

AIN AND OTHER PRECANCEROUS LESIONS OF THE ANAL REGION

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Epithelial tumours of the anal region are uncommon, accounting for only 2% of large intestinal malignancies (1) with an incidence of about 0.7 per 100,000 men and 0.9 per 100,000 women in the USA (2) and 0.5 per 100,000 in the UK (3). Anal squamous neoplasia is increasing in incidence and this is especially so in certain high risk groups, most notably homosexuals with and without HIV/AIDS (4,5) and women with multifocal anogenital neoplasia (6). There is increasing understanding of the pre-neoplastic entities that predispose to the malignant tumours that occur in the anal region. Best understood, in terms of its causation, pathology and natural history is squamous dysplasia, now almost universally classified by the anal intra-epithelial neoplasia (AIN) system (7-9). However we are profoundly ignorant about the pre-neoplastic conditions that lead on to glandular malignancy in the anal region and know little or nothing about melanocytic pre-neoplastic pathology in the anal canal, despite malignant melanoma being a well recognised, if unusual, primary tumour arising at this site. Our lack of knowledge does, of course, reflect the rarity of these tumours. The most common glandular neoplastic condition encountered in this region is Paget's disease, which shows characteristic clinical features, although its pathology has important differential diagnoses that usually require investigation by histochemistry and immunohistochemistry (7).

Anal intraepithelial neoplasia (AIN)

The incidence of AIN remains unknown. There is undoubtedly an increasing incidence, especially in high-risk patients. The highest rates of AIN are in homosexuals who have sex with men (MSM). One study found an AIN rate of 52% in MSM who were also HIV positive although there was also a relatively high rate of 17% in MSM who were HIV negative with rapid advancement of the disease, to high grade AIN, within two years of diagnosis (10). AIN shows epidemiological and pathological parallels with cervical intra-epithelial neoplasia (CIN) and vulval intra-epithelial neoplasia (VIN) (11,12). Women with multifocal genital intra-epithelial neoplasia may demonstrate high rates of AIN (up to 20%) (13). The link between AIN and these high-risk populations is HPV infection. There are strong correlations between HPV infection and anal neoplasia, including AIN (7, 13). High-risk HPV subtypes are present in anal carcinoma, more likely in anal canal neoplasia than in perianal lesions (13). Both AIN 3 and anal canal cancer show high rates of high-risk HPV types, using PCR techniques. It is now known that many high-risk HPV subtypes are involved in the genesis of anal squamous neoplasia and different strains of individual HPV viral types may be associated with different risks (7).

HIV/AIDS is a major risk factor for anal neoplasia (7, 14). In one study, 92% of HIV-positive MSM demonstrated anal HPV infection as against 66% in those MSM who were HIV negative (15). HIV-positive women show much higher rates of HPV infection than HIV-negative women (16,17). Women with multifocal genital intra-epithelial neoplasia show 16 times the rate of AIN if they are HIV-positive (16). It would seem likely that immunosuppression is the major determinant of the dramatic effect of HIV infection in the production of anal neoplasia. Despite this, there is little evidence that highly active anti-

retroviral therapy (HAART) has had an appreciable effect on the incidence of AIN, despite its efficacy in restoring immunological function and reducing opportunistic infection in HIV/AIDS (7, 17). For, the incidence of anal cancer continues to increase in HIV-positive homosexual males, certainly in the USA, despite the widespread uptake of HAART in this group (18).

AIN is more likely to be seen in the upper anal canal and ATZ (anal transitional zone) than in the lower canal and perianal region (8,19,20). However, many patients show involvement of the anal canal and the perianal skin and this underpins the importance of widespread sampling (8, 21). Clinically, AIN does not show any specific features and histology and/or cytology remains the gold standard for its recognition and diagnosis (7). Various clinical/macroscopic appearances have been described for AIN-involved anal mucosa with a raised, red mucosa being the commonest. On the other hand, white scaling mucosa is well described as is pigmented mucosa. A verrucous microscopic appearance of AIN has been associated with the highest risk of malignant transformation (22). Ulceration is a sinister feature and may indicate that malignancy has already supervened. Despite these macroscopic features, it must be emphasised that most AIN disease is effectively sub-clinical, showing few if any macroscopic features to allow the attending physician to make the diagnosis.

Anal colposcopy has been advocated in some centres (7, 23). Using identical techniques to those much more commonly applied to the uterine cervix, AIN is seen as an acetowhite epithelium contrasting with the adjacent iodine-positive (and therefore brown-appearing) non-neoplastic mucosa. Good correlations between anal colposcopic findings and histopathology have been shown, especially at either ends of the dysplastic spectrum (i.e. normal and AIN 3) (23). However, anal colposcopy correlates poorly with histology in the diagnosis of HPV infection alone and of low grade AIN. There has not been widespread uptake of the technique and many centres continue to rely on close clinical surveillance and regular mapping biopsies to assess the presence and extent of AIN disease.

Fenger & Nielsen are credited with the first histological descriptions of AIN disease (8,9,19). AIN shows a variably thickened squamous mucosa in which basal/undifferentiated cells are excessive, show a high nucleo-cytoplasmic ratio and are orientated perpendicular to the basement membrane, failing to demonstrate the transverse orientation of maturing cells. This “dysmaturation” is the most characteristic feature of AIN although nuclear enlargement, irregularity and hyperchromatism are prominent in higher grades of AIN. There is also increased mitotic activity, with suprabasal mitotic activity being a characteristic feature of squamous dysplasia at all sites. Abnormal cell activity is characterised by individual cell keratinisation, dyskeratosis. The latter is more likely to be seen in perianal AIN and has been especially associated with “Bowen’s disease” or “Bowenoid papulosis” of the anal region. However, it has been recommended that the latter terms are not used as they are not causally related to classical Bowen’s disease of the skin and have induced confusion.

The AIN grading is based on the level of involvement by “dysmature” cells in the anal squamous mucous membrane, in a similar way to CIN and VIN (7,19,26). Thus AIN 1 is present when less than one third of the height of the mucosa is involved by dysmature cells and AIN 2 when more than a third but less than two thirds is so involved. AIN 3 is the appropriate designation when more than two thirds of the height of the mucosa is involved by dysmature cells. The term “carcinoma-in-situ” (CIS) has been used when there is full thickness dysmaturity. Most would now regard this as an obsolete term and would incorporate CIS into the AIN 3 category.

The grading of AIN remains controversial. The three-tier grading system of AIN 1, AIN 2 and AIN 3 is in widespread usage although it is associated with considerable inter-observer variation (4,25,27,28). There are better levels of agreement for CIN 3 (4,27) but the levels of disagreement for AIN 1 and AIN 2 have led some to recommend the use of a Bethesda-type

two-tier system. In this system, low grade AIN usually means the combination of AIN 1 and AIN 2 with high grade AIN representing AIN 3 (24). The histological changes of AIN are usually combined with those associated with HPV infection. Dyskeratosis is often a marker of HPV infection whereas koilocytosis is also a common accompaniment of AIN and there may be other histological features to suspect HPV involvement. High grade AIN shows a distinct affinity for involvement of skin adnexal structures, in the perianal region, and of anal gland epithelium, in the anal canal. This deep involvement has particular significance for AIN management as involvement of adnexal structures by AIN may extend to a depth of at least 2mm and may not be detected nor excised or ablated during treatment (29).

In general, most surgical pathologists use standard morphological techniques for the diagnosis of AIN and accept the problems of observer variation. In the UK, cases of "high grade" AIN are discussed in a multidisciplinary team meeting (MDTM) and, in many centres, the diagnosis is approved by a second, often expert, gastro-intestinal pathologist. This tends to offset any potential problems with diagnostic variations. Given the inter-observer variance in the diagnosis of AIN, does the histopathologist have any useful adjunctive techniques in his diagnostic armamentarium? Up until now, there have been no "biomarkers" which have found universal acceptance in the diagnosis of AIN, despite the problems of poor inter-observer reproducibility. This author has, on occasion, found Ki-67 markers useful for the confirmation of significant AIN disease and there is support for the use of Ki-67 in this area. A recent study has shown that Ki-67 and p16 (a protein with a logical influence on AIN presence and grade) immunohistochemistry are both sensitive and reliable markers for the diagnosis of high grade AIN (30). Such markers, in conjunction with standard morphological evaluation, may help to improve the observer reproducibility in the diagnosis of AIN disease.

If AIN 1 or low grade disease is associated with poorer levels of observer reproducibility, are there diagnostic difficulties at the opposite end of the scale, namely in the differentiation of AIN 3 from invasive squamous cell carcinoma? Micro-invasive squamous cell carcinoma is a well accepted concept in cervical and vulval pathology. In the anus, it is not at all well recognised and no criteria have been introduced for its recognition or management. Early invasive carcinoma can be mimicked by tangentially sectioned rete pegs of the dysplastic squamous mucosa. However, isolated epithelium showing characteristic cytoplasmic changes, compared to the overlying CIN 3 disease, probably do represent foci of "microinvasive squamous cell carcinoma" but, as yet, we know little of how to manage this disease.

Low grade AIN, AIN 1 and AIN 2, has been associated, in the literature, with a considerable regression rate of up to 30% (4,12,23). However, it has been argued that this may simply reflect inconsistent interpretation of pathology due to inexperience or poor sample quality (4). In spite of these suggested regression rates, some doubt must be cast on such results as pathological reporting may have been less than consistent and there may be over-reliance on subsequent false negative follow-up (4). There is only a little more evidence of the natural history of AIN 3, particularly in those who are HIV positive. In Scholefield's study in Nottingham, UK, 35 patients with AIN 3 underwent long-term surveillance after all AIN 3 lesions were excised (1). Only six patients, all with multifocal disease, were immunosuppressed. Three of the latter developed invasive carcinoma in the follow-up period (maximally 10 years) but none of the immunocompetent patients did. The authors concluded that the immuno-compromised patients were more likely to have multifocal disease and develop anal cancer earlier (1). Thus, while the rate of progression to invasive carcinoma remains largely unknown, certain high-risk groups, notably the immunosuppressed, seem to have a higher rate of malignancy and demand closer surveillance. The risk of progression of AIN disease in the immunocompetent would seem low. Therefore there are few data on the appropriate management of these patients. There are advocates, on the one hand, for close surveillance only whilst others advise excision/ablation of the diseased epithelium to reduce the malignancy risk.

Paget's disease

Paget's disease of the anus occurs in either sex, in almost equal proportions, and is a disease of the elderly. It manifests clinically as a raised, red, scaling area of the anal canal or perianal skin (7,31,32). In that way, it appears similar to other forms of Paget's disease, especially that of the nipple. Whilst these macroscopic features may suggest Paget's disease, thus justifying biopsy, it is important for clinicians to realise that it may be associated with an underlying malignancy, especially of the rectum and even, on occasion, the sigmoid colon or anus (7). Thus, once the diagnosis is suspected or established, it is mandatory for sigmoidoscopy to be undertaken to confirm or refute such an associated malignancy.

Biopsy usually establishes the diagnosis, although there are important differential diagnoses and immunohistochemical analysis is valuable in confirming the diagnosis and also guiding the clinician as to the underlying cause of the disease. Paget's disease is characterised by an intra-epidermal proliferation of large vacuolated cells, often in clusters but also present discretely. They are typically concentrated at the basal aspect of the epidermis where clustering is more apparent (7). However, spread of clusters and discrete cells to the more superficial parts of the epidermis also occurs: in fact, in other pathological conditions, especially malignant melanoma, this pattern of spread is termed "Pagetoid".

Paget's cells have large, neoplastic-appearing, nuclei and intra-cytoplasmic secretion of mucin, diastase-PAS and alcian blue positive (33,34). Thus histochemistry is valuable in differentiating Paget's disease from its mimics (7,34). So, similar changes are seen as a reactive condition, usually termed Pagetoid dyskeratosis (35). Wart viral involvement, with or without AIN, can mimic Paget's disease. Melanocytic proliferations are also a potential source of mimicry. Paget's disease itself is associated with hyperplasia of the associated squamous epithelium. This "pseudo-epitheliomatous hyperplasia" provides a further potential mimic: if the pathologist fails to appreciate the significance of the vacuolated cells, then a squamo-proliferative pathology, even invasive squamous cell carcinoma, becomes a potential erroneous diagnosis (7). If there is pathological doubt, then (immuno)histochemistry usually serves to confirm or refute the diagnosis.

Similar histological appearances are produced when a primary infiltrative adenocarcinoma of the rectum (and occasionally of the sigmoid colon or anus itself) infiltrates the epidermis of the anus (7). The management of this disease should be primarily directed at that of the primary tumour with treatment of the "Paget's disease" a secondary consideration. It is important to ensure identification of this type of Paget's disease. This is readily achieved by immunohistochemistry with the tumour cells not showing expression of apocrine markers but CK20 with CK7 only rarely (7). Classical Paget's disease cells express CK7 and not, usually, CK20 (7). The immunophenotype of primary anal adenocarcinoma causing Paget's disease will closely match that of classical apocrine-type Paget's disease. In all types of Paget's disease, it is appropriate to use an antibody panel and rely on the results of specific apocrine markers, such as GCDFP, and cytokeratin subset antibodies (7,33).

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