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REVIEW

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The pathology and molecular biology of anal intraepithelial neoplasia: comparisons with cervical and vulvar intraepithelial carcinoma

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Abstract *Background:* Anal intraepithelial neoplasia (AIN) is a well-described pathological precursor of invasive squamous cell carcinoma which has recently been detected with increasing frequency in immunocompromised patients, particularly those with seropositivity for human immunodeficiency virus (HIV). The epidemiology and natural history of this entity is somewhat unclear, since the overall prevalence in the HIV seronegative population is unknown. *Discussion:* There is a clear etiological association between AIN and high-risk human papillomavirus (HPV) subtype infection although there is great variability in HPV DNA detection of cytological and histological material in these patients. It appears that there is an antigen-specific hyporesponsiveness by cytotoxic lymphocytes against HPV peptide sequences or recombinant proteins encoded by oncogenic HPV subtypes in these patients, which is dependent upon the stage of their HIV-associated disease. Although the molecular biology of AIN and cervical or vulvar intraepithelial neoplasia are comparable, in AIN there is less significance of tumor suppressor gene mutations, proto-oncogenic growth factor activation, and genomic instability. *Conclusion:* Current concepts in the epidemiology and etiology of AIN are discussed, as well as its immunological response in the HIV-positive population, drawing parallels where possible between other HPV-related preinvasive disorders, and concluding with a suggested management protocol

Keywords Anal intraepithelial neoplasia · Cervical intraepithelial neoplasia · Homosexuality · Human papillomavirus · Vulvar intraepithelial neoplasia

Introduction

Anal intraepithelial neoplasia (AIN) is a precursor lesion for squamous cell carcinoma of the anus. Although most cases of AIN arise sporadically, it has been described in association with immunosuppression, particularly in patients who are positive for human immunodeficiency virus (HIV), or who have clinical acquired immunodeficiency syndrome. An etiological association between this lesion (and invasive carcinoma) and a sexually transmissible agent was first suggested by Cooper et al. [1] based upon epidemiological case clustering. This was followed by the demonstration of human papillomavirus (HPV) DNA in lesions initially by Southern blot and in situ hybridization of cytological and biopsy material, which identified the presence of oncogenic HPV gene sequences initially implicating HPV types 16 and 18 [2, 3, 4].

The exact natural history of this condition is unknown since the prevalence of HPV in the sexually active asymptomatic community at large is uncertain, and since many of these cutaneous and anal canal lesions appear to undergo spontaneous regression [5, 6]. In theory, its prevalence and behavior may alter with time in the HIV-

positive population with overall improvements in highly active antiretroviral therapy, enabling these patients to live longer with preinvasive lesions capable of progression [7, 8].

It seems appropriate to link the progression of AIN to that seen with the varying histological and cytological grades of both cervical intraepithelial neoplasia (CIN) and vulvar intraepithelial neoplasia (VIN). The clinical link in some patients between invasive cervical cancer and invasive anal cancer obviously suggests a shared cause [9, 10, 11, 12]. Here too cervical and anal preinvasive disease are associated, since a diagnosis of CIN carries a more than threefold increase in the risk of a simultaneous abnormal anal smear, both being diagnosed almost exclusively in the presence of HPV infection [13].

This review discusses the known epidemiology, etiology, immunology, pathology, and molecular biology of AIN, drawing speculative conclusions from the CIN/VIN literature regarding the clinical assessment of AIN, diagnosis, and follow-up in at-risk patient populations.

Epidemiology of AIN

The exact incidence and the predicted likelihood of progression of AIN towards invasive carcinoma in at-risk patients is at present unclear. Equally uncertain is the effect that treatment on squamous cell cancer precursors has on the incidence of invasive disease. In these populations the incidence of serious anal pathology (either high-grade AIN or occult invasive squamous cell carcinoma) is correlated with sexual lifestyle and the presence of HIV seropositivity [7]. In a follow-up of 37 homosexual men with stage IV HIV disease attending an outpatient HIV clinic Palefsky and colleagues [14] showed an increase in the proportion of patients with anal cytological abnormalities (from 27% to 65%), high-grade AIN (from 0% to 16%), or detectable HPV infection (from 60% to 89%) over a mean follow-up of 17 months. Similar findings have been noted in the differential prevalence of high-grade AIN between HIV-affected and non-HIV-affected women, although it is less pronounced when compared with sexually active men (26% vs. 8%, respectively), with HIV-positive women presenting abnormal cervical cytology at a 2.2-fold relative risk over the HIV-negative group having abnormal anal cytology detected at the same visit [15].

There are, however, few prospective studies assessing the progression of AIN in at-risk populations. Although the overall incidence of anal cancer among homosexual men is increasing, and the relative risk of anal cancer in HIV-positive homosexual men is 8–14 times that of HIV-negative men with similar demographics, the association between premonitory lesions such as anal condylomata or AIN and cancer is still unclear [16, 17, 18]. Epidemiologically significant changes in sexual practices may in part be responsible for these findings [19], with a clear association between anal cancer and early homosexual contact [20, 21, 22, 23]. The effect of active antiretroviral therapy on the incidence and progression of AIN in homosexual men is also unclear, given that such progression may take several years, although preliminary data suggest that most high-grade lesions do not regress during such therapy [24].

Etiology of AIN: association with HPV infection

Several lines of evidence point towards HPV infection as the prime causal agent in AIN. There is an epidemiological association between anal cancer and a prior history of anal (but not penile) warts with a close regional proximity of anal cancer to anal condylomata [25, 26]. Although intact HPV virus is difficult to detect in many anal condylomata, varying amounts of nonintegrated low-risk HPV DNA are found in up to 70% of cases using Southern blot hybridization [27, 28]. In a recent study assessing relapse rates of anal condylomata, recurrence was more often associated with HIV positivity and peripheral CD4 T lymphocyte depletion than with HPV persistence [29]. Equally, low-risk subtypes of HPV (principally HPV-6) have been detected in giant condyloma (Buschke-Loewenstein tumor) in the perianal region; a tumor which rarely undergoes malignant change [30, 31].

Variant HPV DNA subtypes have been detected in AIN biopsy material derived from both genders in almost all cases, with HPV RNA detection in many cases of squamous invasive anal cancer [32]. There is considerable variation in the detection rate of HPV DNA in invasive anal cancer, ranging from 50–95% and from our own data, nearly 90% are HPV subtype 16 with 7% HPV 18, 6% HPV 33, 1% HPV 31, and 2% untyped [22, 33]. There are actually few longitudinal studies which have shown a clear progression of AIN to invasive carcinoma in HPV-positive patients. These differences are in part reflected by the greater detection rates reported of HPV RNA, where the level of DNA is too low for detection, since DNA is more readily found in superficial cells undergoing DNA replication. This has led to different levels of DNA detection in basaloid-type tumors, where differentiation is poor and DNA replication is almost absent.

Of the more than 80 different subtypes of HPV which have been characterized at least 23 have been shown to infect the anogenital mucosa. The low-risk variants (including subtypes 6, 11, 42, 43, and 44) are more commonly identified in exophytic and flat condylomata and in low-grade dysplasia or occasionally in verrucous carcinoma. The high-risk subtypes (including 16, 18, 31, 33, 35, 39, 45, 50, 51, 53, 55, 56, 58, 59, and 68) are more often associated with high-grade dysplasia and in-

vasive carinoma. The reported incidence of HPV-subtype detection is somewhat technique dependent, with lower sensitivity achieved using simple in situ hybridization with biotinylated probes and better sensitivity obtained with polymerase chain reaction (PCR) technology or RNA-RNA in situ hybridization with radiolabeled probes [34, 35, 36, 37]. The latter type of riboprobe has several advantages over DNA probe analysis, since single-strandedness increases the sensitivity of viral genome sequence detection without self-annealing, and the thermal stability of RNA-RNA and RNA-DNA hybrids results in lower backgrounds, providing greater access to cytoplasmic RNA sites [38]. More recently the hybrid capture assay (HC-II; Digene, Silver Spring, Md., USA), which requires a higher viral load than PCR to detect HPV, has been used in scrape cytology and appears to be highly sensitive in the detection of multiple HPV subtypes, although there is some evidence to suggest that it is less sensitive in the detection of HPV persistence following surgical excision of areas of AIN or CIN [39]. It is a rapid and relatively simple liquid chemiluminescence hybridization system using a combination of RNA probes and DNA-RNA hybrid microtiter antibodies, having a sensitivity which is only slightly less than the more complex PCR assay [40, 41]. In general for routine use only HC and PCR analyses are sufficiently sensitive and specific for detection of HPV presence. The disadvantage of PCR technology is the relatively high percentage of false-positive tests because of the amplification of extraneous contaminants [42, 43]. Other HPV detection methods including RNA in situ and Southern blot hybridization are too complex for routine use and dot blot, filter in situ, and DNA in situ hybridization are at present too insensitive. Differences in HPV RNA detection with patient age have shown that HPV RNA transcripts are found less commonly in older patients, implying a greater role for HPV in early cancer development and viral elimination rather than in established malignancy. This finding is correlated with the increased prevalence of HPV-negative cervical cancers, which appear to have a worse overall prognosis [44].

The view that knowledge of the natural history of HPV-positive CIN and VIN could provide valuable lessons for the management and surveillance of AIN may be only partially true, since high-risk oncogenic HPV genotypes are detected in 80–90% of CIN grade III lesions as well as in a similar incidence of invasive cervical cancers and metastatic lymph nodes draining invasive disease [45, 46]. There is extensive literature reporting longitudinal studies of CIN and its progression to invasive disease in about 30% of patients over prolonged follow-up [47, 48, 49]. Moreover, up to 60% of cases with vulvar warts, VIN, and invasive vulvar cancer have HPV positivity with a high late development of invasive cervical carcinoma [50, 51]. Many of the early forms of AIN may not be comparable with CIN or VIN (grades I and II), however, since there is a high regression rate in AIN, with less than a 5% patient progression towards invasive malignancy [52, 53].

Immune responses and AIN

More studies assessing peripheral, intralesional and in situ lymphocyte function during AIN progression are needed to determine what immunological factors affect HPV elimination. Isotype-specific IgG responsiveness appears to be strongly associated with high-grade CIN which progresses to invasive carcinoma. In these patients systemic HPV-specific IgG and IgA responses are also correlated with oncogenic viral clearance [54, 55]. There is a high rate of preexisting IgG to high-risk HPV (16, 18, and 31) capsid protein in HIV-positive patients with supervening HPV detected, but although total circulating IgG and IgG1 levels are associated with higher grades of intraepithelial disease (perhaps marking cumulative lifetime HPV exposure and hence the likelihood of disease progression), these data should be viewed with caution as it may represent merely case clustering rather than a true serological differentiation between patients [56]. Despite the fact that there is no clear association between viral elimination and lesion progression in the immunosuppressed or normal populations, HPV persistence is correlated with CIN of higher overall grade [57]. In these patients the presence of HIV infection increases the likelihood of AIN development when HIV-positive patients are compared with non-HIV-infected persons with similar demographic characteristics [58], particularly in HIV-positive patients who have peripheral $CD4^+$ T lymphocyte depletion or a prior history of smoking, or who engage in receptive anal intercourse [26, 59, 60, 61]. This issue is complicated, however, since other studies have been unable to confirm the importance of HIV-associated immunosuppression in HPV-positive AIN, implying that mechanisms other than simple peripheral CD4 depletion are important in the early stages of this lesion [62]. This point is important since HIV seropositivity (particularly in the young sexually active population) may enhance HPV proliferation early in the course of HIV infection through mechanisms which are independent of CD4 T cell immunosuppression [63].

The issue of HPV persistence and its importance is also a complex one, and it appears that HPV positivity despite treatment is detectable in patients who are unable to mount an effective cytotoxic lymphocyte response to the open reading frame of the E6 oncoprotein derived from detectable high-risk HPV 16. This suggests an in vitro method for the determination of surgical response [64]. In these studies T cell clones derived from HIV-positive patients when incubated ex vivo either with HPV-specific sequences or recombinant transforming proteins (E6 and E7 proteins encoded by oncogenic HPV

genes) appear to show subtle antigen-specific proliferation defects. Preliminary evidence suggests that there is higher cytotoxic lymphocyte responsiveness to HPV 16 in the absence of intraepithelial neoplasia, where HPV E6 and E7 fusion proteins are used for the stimulation of peripheral lymphocytes in mixed culture with autologous B lymphoblastoid cell lines infected with a vaccinia virus expressing the E6 and E7 proteins [65].

In HIV-positive patients with HPV-related CIN of high grade, a combination of HIV and HPV positivity has been equated with peripheral conversion of Th1 cytokine-producing lymphocytes (those responsible for cell-mediated immunity, virus eradication, interleukin 2 and interferon-γ production) to a relatively suppressed Th2 phenotype (associated with antibody generation, interleukin 4 and interleukin 10 production). These changes are not detected in those patients with CIN and HIV alone, implying an HPV-specific shift in peripheral blood $CD4^+$ cytokine production in the HIV-infected population away from the cell-mediated responsiveness necessary for viral eradication [66]. This has been coupled with the finding that the degree of eosinophilic infiltrate (a hallmark of Th2 immune responsiveness) is inversely correlated with a worse survival in infiltrative cervical lesions [67]. This issue is complicated by the finding that progression of HIV itself is often associated with peripheral lymphocyte conversion from a Th1 to a Th2 phenotype [68]. Future understanding of the relationship between HPV and the host epithelium as well as the host inflammatory infiltrate may provide important data concerning the subtypes of epithelial cells within the transitional zone which are HPV resistant.

Those who can be rendered HPV-free following definitive therapy are less likely to develop recurrent cytological abnormalities in the cervix, although they may sometimes acquire other low-risk HPV subtypes [69, 70]. It remains to be seen whether the same phenomenon is observed in AIN. The anogenital surface (keratinized and nonkeratinized epithelium) is regarded then as a common field at risk on a background of relative immunosuppression with these patients at higher risk for other cutaneous, visceral, and soft-tissue cancers (including breast carcinoma) over time, showing a high detection rate of oncogenic HPV DNA in these other neoplasms [71, 72, 73]. Although the increase in anal cancer appears chronologically to predate the acquired immunodeficiency syndrome epidemic, there is a clear association between clinical immunodeficiency states, AIN, abnormal anal cytology, and invasive anal cancer [74]. Apart from rare syndromes clinically related to anal cancer, such as Wiskott-Aldrich syndrome and epidermodysplasia verruciformis (both of which have specific attenuation of cell-mediated immunity), much of the clinical detection of anal cancer and AIN has occurred in the transplant population receiving active immunosuppression [75, 76, 77, 78]. In this group AIN and anal cancers tend to occur in younger patients and have a more aggressive course, and patients experience a higher chemotherapy-related morbidity and mortality. Although peripheral natural killer cell reduction has been shown in immunosuppressed patients with combined HPV infection, and AIN and peripheral B cell depletion occurs in HPV infection alone, specific immunological deficiency during progressive disease been little studied in AIN [79].

The pathology of AIN

The pathology of established AIN has been well characterized. It shows morphological abnormalities such as loss of stratification and nuclear polarity, nuclear pleomorphism or hyperchromatism, and occasional koilocytosis. These changes in the anal epithelium occur in the absence of inflammatory infiltrates and without a break in the basement membrane, being described in accordance with the grading system employed for CIN [80]. In order to diagnose AIN a knowledge of the normal appearance of the specialized anal transitional zone epithelium is crucial [69, 81, 82, 83]. Although AIN is associated with all histological variants of anal squamous carcinoma as described in the WHO classification [33, 84], such categorization into large cell keratinizing and nonkeratinizing, basaloid, verrucous, and basal cell variants may have little clinical relevance, particularly since there is a poor reproducibility between experienced histopathologists in subtype agreement [70, 85]. Moreover, many tumors have mixed histological subtypes and small biopsy specimens may not be totally representative of the main tumor mass.

In AIN, HPV positivity appears to be correlated with higher localization within the anal canal [33]. This may imply that, as with invasive cervical cancer, HPV infection is more critical for the development of anal canal cancer, whereas perianal cancer (and its precursors) develops more in parallel with other anogenital skin cancer types such as vulval and penile cancer, where there is an inverse relationship between keratinization and HPV status [86, 87, 88]. Differences in HPV positivity in various reports may have much to do with inadvertent misclassification of anal canal and perianal cutaneous lesions (in accordance with the definition of the anal transitional zone), as with misinterpretation of the significance of keratinization and basaloid differentiation [89, 90].

In AIN I the nuclear abnormalities are restricted to the lower one-third of the epithelium, and in the higher grades epithelial irregularity involves two-thirds (AIN II) or all (AIN III) of the epithelial thickness. This type of lesion was also first described by Fenger et al. [91] in the bordering epithelium of abdominoperineal resection specimens, where AIN was detected by systematic sectioning in 13 of 16 anal canals harboring anal squamous carcinoma, with no AIN found in other resected anal tu-

mors. In interpretation of biopsy material it should be remembered that epithelial keratinization of the anoderm provides many anucleate squames, and that dyskaryosis from this area should be regarded as abnormal only if it is associated with nuclear irregularity, abnormal chromatin distribution pattern, and an abnormal nuclear/cytoplasmic ratio [92]. The detection of low oncogenic risk HPV subtypes in external anal condylomata is usually made in the absence of cellular atypia, whereas dysplasia occurs in up to one-third of internal anal condylomata, where there is a higher incidence of associated AIN of high grade [93]. HPV positivity is more common if the anal tumors have basaloid features, adjacent AIN, poor or absent keratinization, and a predominance either of small or medium sized cells [33]. Recently, high-grade AIN has been shown to display greater angiogenesis and, reciprocally, less apoptosis than with normal anoderm. This is a feature in similarity with higher grades of CIN, suggesting that it is a significant premalignant event [94, 95, 96]. These reciprocal changes show an overall proliferative imbalance in high-grade lesions which has also been observed in mouse models of squamous epithelial carcinogenesis [97, 98].

The molecular biology of AIN

There is little available information concerning the molecular biology of AIN. It is hoped that a better understanding of this aspect of AIN in different patient subgroups will assist in defining cases which are at greater risk of progression. Cellular oncogenes and proto-oncogenes expressed in intraepithelial lesions and invasive cancers are homologous to retroviral genes, with potential cellular transforming capacity. In advanced CIN oncogenic HPV subtypes which encode for transforming proteins capable of complexing with the protein products of local tumor suppressor genes have been identified by in situ hybridization. These include the E6 protein product of HPV types 16 and 18 (see above) which binds to wild-type p53 and enhances its degradation and the E7 protein which binds to the retinoblastoma protein Rb [99, 100]. In CIN expression of these transforming proteins affects immortalization of cervical cancer cell lines in vitro, with wild-type p53 expression suppressing the immortalizing capacity induced by HPV 16 E7 [101]. Recent studies have shown in invasive cervical tumors and cervical tumor cell lines an inverse relationship between HPV sequence expression and p53 mutation, implying an association between oncogenic HPV encoded proteins and the proteins of tumor suppression genes in the pathogenesis of anogenital neoplasms [102, 103].

Extrapolation of this type of data to AIN should, however, be viewed with caution since patterns of mutant p53 expression are similar in both AIN III and invasive anal cancer with little expression in early grades of AIN or condylomata [104]. Recent comparative genomic hybridization analyses of formalin-fixed and paraffin-embedded anal squamous cancers have shown nonrandom copy number increases in chromosomes 17 and 19 and the chromosome arm 3q, with consistent losses mapped to chromosome arms 4p, 11q, 13q, and 18q [105]. Although this has been previously reported as the most common type of alteration in cervical cancer (providing further evidence of a common molecular pathway for HPV-associated anogenital neoplasia), it should be remembered that chromosome 19 changes (which depend upon Alu-repeat sequences, and which result in additional pickups with comparative genomic hybridization techniques) may be nonspecific [106]. As a measure of this nonspecificity, there does not in these reports seem to be any association between chromosomal repeats, tumor ploidy, and HPV infection.

Although genomic instability has been implicated in intraepithelial progression, there are marked differences between CIN and VIN. In CIN, although there is an association between low-grade lesions and genomic instability that may affect the clonal evolution within intraepithelial lesions [107], no correlation has been found between HPV status and the presence of mismatch repair gene expression [108]. This is unlike the changes in skin surrounding vulvar carcinomas and VIN lesions, where a high degree of chromosomal instability has been detected [109]. The loss of heterozygosity and microsatellite instability, however, in vulval skin appears to be associated more with HIV status than with HPV persistence [110]. Most of the reported microsatellite alterations arise in noncoding repeat regions of the genome (so-called minisatellites) and are not part of the direct path of tumorigenesis [111, 112]. Preliminary data suggest that genomic instability is not important in the progression of AIN, although more work in this area is needed.

Although other proto-oncogenic growth factors are likely to be necessary for the development of invasive anogenital malignancy, there is no current evidence for their involvement in AIN. The c-*myc* oncogene, for example, which has been shown to be overexpressed in CIN as well as integrated with papillomavirus sequences close to the c-*myc* locus in some cervical carcinoma cell lines [113], does not appear to play an important role in AIN or in the progression of invasive anal malignancy [114]. This is despite in vitro studies showing that c-*myc* expression is often greater in HPV 16-transformed cell lines resulting in reduced in vitro growth requirements, and that HPV-immortalized keratinocytes overexpressing c-*myc* oncogene sequences result in differentiation resistance and more rapid growth than parent lines lacking the amplified c-*myc* sequences [115]. Similar studies in anal cancer assessing the presence of the K-*ras* oncogene [116] and cyclin D_1 (a cell-cycle regulator and proto-oncogene commonly found in esophageal squamous carcinomas) have failed to show amplification of either

marker or correlation with HPV oncogene expression [117, 118].

The appearance of anal cancer and its potential precursors thus appears to be considerably different from the phenotypic/genotypic correlations in cervical or vulvar carcinogenesis. In adenocarcinomas such as colonic cancer the acquisition of multiple chromosomal aberrations is accompanied by high-level p53 expression, as just one of the molecular changes when premalignant disease becomes invasive [119]. In squamous cell cancers such as cervical carcinoma, however, chromosomal variation acquired during the transition from high-grade dysplasia to invasive cancer appears to be unrelated to mutant p53 expression, although it is effectively HPV dependent [120]. The phenotypic changes during the transition of AIN to invasive cancer are strikingly similar to those observed in cervical cancer, with undetectable p53 (although with less HPV dependency); however, these are unaccompanied by genotypic variations. More studies are required of the molecular biology of prospectively assessed AIN through progression (or regression) in HPV-positive and negative cases in order to understand the importance of growth factors and tumor suppressor genes in defining pathological risk factors.

Diagnosis, follow-up, and treatment of AIN

Anoscopy, anal cytology, and anal colposcopy all aid in the diagnosis and follow-up of AIN in at-risk groups. Anal colposcopy is a specialized procedure which should be performed by a trained colposcopist using 5% acetic acid epithelial painting in accordance with the techniques used for cervical colposcopy [83, 121]. Variations in the density of whitening of dysplastic epithelium, aberrant surface vascular pattern, mucosal cobble-stoning, and mosaicism all guide the interpretation of biopsy material. Abnormal macroscopic appearances include eczematoid, papillomatous, papular, and irregular plaquelike lesions. Abnormal areas may be raised, scaly, white, erythematous, pigmented, or fissured. Induration or ulceration may indicate the presence of invasion. Because of this variability in macroscopic appearance some have recommended that all tissue removed from the region (including hemorrhoidal tissue) should be examined histologically, as the underlying diagnosis of AIN is often unsuspected.

Giving support to the hypothesis that infection with certain strains of HPV predisposes to the development of AIN is the observation that unsuspected or subclinical AIN may also be found in excised perianal condylomata. The rate of this subclinical AIN is significantly higher in those patients who are HIV seropositive. Colquhoun et al. [122] at the Cleveland Clinic Florida have recently examined the rate of this subclinical AIN found in condylomata excised between 1988 and 2000, where 70 patients underwent 97 excisions. The overall incidence of AIN was 31% (30/97) with a median AIN grade of II. Of the HIV seropositive patients 51% were found to have AIN, which was significantly higher then the 17% rate of AIN detected in the HIV seronegative patients. These rates correspond to those independently found by Carter et al. [123] at St Mark's Hospital and Metcalf et al. [124] at the University of Iowa, who reported a 35% and 28% subclinical rate of AIN in all condyloma specimens excised, respectively, with rates of 40% and 60% in their HIV seropositive patients.

Anal cytology should be assessed by a cytopathologist with experience in the assessment of Papanicolaou smears. The adequacy of smears is controversial, particularly when there is coincident fecal contamination, and is based upon the cellularity and the presence of squamous metaplasia. HPV infection is more likely if there is recognizable koilocytosis, anucleate squamous cells (as derived from the anal canal), or multinucleated squamous cells. In general, low-grade AIN may be underreported, as the pathologist may not distinguish between dyskaryosis and HPV infection, particularly away from the anal transitional zone, thus distorting the reported incidence of AIN and the impression regarding its natural history [125, 126, 127].

All of these factors make it difficult to be dogmatic concerning both the treatment and follow-up of AIN particularly in patients whose life expectancy is already compromised by HIV-related immunosuppression. Operative approaches for AIN of high grade include wide local excision with primary skin closure [128], split-skin grafting [129], or advancement flap (V-Y, S, C, or house-style) cutaneous anoplasty [130, 131]. It should be remembered that extensive cutaneous anal surgery in such patients may have considerable morbidity [132]. Simple local therapies such as cautery fulguration [133], CO_2 laser ablation [134], and cryotherapy should be considered for small lesions or in local recurrences around the edge of previous resections. The reported incidence of recurrence (or persistence) of high-grade AIN following wide local excision (however it is reconstructed), is between 10% and 25% even when there is efficient preoperative epithelial mapping [135]. Early nonrandomized studies have suggested that less radical therapies such as laser vaporization are associated with a higher local recurrence rate, also creating difficulties for the pathologist in the assessment of the adequacy of resection margins. Conservative (nonoperative) approaches for lower grade lesions and in those in which there is either widespread perianal and anorectal disease or multifocal recurrence associated with HPV persistence include topical 5-fluorouracil therapy [136], photodynamic therapy [137], and argon beam laser therapy [138].

The involvement by AIN II or AIN III of deeper areas of the perianal skin and its appendages in up to 50% of cases and sweat glands in 25% of biopsy specimens may not to able to be targeted macroscopically by anal colpos-

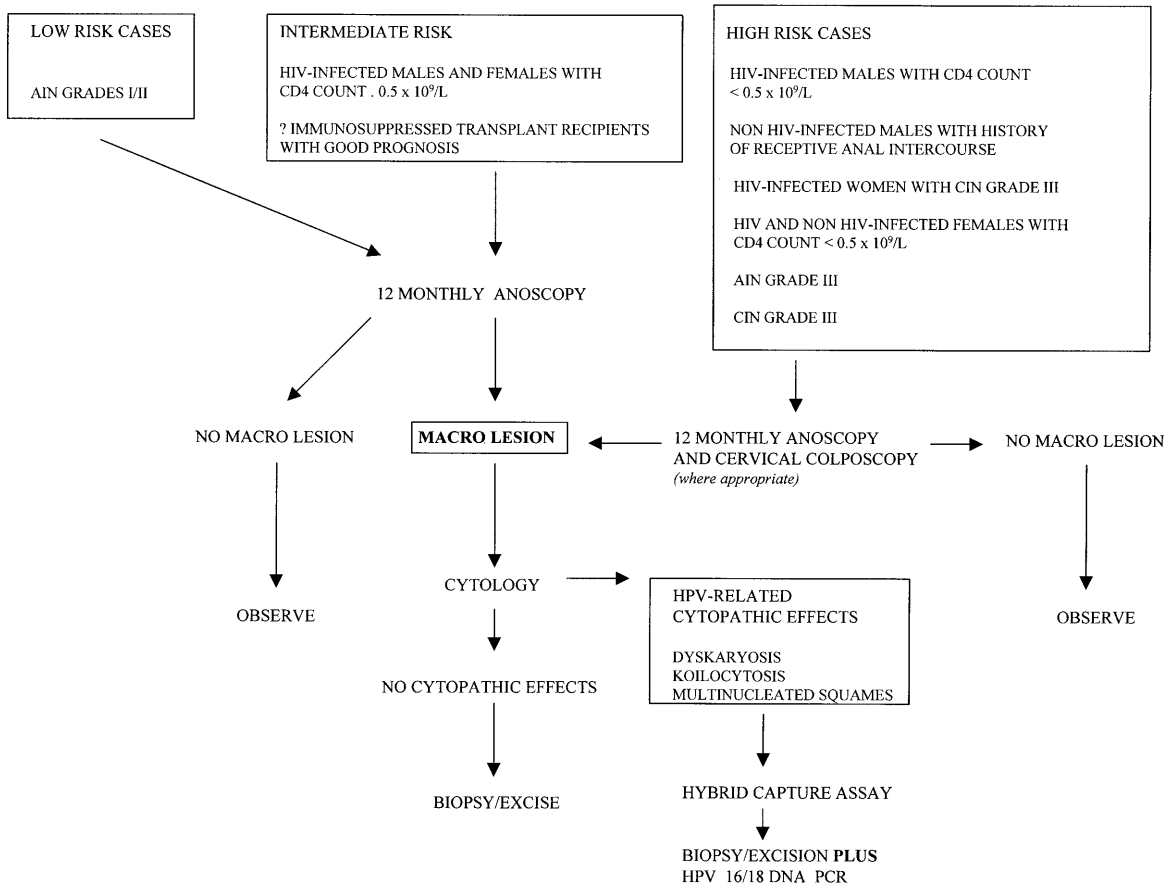

Fig. 1 Suggested follow-up of AIN patient cohorts

copy and may imply that ablative techniques such as cryosurgery, laser vaporization, and electrocautery are inadequate forms of treatment [52]. Since HPV is epitheliotrophic, the entire anogenital surface must be assessed despite the fact that the importance of viral elimination from or reinfection of at risk epithelium is unknown. As it may not be possible to eliminate the virus and the overall incidence of associated genital neoplasia, and since the true incidence of invasive disease and underlying visceral malignancy is low, a policy of observation even in high-grade AIN (unless there is an ulcerated or elevated macroscopic lesion) may be the most sensible course [139]. Both AIN I and AIN II should be treated expectantly since their natural history is unclear, with attendant spontaneous regression on occasion, as well as their greater association with underlying HPV subtypes 6 and 11, both of which are believed to be less oncogenic than HPV 16.

Although long-term follow-up is needed, there are no clear recommended guidelines. Random biopsy sampling at the edge of skin grafts and flaps is suggested with extended follow-up since recurrence may occur more than 5 years following primary surgery [140]. Anal cytology permits screening to be employed in a similar way to that used in cervical cancer. The cytological grading of anal Papanicolaou smears, however, is correlated relatively poorly with the histological grading of confirmed lesions of the anal skin and anal canal, and consequently although departmental experience of cytology in this disease is favored, biopsy of suspicious areas is essential [141, 142]. It is recommended that patients who are HIV-infected undergo 12-monthly assessments with anoscopy for AIN I and AIN II whereas those with AIN III should undergo regular biopsy mapping of grossly irregular (and particularly ulcerated) areas detected at anoscopy. Suggested screening groups for regular anoscopy and cytology might include non-HIV-infected men with a history of receptive anal intercourse, HIV-infected men with low peripheral CD4 counts, HIV and non-HIV-infected women with high-grade CIN, and HIV-infected women with CD4 depletion. A suggested algorithm for AIN follow-up, diagnosis, and treatment in specific patient cohorts is shown in Fig. 1.

There is evidence in preliminary economic studies that screening programs for HIV-positive homosexual and bisexual men using anal cytology alone provides the best quality adjusted life expectancy benefits at a cost which is comparable to that of other accepted clinical preventive interventions during all stages of HIV disease [143]. If assessment of low-grade CIN is anything to go by regarding

the follow-up of AIN, the loss to follow-up of patients with low-grade lesions afforded by simple cytology may suggest an increasing clinical and social importance for simplified testing designed to detect the presence of HPV, particularly through hybrid capture analysis [144, 145].

Prospective comparisons of cytology and histopathology for each unit are necessary to vindicate this approach [146]. This economic issue is complicated by a higher prevalence of significant anal disease in more advanced HIV-related illness and sensitivity analyses of various screening modalities will define the recommended frequency for their use based on the stage of HIV disease. The critical factors which affect the cost effectiveness of screening in such patients are not epidemiologically well defined since they rely on a better understanding of the natural history of AIN (namely its pathological progression or regression with time), a knowledge of the true effectiveness of therapy for AIN (or the effect of antiretroviral treatment on AIN), and the potential survival benefit for patients in detecting AIN at an earlier stage. All of these issues are poorly understood at this time, as are the clinical and economic implications of antiretroviral resistance, patient nonadherence to therapy, and the ability accurately to estimate disease prevalence, where some patients are unable to be fully evaluated by screening tests. All of these factors will influence cost-effectiveness estimates for screening programs [147, 148].

Conclusion

The increase in the incidence of anal cancer reported in the United States does not appear to be evident in the United Kingdom. To reduce the mortality of invasive anal cancer it is essential that high-risk individuals are identified. Prolonged severe immunodeficiency provides the required environment for the development of anogenital neoplasms caused by oncogenic human papillomaviruses, and it is likely that anal cancer and AIN will become a more common problem in HIV-positive patients as a result of improved antiretroviral and antimicrobial therapies.

Multi-institutional, cooperative prospective, epidemiological studies are needed to establish which subgroups undergo AIN progression and regression. Immunological investigations are required to delineate what immunocyte variations are associated with progressive AIN. These should assess T cell clonal responsiveness to defined recombinant HPV sequences and HPV-related transforming peptides using autologous targets transfected with HPV genomes. An improved understanding of molecular biological markers which control for and reflect epithelial cell resistance to HPV infection (and reinfection) in the immunocompromised host will assist in the construction of sensible algorithms for patient follow-up and definitive treatment.

References

1. Cooper HS, Patchevsky AS, Marks G (1979) Cloacogenic carcinoma of the anorectum in homosexual men. Dis Colon Rectum 22:557–558
2. Duggan MA, Boras VF, Inoue M, McGregor SE, Robertson DI (1989) Human papillomavirus DNA determination of anal condylomata, dysplasias and squamous carcinoma with in-situ hybridization. Am J Clin Pathol 92:16–21
3. Palefsky JM, Gonzales J, Greenblatt RM, Ahn DK, Hollander H (1990) Anal intraepithelial neoplasia and anal papillomavirus infection among homosexual males with group IV HIV disease. JAMA 263:2911–2916
4. Palefsky LM, Holly EA, Gonzales J, Berline J, Ahn DK, Greenspan JS (1991) Detection of human papillomavirus DNA in anal intraepithelial neoplasia and anal cancer. Cancer Res 51:1014–1019
5. Syrjanen K, Hakama M, Saarikoski S, Vayrynen M, Yliskoski M, Syrjanen S, Kataja V, Castren O (1990) Prevalence, incidence and estimated life-time risk of cervical human papillomavirus infections in a non-selected Finnish female population. Sex Transm Dis 17:15–19
6. Syrjanen KJ (1996) Spontaneous evolution of intraepithelial lesions according to the grade and type of the implicated human papillomavirus (HPV). Eur J Obstet Gynaecol Reprod Biol 65:45–53
7. Palefsky JM, Holly EA, Gonzales J, Lamborn K, Hollander H (1992) Natural history of anal cytologic abnormalities and papillomavirus infection among homosexual men with group IV HIV disease. J Acquir Immune Defic Syndr Hum Retrovirol 5:1258–1265
8. Hever D, Desmond S, Volberding P, Kahn J (1989) Changing prevalence of malignancies in men at San Francisco General Hospital during the HIV epidemic. Fifth International Conference on AIDS, Montreal. Ottawa International Development Research Centre: WB019
9. Rabkin CS, Biggar RJ, Melbye M, Curtis RE (1992) Second primary cancers following anal and cervical carcinoma: evidence of shared etiologic factors. Am J Epidemiol 136:54–58
10. Scholefield JH, Hickson WG, Smith JH, Rogers K, Sharp F (1992) Anal intraepithelial neoplasia: part of a multifocal disease process. Lancet 340:1271–1273
11. Melbye M, Cote TR, Kessler L, Gail M, Biggar RJ, the AIDS/Cancer Working Group (1994) High incidence of anal cancer among AIDS patients. Lancet 343:636–639
12. Williams AB, Darragh TM, Vranizan K, Ochia C, Moss AR, Palefsky JM (1994) Anal and cervical human papillomavirus infection and risk of anal and cervical epithelial abnormalities in human immunodeficiency virus-infected women. Obstet Gynecol 83:205–211

13. Goldstone SE, Winkler B, Ufford LJ, Alt E, Palefsky JM (2001) High prevalence of anal squamous intraepithelial lesions and squamous-cell carcinoma in men who have sex with men as seen in a surgical practice. Dis Colon Rectum 44:690–698
14. Palefsky JM, Holly EA, Ralston ML, Jay N, Berry JM, Darragh TM (1998) High incidence of anal high-grade squamous intra-epithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. AIDS 12:495–503
15. Holly EA, Ralston ML, Darragh TM, Greenblatt RM, Jay N, Palefsky JM (2001) Prevalence and risk factors for anal squamous intraepithelial lesions in women. J Natl Cancer Inst 93:843–849
16. Frazer IH, Medley G, Crapper RM, Brown TC, Mackay IR (1986) Association between anorectal dysplasia, human papillomavirus and human immunodeficiency virus in homosexual men. Lancet II:657–660
17. Wexner SD, Milsom JW, Dailey TH (1987) The demographics of anal cancers are changing. Identification of a high risk population. Dis Colon Rectum 30:942–946
18. Frisch M, Melbye M, Moller H (1993) Trends in incidence of anal cancer in Denmark. BMJ 306:419–422
19. Melbye M, Biggar RJ (1992) Interactions between persons at risk for AIDS and the general population in Denmark. Am J Epidemiol 135:593–602
20. Judson FN, Penley KA, Robinson MF, Smith JK (1980) Comparative prevalence rates of sexually transmitted diseases in heterosexual and homosexual men. Am J Epidemiol 112:836–843
21. Daling JR, Weiss NS, Klopfenstein LL, Cochrane LE, Chow WH, Daifuku R (1982) Correlates of homosexual behaviour and the incidence of anal cancer. JAMA 247:1988–1990
22. Frisch M, Glimelius B, Van den Brule AJC, Wohlfahrt J, Meijer CJLM, Walboomers JMM, Goldman S, Svensson C, Adami H-O, Melbye M (1997) Sexually transmitted infection as a cause of anal cancer. N Engl J Med 337:1350–1358
23. Daling JR, Weiss NS, Hislop TG, Maden C, Coates RJ, Sherman KJ, Ashley RL, Beagrie M, Ryan JA, Corey I (1987) Sexual practices, sexually transmitted diseases and the incidence of anal cancer. N Engl J Med 317:973–977
24. Palefsky JM (2000) Anal squamous intraepithelial lesions in human immunodeficiency virus-positive men and women. Semin Oncol 27:471–479
25. Taxy JB, Gupta PK, Gupta JW, Shah KV (1989) Anal cancer. Microscopic condyloma and tissue demonstration of human papillomavirus capsid antigen and viral DNA. Arch Pathol Lab Med 113:1127–1131
26. Holly EA, Whittemore AS, Aston DA, Ahn DK, Nickoloff BJ, Kristiansen JJ (1989) Anal cancer incidence: genital warts, anal fissure or fistula, haemorrhoids and smoking. J Natl Cancer Inst 81:1726–1731
27. Krzyzek RA, Watts SL, Anderson DL, Faras AJ, Pass F (1980) Anogenital warts contain several distinct species of human papillomavirus. J Virol 36:236–244
28. Strand A, Andersson S, Zehbe I, Wilander E (1999) HPV prevalence in anal warts tested with the MY09/MY11 SHARP signal system. Acta Derm Venereol 79:226–229
29. Sobhani I, Vuagnat A, Walker F, Vissuzaine C, Mirin B, Hervatin F, Marmuse JP, Cremieux AC, Carbon C, Henin D, Lehy T, Mignon M (2001) Prevalence of high-grade dysplasia and cancer in the anal canal in human papillomavirus-infected individuals. Gastroenterology 120:857–866
30. Bogomoletz WV, Potet F, Molas G (1985) Condylomata acuminata, giant condyloma acuminatum (Buschke-Loewenstein tumour) and verrucous squamous carcinoma of the perianal and anorectal region: a continuous precancerous spectrum? Histopathology 9:155–169
31. Kato N, Ueno H, Tanaka H, Nishikawa T (1993) Human papillomavirus type 6 associated Buschke-Loewenstein tumor (giant condyloma acuminatum). J Dermatol 20:773–778
32. Higgins GD, Uzelin DM, Phillips GE, Pieterse AS, Burrell CJ (1991) Differing characteristics of human papillomavirus RNA-positive and RNA-negative anal carcinomas. Cancer 68:561–567
33. Frisch M, Fenger C, van den Brule AJ, Sorensen P, Meijer CJ, Walboomers JM, Adami HO, Melbye M, Glimelius B (1999) Variants of squamous cell carcinoma of the anal canal and perianal skin and their relation to human papillomaviruses. Cancer Res 59:753–757
34. Gal AA, Saul SH, Stoler MH (1989) In situ hybridization analysis of human papillomavirus in anal squamous cell carcinoma. Mod Pathol 2:439–443
35. Brule AJ van den, Snijders PJ, Gordijn RL, Bleker OP, Meijer CJ, Walboomers JM (1990) General primer-mediated polymerase chain reaction permits the detection of sequenced and still unsequenced human papillomavirus genotypes in cervical scrapes and carcinomas. Int J Cancer 45:644–649
36. Soler C, Allivert P, Chardonnet Y, Cros P, Mandrand B, Thivolet J (1991) Detection of human papillomavirus types 6:11:16 and 18 in mucosal and cutaneous lesions by the multiplex polymerase chain reaction. J Virol Methods 35:143–157
37. Zaki SR, Judd R, Coffield LM, Greer P, Rolston F, Evatt BL (1992) Human papillomavirus infection and anal carcinoma. Retrospective analysis by in-situ hybridization and the polymerase chain reaction. Am J Pathol 140:1345–1355
38. Cox KH, DeLeon DV, Angerer L, Angerer RC (1984) Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. Dev Biol 101:485–502
39. Clavel C, Masure M, Levert M, Putaud I, Mangeonjean C, Lorenzato M, Nazeyrollas P, Gabriel R, Queeux C, Birembaut P (2000) Human papillomavirus detection by the hybrid capture II assay: a reliable test to select women with normal cervical smears at risk for developing cervical lesions. Diagn Mol Pathol 9:145–150
40. Cope JU, Hildesheim A, Schiffman MH, Manos MM, Lörincz AT, Burk RD, Glass AG, Greer C, Buckland J, Helgesen K, Scott DR, Sherman ME, Kurman RJ, Liaw K-L (1997) Comparison of hybrid capture tube test and PCR for detection of human papillomavirus DNA in cervical specimens. J Clin Microbiol 35:2262–2265
41. Fait G, Kupferminc MJ, Danielo Y, Geva E, Ron IG, Lessing JB, Bar-Am A (2000) Contribution of human papillomavirus testing by hybrid capture in the triage of women with repeated abnormal pap smears before colposcopy referral. Gynecol Oncol 79:177–180
42. Wieland U, Meyer T, Stockfleth E, Klaes R, von Knebel, Doeberitz M, Schneider A, Dürst M (1999) Interlaboratory agreement of different human papillomavirus DNA detection and typing assays in cervical scrapes. Int J Cancer 81:666–668
43. Milde-Langosch K, Riethdorf S, Löning T (2000) Association of human papillomavirus infection with carcinoma of the cervix uteri and its precursor lesions: theoretical and practical implications. Virchows Arch 437:227–233
44. Riou G, Favre M, Jeannel D, Bourhis J, Le Doussal V, Orth G (1990) Association between poor prognosis in early-stage invasive cervical carcinomas and non detection of HPV DNA. Lancet 335:1171–1174
45. Scholefield JH, Sonnex C, Talbot IC, Palmer JG, Whatrup C, Mindel A (1989) Anal and cervical intraepithelial neoplasia: possible parallel. Lancet II:765–759

46. Lancaster WD, Castellano C, Santos, Delgado G, Kurman RJ, Jenson AB (1986) Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites. Am J Obstet Gynecol 154:115–119
47. Ostor AG (1993) Natural history of cervical intraepithelial neoplasia – a critical review. Int J Gynecol Pathol 12:186–192
48. Kjaer SK, van den Brule AJ, Brock JE, Poll PA, Engholm G, Sherman ME, Walboomers JM, Meijer CJ (1996) Human papillomavirus – the most significant risk determinant of cervical intraepithelial neoplasia. Int J Cancer 66:601–606
49. Carozzi F, Ronco G, Confortini M, Noferini D, Maddau C, Ciatto S, Segnan N (2000) Prediction of high-grade cervical intraepithelial neoplasia in cytologically normal women by human papillomavirus testing. Br J Cancer 83:1462–1467
50. Buckley C, Butler E, Fox H (1984) Vulvar intra-epithelial neoplasia and micro-invasive carcinoma of the vulva. J Clin Pathol 37:1201–1211
51. Basta A, Adamek K, Pitynski K (1999) Intraepithelial neoplasia and early stage vulvar cancer. Epidemiological, clinical and virological observations. Eur J Gynaecol Oncol 20:111–114
52. Cleary RK, Schaldebrand JD, Fowler JJ, Schuler JM, Lampman RM (1999) Perianal Bowen's disease and anal intraepithelial neoplasia. Dis Colon Rectum 42:945–951
53. Caruso ML, Valentini AM (1999) Different human papillomavirus genotypes in ano-genital lesions. Anticancer Res 19:3049–3053
54. Bontkes HJ, de Gruijl TD, Walboomers JM, Schiller JT, Dillner J, Helmerhorst TJ, Verheijen RH, Scheper RJ, Meijer CJ (1999) Immune responses against human papillomavirus (HPV) type 16 virus-like particles in a cohort study of women with cervical intraepithelial neoplasia. II. Systemic but not local IgA responses correlate with clearance of HPV-16. J Gen Virol 80:409–417
55. Wang ZH, Kjellberg L, Abdalla H, Wiklund F, Eklund C, Knekt P, Lehtinen M, Kallings I, Lenner P, Hallmans G, Mahlck CG, Wadell G, Schiller J, Dillner J (2000) Type specificity and significance of different isotypes of serum antibodies to human papillomavirus capsids. J Infect Dis 181:456–462
56. Petter A, Heim K, Guger M, Ciresa-Ko Nig A, Christensen N, Sarcletti M, Wieland U, Pfister H, Zangerle R, Hopfl R (2000) Specific serum IgG, IgM and IgA antibodies to human papillomavirus types 6:11:16:18 and 31 virus-like particles in human immunodeficiency virus-seropositive women. J Gen Virol 81:701–708
57. Ter Harmsel B, Smedts F, Kuijpers J, van Muyden R, Oosterhuis W, Quint W (1999) Relationship between human papillomavirus type 16 in cervix and intraepithelial neoplasia. Obstet Gynecol 93:46–50
58. Critchlow CW, Surawicz CM, Holmes KK, Kuypers J, Daling JR, Hawes SE, Goldbaum GM, Sayer J, Hurt C, Dunphy C, et al (1995) Prospective study of high grade anal squamous intraepithelial neoplasia in a cohort of homosexual men: influence of HIV infection, immunosuppression and human papillomavirus infection. AIDS 9:1255–1262
59. Surawicz CM, Kiviat NB (1998) A rational approach to anal intraepithelial neoplasia. Semin Colon Rectal Surg 9:99–106
60. Derchain SF, Roteli-Martins CM, Syjanen KJ, de Abreu HJ, Martinez EZ, Alves VA (1999) Association of oncogenic human papillomavirus DNA with high grade cervical intraepithelial neoplasia: the role of cigarette smoking. Sex Transm Infect 75:406–408
61. Palefsky JM, Holly EA, Ralston ML, Da Costa M, Greenblatt RM (2001) Prevalence and risk factors for human papillomavirus infection in human immunodeficiency virus (HIV)-positive and high-risk HIV-negative women. J Infect Dis 183:383–391
62. Melbye M, Palefsky J, Gonzales J, Ryder LP, Nielsen H, Bergmann O, Pindborg J, Biggar RJ (1990) Immune status as a determinant of human papillomavirus detection and its association with anal epithelial abnormalities. Int J Cancer 46:203–206
63. Moscicki AB, Ellenberg JH, Vermund SH, Holland CA, Darragh T, Crowley-Nowick PA, Levin L, Wilson CM (2000) Prevalence of and risks for cervical human papillomavirus infection and squamous intraepithelial lesions in adolescent girls: impact of infection with human immunodeficiency virus. Arch Pediatr Adolesc Med 154:127–134
64. Nakagawa M, Stites DP, Patel S, Farhat S, Scott M, Hills NK, Palefsky JM, Moscicki AB (2000) Persistence of human papillomavirus type 16 infection is associated with lack of cytotoxic T lymphocyte response to the E6 antigens. J Infect Dis 182:595–598
65. Nakagawa M, Stites DP, Palefsky KM, Kneass Z, Moscicki AB (1999) CD4-positive and CD8-positive cytotoxic T lymphocytes contribute to human papillomavirus type 16 E6 and E7 responses. Clin Diagn Lab Immunol 6:494–498
66. Nagai Y, Maehama T, Asato T, Kanazawa K (2000) Persistence of human papillomavirus infection after therapeutic conization for CIN 3: is it an alarm for disease recurrence. Gynecol Oncol 79:294–299
67. Kjellberg L, Wadell G, Bergman F, Isaakson M, Angstrom T, Dillner J (2000) Regular disappearance of the human papillomavirus genome after conization of cervical dysplasia by carbon dioxide laser. Am J Gynecol Obstet 183:1238–1242
68. Syrjanen SM, von Krogh G, Syrjanen KJ (1989) Anal condylomas in men. I. Histopathological and virological assessment. Genitourin Med 65:216–224
69. Hennig EM, Suo Z, Thoresen S, Holm R, Kvinnsland S, Nesland JM (1999) Human papillomavirus 16 in breast cancer of women treated for high grade cervical intraepithelial neoplasia (CIN III). Breast Cancer Res Treat 53:121–135
70. Fenger C (1997) Anal canal. In: Sternberg SS (ed) Histology for pathologists, 2nd edn. Lipincott-Raven, Philadelphia, pp 551–571
71. Penn I (1988) Tumors of the immunocompromised host. Annu Rev Med 39:63–73
72. Majewski S, Skopinska-Rozewska E, Jablonska S, Wasik M, Misiewicz J, Orth G (1986) Partial defects of cell-mediated immunity in patients with epidermodysplasia verruciformis. J Am Acad Dermatol 15:966–973
73. Penn I (1986) Cancer of the anogenital region in renal transplant recipients. Analysis of 65 cases. Cancer 58:611–616
74. Sillman FH, Sedlis A (1987) Anogenital papillomavirus infection and neoplasia in immunodeficient women. Obstet Gynecol Clin North Am 14:537–558
75. Ozsaran AA, Ates T, Dikmen Y, Zeytinoglu A, Terek C, Erhan Y, Ozacar T, Bilgic A (1999) Evaluation of the risk of cervical intraepithelial neoplasia and human papilloma virus infection in renal transplant patients receiving immunosuppressive therapy. Eur J Gynaecol Oncol 20:127–130

76. Stentella P, Frega A, Ciccarone M, Cipriano L, Tinari A, Tzantzoglou S, Pachi A (1998) HPV and intraepithelial neoplasia recurrent lesions of the lower genital tract: assessment of the immune system. Eur J Gynaecol Oncol 19:466–469
77. Lee BN, Follen M, Tortolero-Luna G, Eriksen N, Helfgott A, Hammill H, Shearer WT, Reuben JM (1999) Synthesis of IFN-gamma by CD8 (+) T cells is preserved in HIV-infected women with HPV-related cervical squamous intraepithelial lesions. Gynecol Oncol 75:379–386
78. Driel WJ van, Kievit-Tyson P, van den Broek LC, Zwinderman AH, Trimbos BJ, Fleuren GJ (1999) Presence of an eosinophilic infiltrate in cervical squamous carcinoma results from a type 2 immune responses. Gynecol Oncol 74:188–195
79. Clerici M, Fusi ML, Ruzzante S, Piconi S, Biasin M, Ariente D, Trabattoni D, Villa ML (1997) Type 1 and type 2 cytokines in HIV infection – a possible role in apoptosis and disease progression. Ann Med 29:185–188
80. Fenger C, Nielsen VT (1981) Dysplastic changes in the anal canal epithelium in minor surgical specimens. Acta Pathol Microbiol Scand [A] 89:463–465
81. Fenger C (1978) The anal transitional zone. A method for macroscopic demonstration. Acta Pathol Microbiol Scand [A] 86:225–230
82. Thompson-Fawcett M, Warren BF, Mortensen NJ (1998) A new look at the anal transitional zone with reference to restorative proctocolectomy and the columnar cuff. Br J Surg 85:1517–1521
83. Fenger C, Frisch M, Marti M-C, Parc R (2000) Tumours of the anal canal. In: Hamilton SR, Aaltonen LA (eds) Pathology and genetics of tumours of the digestive system. WHO classification of tumours. International Agency for Research on Cancer, Lyon, pp 146–55
84. Jass JR, Sobin LH (1989) Histological typing of intestinal tumours, 2nd edn. Springer, Berlin Heidelberg New York, pp 41–47
85. Fenger C, Frisch M, Jass JJ, Williams GT, Hilden J (2000) Anal cancer subtype reproducibility study. Virchows Arch 436:229–233
86. Toki T, Kurman RJ, Park JS, Kessis T, Daniel RW, Shah KV (1991) Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva of older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. Int J Gynecol Pathol 10:107–125
87. Gregoire L, Cubilla AL, Reuter VE, Haas GP, Lancaster WD (1995) Preferential association of human papillomavirus with high-grade histologic variants of penile-invasive squamous cell carcinoma. J Natl Cancer Inst 87:1705–1709
88. Trimble CL, Hildesheim A, Brinton LA, Shah KV, Kurman RJ (1996) Heterogeneous etiology of squamous carcinoma of the vulva. Obstet Gynecol 87:59–64
89. Fenger C (1979) The anal transitional zone. Location and extent. Acta Pathol Microbiol Scand [A] 87:379–386
90. Williams GR, Love SB, Talbot IC, Northover JMA (1996) HPV-positive and HPV-negative anal cancers. J Pathol 180:378–382
91. Fenger C, Nielsen V (1986) Precancerous changes in the anal canal epithelium in resection specimens. Acta Pathol Microbiol Scand [A] 94:63–69
92. Carter PS, Sheffield JP, Shepherd DH, Melcher DH, Jenkins D, Ewings P, Talbot I, Northover JM (1994) Interobserver variation in the reporting of the histopathological grading of anal intraepithelial neoplasia. J Clin Pathol 47:1032–1034
93. Caruso ML, Valentini AM (1999) Different human papillomavirus genotypes in ano-genital lesions. Anticancer Res 19:3049–3053
94. Litle VR, Leavenworth JD, Darragh TM, Kosinski LA, Moore DH, Smith-McCune KK, Warren RS, Palefsky JM, Welton ML (2000) Angiogenesis, proliferation and apoptosis in anal high-grade squamous intraepithelial lesions. Dis Colon Rectum 43:346–352
95. Maxwell GL, Sosson AP, Oster C, Miles P, Webb J, Carlson J (1998) Subepithelial angiogenesis in cervical intraepithelial neoplasia. J Lower Genital Tract Dis 2:191–194
96. Dellas A, Moch H, Schultheiss E, Feichter G, Almendral AC, Gudat F, Torhorst J (1997) Angiogenesis in cervical neoplasia: microvessel quantification in precancerous lesions and invasive carcinomas with clinicopathological correlations. Gynecol Oncol 67:27–33
97. Hanahan D, Christofori G, Naik P, Arbeit J (1996) Transgenic mouse models of tumour angiogenesis: the angiogenic switch, its molecular controls and prospects for preclinical therapeutic models. Eur J Cancer 32A:2386–2393
98. Bergers G, Hanahan D, Coussens LM (1998) Angiogenesis and apoptosis are cellular parameters of neoplastic progression in transgenic mouse models of tumourigenesis. Int J Dev Biol 42:995–1002
99. Werness BA, Levine AJ, Howley PM (1990) Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 248:76–79
100. Dyson N, Howley PM, Munger K, Harlow E (1989) The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 243:934–947
101. Crook T, Wrede D, Tidy J, Scholefield J, Crawford L, Vousden KH (1991) Status of *c-myc*, p53 and retinoblastoma genes in human papillomavirus positive and negative squamous cell carcinoma of the anus. Oncogene 6:1251–127
102. Munger K, Phelps WC, Bubb V, Howley PM, Schlegel R (1989) The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. J Virol 63:4417–4421
103. Watanabe S, Kanda T, Yoshiike K (1989) Human papillomavirus type 16 transformation of primary human embryonic fibroblasts requires expression of open reading frames E6 and E7. J Virol 63:965–969
104. Ogunbiyi OA, Scholefield JH, Smith JHF, Polacarz SV, Rogers K, Sharp F (1993) Immunohistochemical analysis of p53 expression in anal squamous neoplasia. J Clin Pathol 46:507–512
105. Haga T, Kim SH, Jensen RH, Darragh T, Palefsky JM (2001) Detection of genetic changes in anal intraepithelial neoplasia (AIN) of HIV-positive and HIV-negative men. J Acquir Immune Defic Syndr 26:256–262
106. Heselmeyer K, du Manoir S, Blegen H, Friberg B, Svensson C, Schrock E, Veldman T, Shah K, Auer G, Ried T (1997) A recurrent pattern of chromosomal aberrations and immunophenotypic appearance defines anal squamous cell carcinomas. Br J Cancer 76:1271–1278
107. Chu TY, Shen CY, Lee HS, Liu HS (1999) Monoclonality and surface lesion-specific microsatellite alterations in premalignant and malignant neoplasia of the uterine cervix: a local field effect of genomic instability and clonal evolution. Genes Chromosomes Cancer 24:127–134
108. Jimenez P, Canton J, Concha A, Torres LM, Abril E, Real LM, Garcia A, Garrido F, Ruiz-Cabello F (1998) Microsatellite instability in cervical intraepithelial neoplasia. J Exp Clin Cancer Res 17:361–366

109. Carlson JA, Healy K, Tran TA, Malfetano J, Wilson VL, Rohwedded A, Ross JS (2000) Chromosome 17 aneusomy detected by fluorescence in situ hybridization in vulvar squamous cell carcinomas and synchronous vulvar skin. Am J Pathol 157:973–983
110. Wistuba II, Syed S, Behrens C, Duong M, Milchgrub S, Muller CY, Jagirdar J, Gazdar AF (1999) Comparison of molecular changes in cervical intraepithelial neoplasia in HIV positive and HIV indeterminate subjects. Gynecol Oncol 74:519–526
111. Shibata D (1997) Molecular tumor clocks and dynamic phenotype. Am J Pathol 151:643–646
112. Imai H, Nakagama H, Komatsu K, Shiraishi T, Fukuda H, Sugimura T, Nagao M (1997) Minisatellite instability in severe combined immunodeficiency mouse cells. Proc Natl Acad Sci USA 94:10817–820
113. Polacarz SV, Darne J, Sheridan EG, Ginsberg R, Sharp F (1991) Endocervical carcinoma and precursor lesions: c-myc expression and the demonstration of field change effects. J Clin Pathol 44:896–899
114. Ogunbiyi OA, Scholefield JH, Rogers K, Sharp F, Smith JHF, Polacarz SV (1993) C-myc oncogene expression in anal squamous neoplasia. J Clin Pathol 46:23–27
115. Crook T, Greenfield I, Howard J, Stanley M (1990) Alterations in growth properties of human papillomavirus type 16 immortalised human cervical keratinocyte cell line correlate with amplification and overexpression of c-myc oncogene. Oncogene 5:619–622
116. Hiorns LR, Scholefield JH, Palmer JG, Shepherd NA, Kerr IB (1990) Kiras oncogene mutations in non-HPV-associated anal carcinoma. J Pathol 161:99–103
117. Sheyn I, Noffsinger AE, Heffelfinger S, Davis B, Miller MA, Fenoglio-Preiser CM (1997) Amplification and expression of the cyclin D1 gene in anal and esophageal squamous cell carcinomas. Hum Pathol 28:270–276
118. Shinozaki H, Ozawa S, Ando N, Tsuruta H, Terada M, Ueda M, Kitajima M (1996) Cyclin D1 amplification as a new predictive classification for squamous cell carcinoma of the esophagus, adding gene information. Clin Cancer Res 2:1155–1161
119. Ried T, Knutzen R, Steinbeck R, Blegen H, Schrock E, Heselmeyer K, DuManoir S, Auer G (1996) Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumours. Genes Chromosomes Cancer 15:234–245
120. Heselmeyer K, Schrock E, DuManoir S, Blegen H, Shah K, Steinbeck R, Auer G, Ried T (1996) Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. Proc Natl Acad Sci USA 93:497–484
121. Reid R, Stanhope CR, Agronov SJ (1984) Genital warts and cervical cancer. IV. A colposcopic index for differentiating subclinical papillomavirus infection from cervical intraepithelial neoplasia. Am J Obstet Gynecol 149:815–819
122. Colquhoun PHD, Efron JE, Eze G, Weiss EG, Vernava AM III, Nogueras JJ, Wexner SDW (2001) The incidence of subclinical anal intraepithelial neoplasia (AIN) in patients with anal condylomata. Dis Colon Rectum 44 (in press)
123. Carter PS, de Ruiter A, Whatrup C, Katz DR, Exings P, Mindel A, Northover JM (1995) Human immunodeficiency virus infection and genital warts as risk factors for anal intraepithelial neoplasia in homosexual men. Br J Surg 82:473–474
124. Metcalf AM, Dean T (1955) Risk of dysplasia in anal condyloma. Surgery 118:724–726
125. Chanen W (1990) The CIN saga-the biological and clinical significance of cervical intraepithelial neoplasia. Aust N Z J Obstet Gynaecol 30:18–23
126. Sherman ME, Schiffman MH, Erozan YS, Wacholder S, Kurman RJ (1992) The Bethesda system, a proposal for reporting abnormal cervical smears based on the reproducibility of cytopathologic diagnoses. Arch Pathol Lab Med 116:1155–1158
127. Scholefield JH, Johnson J, Hitchcock A, Kocjan G, Smith JHF, Smith PA, Ferryman S, Byass P (1998) Guidelines for anal cytology – to make cytological diagnosis and follow-up much more reliable. Cytopathology 9:15–22
128. Rasmussen OO, Christiansen J (1989) Conservative management of Bowen's disease of the anus. Int J Colorectal Dis 4:164–166
129. Seckel BR, Schoetz DJ Jr, Coller JA (1985) Skin grafts for circumferential coverage of perianal wounds. Surg Clin North Am 65:365–371
130. Blatchford GJ, Pitsch RM (1996) Operative results of house advancement anoplasty. Br J Surg 83:1242–1244
131. Core GB, Bite U, Pemberton JH, Petty PM (1994) Sliding V-Y perineal island flaps for large perianal defects. Ann Plast Surg 32:328–331
132. Carr N, Mercey D, Slack W (1989) Non condylomatous perianal disease in homosexual men. Br J Surg 76:1064–1066
133. Northfelt DW, Swift PS, Palefsky JM (1996) Anal neoplasia. Pathogenesis, diagnosis and management. Hematol Oncol Clin North Am 10:1177–1187
134. Bandieramonte G, Bono A, Zurrida S, Bartoli C, de Palo G (1993) Laser therapy for small perianal neoplasms. Eur J Cancer 29:1528–1531
135. Holt PJ (1988) Cryotherapy for skin cancer: results over a 5 year period using liquid nitrogen spray cryosurgery. Br J Dermatol 119:231–240
136. Beck DE, Fazio VW (1989) Premalignant lesions of the anal margin. South Med J 82:470–474
137. Petrelli NJ, Cabollero JA, Rodriquez-Bigas M, Mang T (1992) Photodynamic therapy in the management of neoplasms of the perianal skin. Arch Surg 127:1436–1438
138. Boynton KK, Bjorkman DJ (1991) Argon laser therapy for perianal Bowen's disease: a case report. Lasers Surg Med 11:385–387
139. Cleary R, Schaldenbrand J, Fowler J, Schuler J, Lampman R (1999) Treatment options for perianal Bowen's disease: survey of members of the American Society of Colon and Rectal Surgeons. Dis Colon Rectum 42:37(A)
140. Scholefield JH (1991) Studies in the pathogenesis of HPV associated anal squamous cell carcinoma. Thesis, University of Liverpool
141. Beck DE (1995) Paget's disease and Bowen's disease of the anus. Semin Colon Rectal Surg 6:143–149
142. Marchesa P, Fazio VW, Oliart S, Goldblum JR, Lavery IC (1997) Perianal Bowen's disease: a clinicopathologic study of 47 patients. Dis Colon Rectum 40:1286–1293
143. Goldie SJ, Kuntz KM, Weinstein MC, Freedberg KA, Welton ML, Palefsky JM (1999) The clinical effectiveness and cost-effectiveness of screening for anal squamous intraepithelial lesions in homosexual and bisexual HIV-positive men. JAMA 281:1822–1829

144. Lytwyn A, Sellors JW, Mahony JB, Daya D, Chapman W, Ellis N, Roth P, Lorincz AT, Gafni A (2000) Comparison of human papillomavirus DNA testing and repeat Papnicolaou test in women with low-grade cervical cytologic abnormalities: a randomized trial. HPV Effectiveness in lowgrade Paps (HELP) study no. 1 Group. Can Med Assoc J 163:701–707
145. Milde-Langosch K, Riethdorf S, Loning T (2000) Association of human papillomavirus infection with carcinoma of the cervix uteri and its precursor lesions: theoretical and practical implications. Virchows Arch 437:227–233
146. Ruiter A de, Carter P, Katz DR, Kocjan G, Whatrup C, Northover J, Mindel A (1994) A comparison between cytology and histology to detect intraepithelial neoplasia. Genitourin Med 70:22–25
147. Condra JH (1998) Resisting resistance: maximizing the durability of antiretroviral therapy. Ann Intern Med 128:951–954
148. Sasieni P (2001) Estimating prevalence when the true disease status is incompletely ascertained. Stat Med 20:935–949