Anal intraepithelial neoplasia in HIV+ men
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High Resolution Anoscopy: Clinical Features of Anal Intraepithelial Neoplasia in HIV-positive Men

Olivier Richel, Nora D.L. Hallensleben, Alexander Kreuter, Carel J.M. van Noesel, Jan M. Prins, Henry J.C. de Vries

Abstract

Background High resolution anoscopy (HRA) is increasingly advocated to screen HIV+ men who have sex with men (MSM) for anal cancer and its precursor lesions, anal intraepithelial neoplasia (AIN). A systematic comparison between clinical features and the histopathology of suspect lesions is lacking.

Objective To analyse interobserver agreement in classifying features of intra-anal lesions suspect for AIN and to compare these features with their histopathological outcome.

Design Cross sectional survey regarding high resolution anoscopy (HRA) with images and biopsies of suspect lesions. Two HRA-experienced dermatologists, blinded for histopathological outcome, independently classified the lesions on clinical features.

Setting Dermatology outpatient clinic of the Academic Medical Center in Amsterdam, The Netherlands

Patients 163 HIV+ men who have sex with men (MSM), older than 18 years, with no history of anal cancer

Main outcome measures Kappa coefficient for interobserver agreement and proportions of AIN per clinical feature.

Results In 163 patients 304 biopsies were taken. 168 biopsies (55%) showed AIN and 67/304 (22%) high grade (HG)AIN. The kappa-coefficient was 0.65 for condylomatous lesions, 0.14 for surface configuration, 0.54 for punctation, 0.08 for mosaicism, 0.43 for atypical vessels. Condylomatous lesions showed HGAIN in 18% (95%CI 11-27%). In lesions with flat leukoplakia, punctation and atypical vessels, HGAIN was seen in 25%, 30% and 23% respectively. In lesions with the combination punctation/atypical vessels and punctation/flat leukoplakia/atypical vessels HGAIN was found in 38% and 40% respectively.

Limitations We did not take biopsies of healthy looking mucosa. Further, real time description of features during HRA, instead of using images, would improve the recognition of subtle mucosal abnormalities.

Conclusions A moderate to substantial interobserver agreement was demonstrated in recognising condylomas, punctation and atypical vessels. Further, HGAIN is present in a high proportion of intra-anal condylomata. A combination of punctation, flat leukoplakia and atypical vessels is the best predictor for HGAIN.
Introduction

Anal cancer is an increasing problem among patients with HIV, especially among HIV positive men who have sex with men (MSM) with incidence rates of 65-109 per 100,000 person-years.\(^1\) This is much higher than the incidence of cervical cancer in HIV-negative women before standard cytological screening was introduced, and therefore routine screening for anal premalignant lesions is subject of discussion.\(^2\) Like cervical cancer, anal cancer is preceded by a precursor, called anal intraepithelial neoplasia (AIN). This is graded as AIN 1 (mild), AIN 2 (moderate) and AIN 3 (severe).\(^3\)\(^-\)\(^5\) AIN 1 is increasingly being referred to as low grade AIN (LGAIN) and AIN 2/3 as high grade AIN (HGAIN). The majority of HIV+ MSM has AIN and HGAIN is seen in 25-52%.\(^6\)\(^-\)\(^8\) For HGAIN progression rates to anal cancer have been reported to be 14 and 16% in HIV+ MSM, with median follow-ups of 2 and 5 years.\(^9\)\(^)\(^10\) The gold standard for AIN detection is high resolution anoscopy (HRA) in combination with biopsies and histopathological analysis of suspect lesions.\(^11\) Given the high prevalence of HGAIN and low specificity of anal cytology, HRA in combination with histopathological examination of lesional biopsies is likely to be the preferable first line (HG)AIN screening method in HIV+ MSM. Currently only a limited number of physicians are experienced in HRA, and training programs should be implemented to facilitate future AIN screening. However, HRA is a complicated procedure and no guidelines exist to identify lesions suspect for AIN. To our knowledge, only one clinically relevant paper on systemic comparison of clinical features with pathological outcome has been published.\(^12\)

More information on challenges and pitfalls in HRA are needed for the development of screening algorithms and guidelines. Until now, HRA evaluation of anal mucosa is based on guidelines for the colposcopic detection of cervical intraepithelial neoplasia (CIN). Yet experience from CIN screening cervical colposcopy is of limited use for HRA. For example, in the evaluation of CIN lesions, an overview image of the complete cervix can be obtained, allowing the evaluation of suspected lesions in one picture. In contrast, in the evaluation of AIN lesions an overview of the complete transformation zone in the anal canal cannot be obtained due to the folded nature of the anal mucosa. As a consequence, the anal transformation zone has to be evaluated in consecutive images to get a full picture of possible AIN involvement.
In this study of HRA in HIV+ MSM, we analysed interobserver agreement in classifying features of intra-anal lesions suspect for AIN and compared the features of lesions suspect for AIN with their histopathological outcome.

**Methods**

The study was performed at the HIV and dermatology outpatient clinics of the Academic Medical Center in Amsterdam, the Netherlands. HIV+ MSM older than 18 years were eligible if they did not have a history of anal cancer or current active inflammatory bowel disease. Patients who fulfilled the criteria were asked by their HIV physician to participate in our HRA cohort. They were not specifically referred for anal complains or previous anal pathology. Eligible for the present sub-study were participants in whom biopsies were taken from suspect lesions and good-quality images were available. All patients provided written informed consent and the study was approved by the local ethics committee.

A single HRA-experienced physician (OR) performed HRA as described previously, with magnification up to 17.7x. After application of acetic acid 3%, suspect intra-anal lesions (if present) were photographed and biopsied for histopathological analysis. A single pathologist (CVN) evaluated all biopsies, using Ki-67 and p16 immunostaining. The physician who performed HRA was not participating in the analysis of interobserver agreement, because of his knowledge of the participants and pathological outcomes. He blinded all images and marked the locations where biopsies were taken. Images that were out of focus were left out of the analysis. Two dermatologists experienced in HRA (HDV, AK), from two different centres, judged the images on macroscopic appearance of the suspect lesions without knowledge of the pathological outcome. Lesions were classified as being condylomatous or flat (non-condylomatous). This was followed by a sub-classification of flat lesions, distinguishing abnormal surface configuration (acetowhitening, flat leukoplakia, hyperkeratotic leukoplakia), punctation, mosaicism and atypical vessels as previously described for cervical suspect lesions.

In a first round, classification was performed by the two dermatologists independently from each other. These data were used in the interobserver agreement analysis. In a second classification round (consensus meeting), the two dermatologists jointly characterised the lesions they disagreed on in the
first round. After this second round, all lesions they agreed upon were linked to their pathological outcome. If no agreement was reached, lesions were excluded from further analyses. For each characteristic interobserver agreement was analysed by Cohen’s kappa coefficient, with standard error.\textsuperscript{17} For all lesional characteristics on which agreement was reached, the positive predictive value (PPV) for LGAIN and HGAIN was calculated, with 95% confidence intervals. This was repeated for clusters of characteristics. Statistical analyses were performed using SPSS software (version 16.0.2 for Windows; SPSS Inc., Chicago, IL, U.S.A.).

Results

Between March 2008 and December 2010, 650 HIV+ MSM were screened for eligibility, of which 191 were not interested, 14 fulfilled the exclusion criteria and 57 had significant comorbidity according to their treating physician. The remaining 388/650 (60%) HIV+ MSM were screened for AIN. Patients in whom no biopsies were taken (n=19) were left out, as well as patients in whom no HRA-guided images were taken (n=180) because no visual recording equipment was yet available, or images were of insufficient quality (n=26). The remaining 163/388 (42%) patients were included in the present analysis (Figure 1).

Figure 1. Inclusion flowchart. HRA=High Resolution Anoscopy
AIN was found in 116/163 patients (71%), HGAIN in 62/163 (38%). Baseline characteristics of the patients can be found in Table 1. In total 304 biopsies were taken. One-hundred and sixty eight (55%) showed AIN, and HGAIN was seen in 67/304 (22%). 301 pictures of the 304 lesions were analysed. The study flowchart is presented in Figure 2.

Table 1 Characteristics of participants. Data are medians (interquartile range) or proportions. cART=combined antiretroviral therapy; aas measured around the time of HRA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>163</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46 (41-52)</td>
</tr>
<tr>
<td>Duration since HIV diagnosis (years)</td>
<td>8 (3-14)</td>
</tr>
<tr>
<td>Percentage on cART</td>
<td>140/163 (86%)</td>
</tr>
<tr>
<td>CD4, in cells/μl</td>
<td>550 (430-710)</td>
</tr>
<tr>
<td>Detectable plasma HIV-RNA loada</td>
<td>34/163 (21%)</td>
</tr>
<tr>
<td>Plasma HIV-RNA load, in copies/mla</td>
<td>4702 (243-28615)</td>
</tr>
<tr>
<td>AIN</td>
<td>116/163 (71%)</td>
</tr>
<tr>
<td>HGAIN</td>
<td>62/163 (38%)</td>
</tr>
</tbody>
</table>

In the first, independent classification round, the two dermatologists agreed in 85% (259 of 304) of cases in classifying a lesion as condylomatous or flat. After correction for expected agreement this leads to a kappa coefficient of 0.65. In non-condylomatous (flat) lesions, surface configuration, punctuation, mosaicism and atypical vessels showed kappa coefficients of 0.14, 0.54, 0.08 and 0.43 respectively. The observed agreement for both surface configuration and mosaicism was 72% and 92% respectively, but the expected agreement was almost similar, leading to the poor kappa coefficient (Table 2).
Clinical features of AIN

Table 2 Kappa-coefficient between the two dermatologists per characteristic. SE=Standard Error; Surface configuration = acetowhitening, flat leukoplakia, hyperkeratotic leukoplakia.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Observed agreement</th>
<th>Kappa</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condyloma yes/no (n=304)</td>
<td>85%</td>
<td>0.65</td>
<td>0.05</td>
</tr>
<tr>
<td>Agreed non-condyloma (n=190):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface configuration</td>
<td>72%</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Punctation</td>
<td>83%</td>
<td>0.54</td>
<td>0.05</td>
</tr>
<tr>
<td>Mosaicism</td>
<td>92%</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Atypical Vessels</td>
<td>83%</td>
<td>0.43</td>
<td>0.08</td>
</tr>
</tbody>
</table>

In the second, joint round of classification the two dermatologists agreed upon all lesions but two (both healthy mucosa on histopathological analysis), which were excluded from the following analyses. Of the 302 lesions, 90 (30%) were classified as condylomatous. Of those, 13 (14%) did not show AIN, 61 (68%) showed LGAIN and HGAIN was seen in 16 (18%). Reversely, 24% of all HGAIN lesions were condylomatous.

Two hundred and twelve lesions of 302 (70%) were classified as flat. Of those, acetowhitening was seen in 28 (13%), flat leukoplakia in 161 (76%), hyperkeratotic leukoplakia in 20 (9%), punctation in 151 (71%), mosaicism in 9 (4%) and atypical vessels in 44 (21%). Histopathological analysis showed HGAIN in 51/212 of all flat lesions (PPV: 24%). None of the lesions with mosaicism showed HGAIN, whereas lesions with punctation showed a PPV of 30% for HGAIN, lesions with flat leukoplakia a PPV of 25% for HGAIN and atypical vessels a PPV of 23% for HGAIN. Reversely, 70% of all HGAIN lesions showed punctation and 61% flat leukoplakia. Among flat (non-condylomatous) HGAIN lesions only, this was even 92% and 80% respectively. When combining characteristics, the combination of punctation, flat leukoplakia and atypical vessels showed the highest PPV for HGAIN (40%) (Table 3). Examples of clinical features with pathological diagnosis are presented in figure 3.
Table 3 Positive predictive value (PPV) for LGAIN (low grade AIN) and HGAIN (high grade AIN) per clinical feature.

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Total N</th>
<th>PPV for LGAIN per feature (95%CI)</th>
<th>PPV for HGAIN per feature (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condylomatous lesions</td>
<td>90</td>
<td>68% (57-77)</td>
<td>18% (11-27)</td>
</tr>
<tr>
<td>Flat lesions</td>
<td>212</td>
<td>19% (14-25)</td>
<td>24% (18-30)</td>
</tr>
<tr>
<td>Acetowhitening</td>
<td>28</td>
<td>21% (10-40)</td>
<td>21% (10-40)</td>
</tr>
<tr>
<td>Flat Leukoplakia</td>
<td>161</td>
<td>17% (12-24)</td>
<td>25% (19-32)</td>
</tr>
<tr>
<td>Hyperkeratonic Leukoplakia</td>
<td>20</td>
<td>25% (11-47)</td>
<td>15% (4-37)</td>
</tr>
<tr>
<td>Punctation</td>
<td>151</td>
<td>19% (13-26)</td>
<td>30% (24-38)</td>
</tr>
<tr>
<td>Mosaicism</td>
<td>9</td>
<td>56% (27-81)</td>
<td>0% (0-27)</td>
</tr>
<tr>
<td>Atypical vessels</td>
<td>44</td>
<td>18% (9-32)</td>
<td>23% (13-37)</td>
</tr>
<tr>
<td>Punctation and flat leukoplakia</td>
<td>119</td>
<td>18% (12-26)</td>
<td>32% (24-41)</td>
</tr>
<tr>
<td>Punctation and atypical vessels</td>
<td>21</td>
<td>24% (10-45)</td>
<td>38% (21-59)</td>
</tr>
<tr>
<td>Punctation, flat leukoplakia and atypical vessels</td>
<td>15</td>
<td>13% (2-39)</td>
<td>40% (20-64)</td>
</tr>
</tbody>
</table>

Discussion

In this study, we analysed the inter-observer agreement in classifying features of intra-anal lesions suspect for AIN, and compared these features with their histopathological outcome.

To our knowledge, no previous studies exist for HRA evaluating the inter-observer agreement in classifying lesions. Here, we found a substantial inter-observer agreement on condylomatous lesions, with a kappa coefficient of 0.65. In flat lesions, the kappa varied between poor to moderate agreement (kappa from 0.08-0.54), with moderate consensus being reached for punctation and atypical vessels. This is better than the poor agreement seen in classifying cervical lesions suspect for CIN.18;19

After the independent classification, consensus between the 2 observers was reached on all lesions but 2. In the 90 condylomatous lesions found in this study, 18% showed HGAIN, which is considerable for an assumed benign abnormality. Two previous studies retrospectively looked at surgically removed anal condylomata in HIV+ MSM and found HGAIN in 47% and 52% respectively.20;21 However, these studies only involved surgically removed condylomata classified as ‘large, multiple’ and ‘severe condylomatous disease’. In our study, all condylomatous lesions were intra-anal and smaller than 1 cm.
Clinical features of AIN

Figure 3. High resolution anoscopy-guided images of suspect lesions with clinical features and pathological outcome

A: condylomatous; LGAIN
B: condylomatous; HGAIN
C: hyperkeratotic leukoplakia and punctation; HGAIN
D: acetowhiteness and punctation; HGAIN
E: Punctuation and atypical vessels; HGAIN
F: Mosaic; LGAIN
A third study showed HGAIN in 22% of recurrent condylomata in HIV+ patients.\textsuperscript{22} We found one study comparable to ours, which showed a HGAIN rate of 22% (8/37) in discrete warts and 30% (7/23) in extensive warts, in asymptomatic HIV+ and HIV- men.\textsuperscript{23} Our study confirms this considerable degree of HGAIN in discrete, asymptomatic condylomata in a large cohort HIV+ MSM. The implication of these observations is that condylomata in this group should always be biopsied and examined by histopathology. It seems justified to always treat asymptomatic condylomata in HIV+ individuals, not to miss HGAIN. Presently, both the 2010 CDC guideline on anogenital warts and the 2012 IUSTI European guideline on anogenital warts do not recommend this.\textsuperscript{24,25}

In flat (non-condylomatous) lesions, punctation as a single characteristic was the best predictor for HGAIN, with a percentage of 30%, followed by flat leukoplakia (25%) and atypical vessels (23%) as single characteristics. A combination of punctation, flat leukoplakia and atypical vessels had the highest PPV of HGAIN (40%), followed by punctation and atypical vessels (38%). Alternatively, the vast majority of HGAIN lesions had punctation.

A recent study in HIV+ patients showed that HGAIN was associated with dense acetowhitening and flat, smooth, non-papillary lesions. However, HGAIN was only seen in 18 of 128 participants and the ratio of men and women was not specified.\textsuperscript{26} In a much larger study from 1997, macroscopic features of 385 biopsies of 152 MSM (vast majority HIV+) were compared with pathological outcome.\textsuperscript{12} It showed HGAIN in 43% of lesions with punctation. A combination of flat acetowhitening and fine punctation showed HGAIN in 50% and in case of flat acetowhitening and coarse punctation 49% contained HGAIN. In the same study, 8% of lesions with punctation represented normal mucosa, while in our study 51% was non-dysplastic. We took more biopsies of fine, regular punctation, which, like on the cervix, can be a feature of normal metaplastic epithelium of the transformation zone. In our experience, patterns of punctation are difficult to recognise and sub-characterising could lead to lower reproducibility. Another difference with our study is the frequency of mosaicism. We only found mosaicism in 3%, none of which represented HGAIN. In contrast, Jay et al found mosaicism in 20%, 47% of which was HGAIN. We think that this difference, as well as the difference in non-dysplastic punctation mentioned above, might be explained by the fact that in our study features were described using images of abnormalities, while in Jay’s study features were described during HRA. In this
case the lesion can be looked at in real time, which obviously leads to better recognition of subtle vascular abnormalities.\(^{12}\)

Strong points of our study are the high number of patients, a single, well trained pathologist evaluating all biopsies and the fact that two HRA experienced dermatologists independently characterised all lesions. A limitation of our study is that we did not take biopsies of healthy looking mucosa. In addition, we did not evaluate the use of Lugol-staining of the mucosa, which is an alternative for acetic acid. Further, describing features in real time during high resolution anoscopy, instead of using images, would improve the recognition of subtle mucosal abnormalities.

In conclusion, our study shows that moderate to substantial inter-observer agreement between two HRA-experienced physicians can be reached. Secondly, our study underscores the need of histopathological evaluation of anal condylomatous lesions in HIV+ men and shows that flat lesions, punctation and flat leukoplakia are important signs for HGAIN. These findings may be helpful in establishing evidence-based HRA screening guidelines. To improve knowledge on clinical features of AIN, we think that future studies should focus on real time description of lesions with more than two independent observers. Furthermore, besides suspect lesions, also biopsies of normal looking mucosa should be taken for the development of a solid predictive model for AIN.
Chapter 5

References


