianal lesions and does not apply to intra-anal lesions. Genital and perianal basal cell carcinoma is rare, occurs in older patients (mean age, 65-70 years), and exhibits no clear sex predilection. It typically presents as a nodular or ulcerated nodular lesion, 0.5 to 5 cm in size, although infiltrative and superficial patterns are also seen. There is no association with HPV. However, patients with polypoid (acrochordon-like) basal cell carcinomas may have basal cell carcinomas elsewhere (basal cell nevus syndrome). Complete excision is curative.

**Small Cell Carcinoma**

Distinction between anal canal small cell carcinoma and poorly differentiated invasive squamous cell carcinoma can be difficult on small biopsy specimens, but treatment differs for the two processes. Demonstration of synaptophysin and chromogranin expression in small cell carcinoma on the one hand, and p63 expression in invasive squamous cell carcinoma on the other hand may be useful. The latter marker is also useful in excluding a poorly differentiated adenocarcinoma (either arising from an anal duct or more commonly, from a low seated rectal adenocarcinoma) in small biopsy samples.

If a problematic lesion expresses neuroendocrine markers, care should be taken to exclude a well differentiated neuroendocrine tumor with biopsy crush artifact – high Ki-67 expression will confirm a high proliferative rate in small cell carcinoma.

**Melanoma**

Anal melanomas typically arise near the dentate line, but advanced cases may appear in biopsies obtained more distally. The diagnosis is rarely suspected clinically. Paget’s disease and poorly differentiated carcinoma are the chief differential diagnostic considerations; standard immunohistochemistry will resolve almost all problematic cases.
Metastasis

Since primary squamous cell carcinoma is uncommon in the anal canal, the absence of an adjacent in situ lesion should prompt consideration of metastasis or extension from another undetected source, especially when a diagnosis of poorly differentiated carcinoma is being considered.

Summary

HPV exposure and infection are highly prevalent. Although the cervical model of pathogenesis can be applied to anal squamous cell carcinoma, screening programs for anal dysplasia pose unique problems. Neither the Pap smear nor the oncogenic HPV test has been subject to the same degree of evaluation in anal canal lesions as in the uterine cervix. Issues of positive predictive value, negative predictive value, cost-effectiveness, and overall efficacy of screening are still being answered. Screening programs are currently directed at high risk HIV seropositive patients, but there is evidence that other patients, particularly those with chronic immunosuppression, may also be at risk. The evaluation and management of the individual patient with suspected anal dysplasia is significantly aided by clear communication between the pathologist and the treating clinician.

Table 1. Proposed Common Terminology for Anatomic Location of Anal Lesions*

<table>
<thead>
<tr>
<th>Proposed terminology</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-anal (= anal canal)</td>
<td>Cannot be visualized or cannot be visualized in entirety when gentle traction is placed on the buttocks because it passes into the anal opening</td>
</tr>
<tr>
<td>Perianal (= anal margin)</td>
<td>Can be completely visualized with gentle traction on the buttocks and lies within 5 cm of the anal opening</td>
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<tr>
<td>Skin (= cutaneous)</td>
<td>Is &gt;5 cm from the anal opening visualized with gentle traction on the buttocks</td>
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<tr>
<td>Transformation zone</td>
<td>Region above dentate line of variable height where squamous metaplasia is found as a normal anatomic variant</td>
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*Modified from 48
Table 2. Proposed Common Terminology for Cytologic & Histologic Classification of Anal Lesions

<table>
<thead>
<tr>
<th>Proposed terminology</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Low-grade squamous intraepithelial lesion (LSIL)</td>
<td>AIN I, low-grade dysplasia</td>
</tr>
<tr>
<td>High-grade squamous intraepithelial lesion (HSIL)</td>
<td>AIN II, AIN III, CIS, Bowen’s disease, moderate dysplasia, severe dysplasia</td>
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AIN, anal intraepithelial lesion; CIS, carcinoma in situ

Table 3. Risk Factors for Anal Squamous Cell Carcinoma

<table>
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<tr>
<th>Risk Factor</th>
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<tbody>
<tr>
<td>History of lower genital tract neoplasia (esp. HSIL, cancer)*</td>
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<tr>
<td>HIV seropositivity</td>
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<tr>
<td>Low CD4 count</td>
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<td>Immunosuppression (esp. solid organ allograft, cyclosporine therapy)</td>
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<td>Heavy cigarette smoking</td>
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<tr>
<td>Anoreceptive intercourse</td>
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<tr>
<td>Infection with multiple HPV genotypes</td>
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<tr>
<td>Persistent high risk genotype HPV infection</td>
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<tr>
<td>Radiation</td>
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*Women with anal HPV infections are at high risk for HPV infection in the uterine cervix as well; screening for such lesions should also be carried out when anal lesions are identified.
Figure 1. Screening program for patients with anal condyloma (or otherwise at risk for anal dysplasia-carcinoma)*

*Modified from 31

References


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Diagnostic Problems in Anal Pathology

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Introduction: Anal Squamous Dysplasia & Carcinoma

• Epidemiology of anal squamous cell carcinoma
• HPV-mediated dysplasia-carcinoma sequence in anorectum & female genital tract: are there differences?
• Terminology: topographic & diagnostic
• Surrogate markers in detecting dysplasia: p16, Ki-67, ProEx C
• Screening of anal/rectal squamous intraepithelial lesions
Epidemiology of Anal Squamous Cell Carcinoma

- Anal squamous intraepithelial lesions & squamous cancer has been increasing in incidence in US since at least 1973 (predates HIV/AIDS)
- In 2006, approx 4,650 anal squamous cell carcinomas/year vs 11,150 cervical squamous cell carcinomas/year

Maggard et al Dis Colon Rectum 2003;46:1517-23
CA Cancer J Clin 2007;57:43-46
Epidemiology of Anal Squamous Cell Carcinoma

- Anal cancer diagnoses (2006): female to male: 1.4:1
- Anal cancer deaths (2006): female to male: 1.7:1
- Who gets screened? How should we screen?

CA Cancer J Clin 2007;57:43-46
Epidemiology of Anal Squamous Cell Carcinoma

Anal Canal (Transformation Zone)

- Women > men
- Most invasive at diagnosis

Peri-anal

- Men > women
- Assoc with recognizable precursor lesions (screening effect?)
- Most noninvasive at diagnosis
Anal Canal Squamous Cell Carcinoma

- Keratinizing
- Nonkeratinizing
- Cloacogenic
- Transitional
- Basaloid
- Adenoid cystic
- Mucoepidermoid or microcystic

No clear difference in prognosis or response to therapy
Anal Canal Squamous Cell Carcinoma
• Keratinizing
• Nonkeratinizing
• Cloacogenic
• Transitional
• Basaloid
• Adenoid cystic
• Mucoepidermoid or microcystic

All harbor high risk HPV
Risk Factors for Anal Squamous Cell Carcinoma

- History of HPV-mediated genital tract disease – esp. multifocal (esp. HSIL, cancer)
- HIV seropositivity
- Low CD4 count
- Other immunosuppression: solid organ transplant, ulcerative colitis
- Heavy cigarette smoking
Risk Factors for Anal Squamous Cell Carcinoma

- Frequent anoreceptive intercourse – *not required*
- Multiple HPV genotypes
- Persistent high risk HPV genotype infection
- Radiation
- Chronic irritation: persistent hemorrhoids
Cervical HSIL vs Anal HSIL

- High risk HPV types in cervix more varied & multiple: HPV 16, 18, 31, 35
- High risk HPV types in anal canal disproportionately HPV 16, with HPV 18 distant second
- Prevalence of high risk HPV in cervix high but most cleared – rates of progression/regression well defined
- Prevalence of high risk HPV in anal canal unknown – rates of progression/regression largely unknown
Anal SIL Regression & Progression Rates

- Anal LSIL may regress in up to 30% of cases, but regression uncommon in HIV
- Anal HSIL, when associated with systemic immunosuppression, may progress to invasive carcinoma in up to 50% of cases
- Progression rate probably depends on site: skin & perianal (low) vs anal canal (high)

Proposed Common Terminology

- **Intra-anal** = not visualized or incompletely visualized (anal canal)
- **Perianal** = completely visualized & within 5 cm of anal opening (anal margin)
- **Skin** = completely visualized & > 5 cm from anal opening (cutaneous)
- **Transformation zone** = region above dentate line where squamous metaplasia is found as normal variant

Common Terminology: Benefits

- Anatomic correlate of anal margin ill defined
- Skin condyloma differ from anal canal condyloma
- Terminology directly conveys whether lesion(s) are completely visualized – important in management

## Classification of Anal Lesions: Suggested Common Cytological and Histological Terminology

<table>
<thead>
<tr>
<th>Terminology</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade squamous intraepithelial lesion (LSIL)</td>
<td>AIN I, low grade dysplasia, mild dysplasia, condyloma</td>
</tr>
<tr>
<td>High grade squamous intraepithelial lesion (HSIL)</td>
<td>AIN II, AIN III, CIS, Bowen’s disease, moderate dysplasia, severe dysplasia</td>
</tr>
</tbody>
</table>

Common Terminology: Benefits

- Same terminology as cervix
- Same terminology as anal Pap smear - allows direct correlation
- Improved interobserver agreement in classification
- Avoids confusion of condyloma vs AIN I
- In line with low grade - high grade dysplasia classification elsewhere in GI tract
Reproducibility of 3-Tier AIN vs 2-Tier SIL in Biopsy Specimens

AIN
• Moderate at best (kappa=0.17-0.70)

SIL
• At least moderate (kappa=0.70-0.94)

*Interobserver agreement for HPV changes fair at best (kappa=0.4-0.53)*

Lytwyn et al Cancer 2005;103:1447-56
Colquhoun et al Dis Colon Rectum 2003;46:1332-8
## LSIL vs HSIL (The AIN I-II Problem)

<table>
<thead>
<tr>
<th>LSIL</th>
<th>HSIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Mitotic activity in lower 2/3 mucosa</td>
<td>• Mitotic activity in upper 1/3 mucosa</td>
</tr>
<tr>
<td>• Koilocytosis – often involves upper 1/3 mucosa</td>
<td>• High N:C ratio</td>
</tr>
<tr>
<td>• No atypical mitotic figures</td>
<td>• Loss of surface maturation</td>
</tr>
<tr>
<td></td>
<td>• Atypical mitotic figures</td>
</tr>
</tbody>
</table>
Tests For High Risk HPV

- PCR
- In situ hybridization
- Surrogate markers
PCR: The “Gold Standard”

• May be too sensitive – presence does not indicate integration
• Problems with contamination
• Many home brews
• Currently no FDA approved assay
• Expensive
HPV In Situ Hybridization

- Slide based, non-amplified
- Direct visualization of positive cells
- High specificity
- Can determine viral integration status: diffuse vs episomal pattern
- Use on cytology or histology specimens
- Benchmark: Multiple testing capabilities
Diffuse Nuclear Staining = Episomal Pattern
Punctate Nuclear Staining = Integrated Pattern
HPV In Situ Hybridization

- No large-scale studies validating use for triage
- Can be difficult to interpret
- False positives due to artifact
- Overstaining can cause punctate pattern to appear diffuse
- Variable, often low sensitivity with current commercially available technology
Surrogate Markers for High Risk Lesions

- Ki-67 (mib-1)
- p16$^{INK4a}$
- ProEx C
**Ki-67**

- Localized to basal layer in normal mucosa
- Present throughout mucosal layer in high grade SIL
- Increased % and upper (2/3) mucosal distribution considered abnormal
- Increased in inflammation, hyperplasia, some condyloma; dependent on orientation
Stimulation of Cell-Cycle Progression by High-Risk HPV

A Uninfected Epithelium

B High Risk HPV Infection

Clin. Sci. 2006:110;525-541
**p16**\textsuperscript{INK4a} **Immunohistochemistry**

"Gold Standard": HPV ISH

- Diffuse, strong staining of basal layer correlates with high risk HPV and HSIL
- Useful in problematic cases: condyloma vs HSIL, atypical squamous epithelium vs HSIL (*most* cases)
- Can be used in conjunction with Ki-67 (>50%)

### Role of p16\(^{INK4a}\) in Anal Lesions

<table>
<thead>
<tr>
<th></th>
<th>Band (&gt;90%)</th>
<th>Spotty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>(1 case)</td>
<td>8.1%</td>
</tr>
<tr>
<td>Condyloma</td>
<td>0%</td>
<td>8.3%</td>
</tr>
<tr>
<td>AIN I</td>
<td>21.4%</td>
<td>14.3%</td>
</tr>
<tr>
<td>AIN II</td>
<td>80%</td>
<td>12%</td>
</tr>
<tr>
<td>AIN III</td>
<td>87.5%</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

p16\textsuperscript{INK4a} Immunohistochemistry
“Gold Standard”: Consensus Diagnosis

- Diffuse/continuous p16 in > 1/3 of mucosa correlates with AIN II-III
- Ki-67 in > 25% in > 1/3 of mucosa correlates with AIN II-III
- p16 and Ki-67 so defined are highly correlated & complementary

p16\textsubscript{INK4a} Immunohistochemistry

“Gold Standard”: PCR

- p16 excellent surrogate marker for high risk HPV in HSIL
- Significant % LSIL & borderline LSIL-HSIL harbor high risk HPV not detected by p16
- HPV ISH is highly specific but insensitive in detecting high risk HPV

Balasubramaniam et al, # 48 Wednesday
Problems With p16

- Diffuse, strong (band) staining may be seen in some LSIL & rarely, normal squamous lesions
- Focal strong or spotty staining may be seen in some HSIL
- Highly dependent on antibody and titer
- Requires experience
When Is p16 Useful?

- Condyloma with possible HSIL
- AIN I – AIN II (esp flat lesions)
- Tangential sectioning or suboptimal orientation
- Transitional zone
- Inflammation with atypia
Condyloma (LSIL) vs HSIL: “The AIN I-II Problem”
Condyloma (LSIL) vs HSIL: “The AIN I-II Problem”

Condyloma (LSIL)  ? Focal HSIL (AIN II)
Condyloma (LSIL) vs HSIL: “The AIN I-II Problem”

Condyloma (LSIL)  ? Focal HSIL (AIN II)

Ki-67 (mib-1)
Transitional Mucosa vs HSIL

H&E
Condyloma, at right

p16\text{INK4a}
When is p16 Not Useful?

• May not detect high risk HPV in LSIL & LSIL-HSIL lesions
• May disappear in imiquimode treated high risk lesions
• May not help in verrucous carcinoma (condyloma of Buschke-Lowenstein) - HPV 6/11 often reported in these lesions
ProEx C Immunohistochemistry

- Targets 2 cell cycle proteins
- Topoisomerase II-alpha & minichromosome maintenance protein-2
- May be useful as adjunct to p16, Ki-67
- Characteristics not as well defined as p16
- Expensive

Shi et al, Hum Pathol 2007;38:1335-44
Discordant Results Among Various Tests: Some Possible Explanations

- Small lesion
- Focal HPV integration
- Focal dysplasia – many anal LSIL are associated with HSIL elsewhere in anal canal or on follow up
- "Gold standard": H&E vs PCR vs ISH vs high risk DNA (hybrid capture)
Who Gets Screened?

• HIV seropositive with high risk HPV - either LSIL (incl. condyloma) or HSIL
• Solid organ transplant with high risk HPV (?)
• Chronic immunosuppressive therapy with high risk HPV (?)
• History of lower genital tract HPV-related disease, esp. HSIL cancer (?)
Who Gets Screened: Some Considerations

- Prevalence of AIN in renal transplant patients approx 20% [relative risk for squamous cell carcinoma = 10]
- Prevalence of HPV infection in new transplant patients = 23%
- Prevalence of HPV infection in established renal transplant patients = 47%
- Screen or vaccine?

Who Gets Screened: Some Considerations

- Higher rate of cervical dysplasia in patients with UC (35.5% vs 14.6%)
- Higher rate of cervical dysplasia in patients with UC on chronic immunomodulators (44.6% vs 22%)
- Recent reports of anal-rectal HSIL & squamous cell carcinoma in UC
- Should these patients be screened?

Gastroenterology 2006;130:4, Suppl 2:A2, A3
Screening For Anal-Rectal Dysplasia

- High resolution anoscopy with biopsy & mapping – may not detect all flat lesions
- Anal Papanicolaou smear – doesn’t detect extent or distribution of disease
- Oncogenic HPV test – indicates presence of high risk HPV types (high risk probe)
Anal Condyloma: Screening Program

- Anorectal Cytology + Oncogenic HPV Test
  - High Risk Lesion
    - HSIL or (+) Oncogenic HPV-Test
      - High Resolution Anoscopy & Perianal Mapping with Biopsies
        - Definitive Therapy
  - Repeat Surveillance (3 months)
  - Low Risk Lesion
    - LSIL and (-) Oncogenic HPV-Test
      - Topical Therapy +/- Fulguration

Is There a Wider Role For High Risk HPV DNA Testing?

• Depends on prevalence of high risk HPV
• In cervix, where prevalence is high but virus is often cleared, only reflex testing for atypical Pap (ASCUS) is useful
• In anal canal, prevalence is not well defined and so role is not well defined
• Additional studies warranted as current screening may be insufficient for anal canal lesions