A comparison between cytology and histology to detect anal intraepithelial neoplasia

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Abstract

Introduction—Anal intraepithelial neoplasia (AIN), which may be a precursor of anal carcinoma, has been identified on histology following minor anal surgical procedures, in particular the removal of perianal condylomata, in increasing numbers of homosexual and bisexual men. Anal cytology has recently been proposed as a useful method of identifying AIN lesions.

Objective—To compare anal cytology with histology as a method of detecting AIN.

Methods—215 homosexual and bisexual men attending a central London sexually transmitted diseases clinic had an anal cytological smear performed under standard conditions. The perianal area and anal canal were then examined using a colposcope, and areas macroscopically suggestive of intraepithelial neoplasia were biopsied.

Results—176 of the 215 patients were biopsied of whom 76 had AIN on histology. 154 of the 215 patients had an adequate anal smear of whom 46 and 85 had cytological features of both HPV and AIN, or HPV alone respectively. Including features of HPV alone as an abnormal smear, anal cytology, when compared with anoscopy and histology as the gold standard for diagnosing AIN, resulted in a sensitivity of 87.5%, a specificity of 16.3%, a positive predictive value of 37.4% and a negative predictive value of 69.6%. Restricting abnormal smears to those with features of both HPV and AIN resulted in a sensitivity of 33.9%, a specificity of 72.5%, a positive predictive value of 41.3% and a negative predictive value of 65.7%.

Conclusion—Anal cytology is a sensitive but nonspecific method of identifying patients with biopsy proven AIN if cytological features of HPV alone are included as abnormal smears. Specificity is improved by restricting abnormal smears to those with features of both HPV and AIN but this markedly lowers the sensitivity of the test. At present, anoscopy and histology are required in addition to anal cytology to differentiate between patients who simply have anal condylomata and those who also have AIN.

Introduction

Anal intraepithelial neoplasia (AIN) may be defined as the presence of nuclear abnormalities in the epithelium, without breach of the basement membrane. The natural history of AIN, its prevalence and significance and ability to progress to invasive carcinoma in either immunocompetent or immunocompromised patients is as yet unknown.

In recent years there have been a number of reports of AIN, often as an incidental finding following the histological evaluation of surgical specimens from minor anal surgical procedures such as the removal of anal condylomata.¹⁻⁶ Most AIN lesions are situated at the anal transitional zone, an area of epithelium with rectal columnar epithelium above and squamous epithelium below, but lesions have also been reported from the perianal region.

Although AIN has been found in both men and women, and in the latter may be regarded as part of a so-called "field effect" of multifocal intraepithelial neoplasia,⁶⁻⁸ the majority of reports describe lesions occurring in homosexual and bisexual men: a group in which there is a known high prevalence of anal condylomata.³⁻⁶ Of interest is the suggestion from epidemiological studies that homosexual and bisexual men may be at increased risk of anal carcinoma and that this malignancy is becoming more prevalent in this risk group.⁹⁻¹²

Anal cytology has been used by several investigators to demonstrate the presence of HPV, and more recently AIN.¹³⁻¹⁸ Although widely accepted as an adequate means of detecting intraepithelial lesions of the cervix, cytology is an untested technique when it comes to intraepithelial neoplasia of the anal canal, and its sensitivity and specificity for the detection of AIN lesions are unknown. Prevalence and natural history studies are required to determine whether AIN is a precursor of anal carcinoma, and it is essential that methods used to detect and follow up patients harbouring this lesion are reliable and reproducible. The aim of this study was to compare anal cytology with histology as a method of detecting AIN.

Methods

Homosexual and bisexual men attending a department of genitourinary medicine for routine follow up of their HIV infection or with newly diagnosed anal condylomata were recruited. Informed consent was obtained.

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This group forms part of an ongoing study of the prevalence and natural history of AIN.

Cytology

Patients were examined in the lithotomy position. Anal smears were obtained by blindly inserting a cytobrush (Medscand, Sweden) 1.5 to 2.0 cm into the anal canal, rotating through 360 degrees, transferring to a glass slide and fixing with 96% ethanol. Fixed smears were stained according to the Papanicolaou method. All smears were examined by one observer (GK) and coded blind without knowledge of the clinical findings. The adequacy of the smear was assessed on the basis of: (a) presence of columnar and/or metaplastic cells from the squamocolumnar junction, (b) cellularity of the smear, (c) technical adequacy (fixation and smearing artefacts, contamination with faecal contaminants and unusual flora).

Morphological criteria for establishing the presence of HPV infection were: (a) widespread parakeratosis, (b) presence of anucleate squamous cells, (c) dyskeratotic cells, (d) multinucleation, (e) koilocytosis. Koilocytosis was notably absent in the majority of the smears examined.

The presence of AIN in the cytological smears was assessed on the basis of abnormality of the nucleus (dyskaryosis), and according to the criteria established in cervical intraepithelial neoplasia (CIN) these changes were reported as AIN grades 1 to 3: AIN 1 with the mildest of nuclear changes such as enlargement to approximately one third of the total cell diameter without significant change in the chromatin pattern, to AIN 3 where nuclear abnormality included enlargement to greater than two thirds of the cell diameter with irregularity of nuclear contour and chromatin expressed as hyperchromasia.

Similar to cervical cytology, changes associated with HPV infection (so called "borderline" nuclear changes) were often difficult to distinguish from those associated with AIN 1 (so called "mild" nuclear changes).

Histology

An oblique viewing Graeme Anderson proctoscope was inserted into the anal canal and a Zeiss colposcope was used to examine the transitional zone, the anal canal and the perianal area both before and after the application of 5% acetic acid. Biopsy specimens were taken from anal condylomata and, where present, from areas macroscopically suggestive of intraepithelial neoplasia with abnormal colour, vasculature or surface pattern using modified Ajax punch biopsy forceps following infiltration of the submucosa with 2% xylocaine with adrenaline for anal canal lesions, and scissor excision for perianal lesions. Samples were fixed in modified formal calcium for 16 hours at room temperature followed by immediate processing. Routine haematoxylin and eosin stained sections were used throughout. Not less than three sections were reviewed from each sample by one

observer (DRK) who was blinded to the clinical and cytological findings.

Histological criteria for diagnosing HPV and AIN were based on those routinely applied to the cervix with the degree of AIN being determined by the proportion of the epithelium occupied by basaloid undifferentiated cells with loss of the normal epithelial maturation and decreased glycogenation. The lesions were graded into AIN 1 (basaloid cells in the lower third of epithelium), AIN 2 (lower to middle third) and AIN 3 (middle third to full thickness).

Results

For the analysis, histology results were paired with the smear taken at the same visit, or, in the case of an inadequate smear, with the result of the smear taken at the previous visit provided that no biopsy had been taken at that time.

Histology

Two hundred and fifteen homosexual and bisexual men were examined fully. One hundred and sixty nine (78.6%) had macroscopic anal condylomata, 46 (21.4%) did not have macroscopic anal condylomata and 176 (81.9%) were biopsied. Seventy six patients (35.4%) had histological evidence of both HPV and AIN, 91 (42.3%) had features suggestive of HPV alone, and nine (4.2%) were normal. The distribution of AIN grades 1, 2 and 3 among the 76 patients was 48, 18, and 10 respectively. Fifty six patients had lesions located at the level of the transitional zone, 12 in the perianal area, and eight patients were found to have lesions at both sites.

Cytology

One hundred and fifty four (71.6%) of the 215 patients had an adequate smear of which 140 (90.9%) were taken at the time of their biopsy or normal examination, and 14 (9.1%) were taken a median of three months earlier (range 1–6). One hundred and twenty six (81.8%) of these 154 patients had macroscopic anal condylomata and 28 (18.2%) had no macroscopic evidence of anal condylomata. Forty six patients (29.9%) were found to have features suggestive of both HPV and AIN, 85 (55.2%) had evidence of HPV alone, and 23 (14.9%) were negative. The distribution of smears showing AIN 1, AIN 1–2, AIN 2, AIN 2–3 and AIN 3, were 31, eight, six, one, and zero respectively.

The cytology results of the 39 patients who were anoscopically normal and who were not biopsied were as follows: Inadequate 15, negative three, HPV alone 15, HPV and AIN six. These patients were subsequently reexamined within three months, but as no abnormality was seen, no biopsy was performed.

Comparison between anal cytology and histology

Of the 154 patients with an adequate anal smear, 56 (36.4%) had both HPV and AIN on histology (34 AIN 1, 13 AIN 2, 9 AIN 3), 67 (43.5%) had evidence of HPV alone, and

Table Histology and cytology results in the 154 patients with an adequate anal smear

Cytology	Histology					Total
	Negative	HPV	AIN 1	AIN 2	AIN 3	
Negative	5	11	5	1	1	23
HPV	20	35	17	8	5	85
AIN 1	5	15	8	3	0	31
AIN 1-2	1	4	2	0	1	8
AIN 2	0	1	2	1	2	6
AIN 2-3	0	1	0	0	0	1
AIN 3	0	0	0	0	0	0
Total	31	67	34	13	9	154

31 (20.1%) were negative (table). In order to validate anal cytology it was assumed that microanoscropy, with histology when any abnormality was seen was the gold standard for diagnosing or excluding AIN.

The results were analysed using two separate methods. In the first, Method 1, histology results were deemed positive if there was any degree of AIN and negative if the result was suggestive of HPV alone, negative, or no abnormality was seen on microanoscropy and no biopsy was taken. Anal cytology results were deemed positive if the smear showed any degree of AIN or features suggestive of HPV alone, and negative if the smear was completely normal. Using this method, the number of true positive anal smears was 49 (31.8%), false positives 82 (53.2%), false negatives seven (4.5%), and true negatives 16 (10.4%) giving a sensitivity of 87.5%, a specificity of 16.3%, a positive predictive value of 37.4% and a negative predictive value of 69.6%.

In Method 2, histology results were classified in the same manner as in Method 1, but cytology results were only deemed positive if the result showed any degree of AIN, and negative if the result was suggestive of HPV alone or negative. Using these criteria the number of true positive anal smears was 19 (12.3%), false positives 27 (17.5%), false negatives 37 (24.0%) and true negatives 71 (46.1%) resulting in a sensitivity of 33.9%, a specificity of 72.5%, a positive predictive value of 41.3% and a negative predictive value of 65.7%.

In a subanalysis of 169 patients of known HIV status there was no significant difference in the sensitivity of anal cytology when comparing those who were HIV positive with those who were HIV negative (data not shown).

Of the nine patients with HPV and AIN 3 on biopsy who had an adequate smear, eight would have been detected using Method 1, and only three using Method 2.

Discussion

In this study there was a high prevalence of biopsy-proven AIN with 35.4% of the study group found to harbour this lesion.

With regards to the ability of anal cytology to detect patients with AIN on biopsy, Method 1, which includes cytological features of HPV alone as a “positive” or “abnormal” smear requiring anoscopy, and is the method employed by other investigators to identify

patients with HPV and AIN lesions, resulted in a high sensitivity of 87.5%, allowing the detection of the majority of patients with biopsy-proven AIN, including eight of the nine patients with AIN 3, but an extremely low specificity of 16.3%. As AIN is associated with anal condylomata, a condition highly prevalent in homosexual men in whom the high prevalence of AIN and the increasing incidence of anal carcinoma is causing concern, if the cytological criteria of Method 1 were applied as a screening test for AIN in HIV positive and negative homosexual and bisexual men, particularly those with anal condylomata, the low specificity of this method in identifying patients with AIN as opposed to lesions containing HPV alone, would result in the overinvestigation of large numbers of patients in this risk group.

It is for this reason that the results were also analysed using Method 2, in which anal smears were only deemed “positive” or “abnormal” if they showed any degree of AIN, and “negative” or “normal” if the smear showed cytological features of HPV alone or was negative. Ideally, in this way, a simple noninvasive technique would differentiate between those patients who simply had anal condylomata, and those who harboured AIN lesions who could then be referred for anoscopy. The low sensitivity of 33.9% obtained in our study using this method, and in particular the failure to identify six out of the nine patients with biopsy-proven AIN 3 who had an adequate smear, was therefore disappointing.

There are several possible sources of error in the methods employed in our study, and a number of assumptions made which could lead to inaccurate results.

Regarding anal cytology there are a number of possible sources of inaccuracy and possible explanations for the apparent low sensitivity of Method 2. Firstly, the cytobrush was blindly inserted into the anal canal without prior visualisation of the transitional zone which would normally be the case in cervical cytological sampling. Although satisfied that we were sampling the transitional zone, as all samples without columnar and/or metaplastic cells were considered inadequate, it is conceivable, bearing in mind the anatomy of the anal canal, that the cytobrush may have been trapped in a fold of tissue and that only part of the transitional zone was sampled. Secondly, 12 patients were found to have AIN lesions in the perianal area alone which would not have been detected by sampling the transitional zone alone. Thirdly, a cytobrush was used to obtain cellular material in this study. Other investigators have used wooden spatulas or cotton-wool tipped or Dacron swabs and it is possible that these would have yielded more consistent and comparable results.

We have assumed that anoscopy with biopsy of abnormal lesions is the gold standard for the diagnosis of AIN. Errors in either of these techniques with inadequate anoscopy and misdirected biopsy or inaccurate

histopathological diagnosis could lead to inaccurate results. Regarding the former, identical colposcopic criteria routinely used for the diagnosis of CIN were applied to our study for the diagnosis of AIN and bar perhaps increased anoscopic experience it is difficult to see how this could be improved. Thirty-nine patients in whom no anoscopic abnormality was detected were not biopsied. As previously mentioned all 39 were reexamined within three months, but as no abnormality was seen, no biopsy was taken. For the purposes of the calculation it was assumed that the subgroup of 21 patients with either HPV alone ($n = 15$) or HPV and AIN ($n = 6$) were false positives according to Method 1, and the six with both HPV and AIN were false positives in Method 2. The absence of histology in all these 39 patients remains a potential source of error. Regarding possible inaccurate histopathological diagnosis it has been demonstrated that there may be considerable interobserver and intraobserver variability in the histopathological assessment of cervical biopsy specimens, particularly when differentiating between changes suggestive of HPV lesions and lower grade CIN lesions.¹⁹ In our study although intraobserver error is not ruled out, this is a potential problem that affects cervical histological diagnosis in general and not just our study. At present, histological review of colposcopically directed biopsies remains the gold standard in the diagnosis of CIN.

Cytological criteria for diagnosing anal HPV and grading AIN lesions are not as well established as those used for interpreting cervical smears although the same principles apply.^{20 21} Differentiating between features suggestive of HPV infection and low grade AIN lesions may be difficult as it can be in the interpretation of cervical cytology. The absence of koilocytosis in the presence of other features suggestive of HPV on anal smears concurred with the findings of other studies.^{14 15 17 18} It is conceivable that there may be additional ways in which anal and cervical cytology differ and that further experience may bring these to light.

What conclusions can we draw from this study? Our results suggest that although anal cytology is a sensitive technique for identifying patients with HPV related anal disease, on its own it is only infrequently able to differentiate between those patients who simply have anal condylomata and those who also have AIN, a task which requires anoscopy and biopsy. These results have important implications. At present the natural history of AIN and its possible ability to progress to anal carcinoma are unknown and there is a pressing need for well conducted natural history studies to determine the management of patients with this lesion. The ideal natural history study would use a non-invasive technique such as anal cytology both for the identification and follow-up of patients with AIN. If biopsy is required to differentiate patients with AIN from those who have HPV only, this could result in an alteration of the natural

history and perhaps even remove the lesion altogether. Studies of the natural history of CIN have demonstrated a significantly higher rate of regression and a lower rate of progression if histology as opposed to cytology is used as the diagnostic method.²²

Additional studies with anal cytology and anoscopy and biopsy performed in parallel on larger patient numbers possibly with different cytological sampling techniques are required to examine further the relative roles of anal cytology and histology in the detection of AIN. In the meantime we would suggest caution in the interpretation of different grades of abnormal anal cytological smears in this context if used in isolation without confirmatory anoscopy and biopsy.

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