Anal chromoendoscopy using gastroenterological video-endoscopes: A new method to perform high resolution anoscopy for diagnosing intraepithelial neoplasia and anal carcinoma in HIV-infected patients

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Anal chromoendoscopy using gastroenterological video-endoscopes: A new method to perform high resolution anoscopy for diagnosing intraepithelial neoplasia and anal carcinoma in HIV-infected patients

Anale Chromoendoskopie mittels gastroenterologischer Video-Endoskopie: Eine neue Methode zur Durchführung der hochauflösenden Anoskopie in der Diagnostik der intraepithelialen Neoplasie und des Analkarzinoms bei HIV-infizierten Patienten

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ZUSAMMENFASSUNG

Einleitung Das Analkarzinom stellt ein zunehmendes Problem bei HIV-infizierten Patienten dar. Seine Vorläuferläsion, die anale intraepitheliale Neoplasie (AIN), wird gegenwärtig durch die hochauflösende Anoskopie (HRA) diagnostiziert, bei der eine gynäkologische Vergrößerungsoptik eingesetzt wird (Kolposkopie). Die vorliegende prospektive Studie untersucht die anale Chromoendoskopie (ACE) mit gastroenterologischen Video-Endoskopen in der Diagnostik der AIN.
Methoden Nach rektal-digitaler Austastung, einer Proktoskopie sowie der Oberflächenfärbung mit Essigsäure und Lugol'scher Lösung wurde die ACE mithilfe einer auf die Endoskopspitze aufgesetzten Mukosektomiekappe durchgeführt. Biopsien wurden von Arealen mit pathologischem Färbeverhalten und von unauffälligen Bezirken entnommen, als Referenz diente die kombinierte Analyse der Biopsiebefunde.
Ergebnisse Von 2007 bis 2013 wurden 211 Patienten eingeschlossen. 95,7 % waren männlich, das mediane Alter betrug 45 Jahre, bei 86,7 % war die HIV-Infektion durch Sex unter Männern erworben, eine kombinierte antiretrovirale Therapie wurde bei 75,8 % angewandt. Die Sensitivität der ACE für die Diagnose der AIN betrug 0,85, die Spezifität 0,55, der positive prädiktive Wert lag bei 0,50 und der negative prädiktive Wert (NPV) bei 0,87. Die diagnostische Ausbeute nahm für Patienten mit hochgradiger Neoplasie (NPV: 0,99) und in der zweiten Studienperiode von 2011 bis 2013 zu. Nebenwirkungen waren selten und von geringer klinischer Relevanz.
Schlussfolgerung Die ACE ist sicher und nützlich in der Diagnostik der AIN bei HIV-Infizierten. Sie ist insbesondere geeignet, um hochgradige anale Läsionen auszuschließen, die mit dem höchsten Risiko eines Progresses zum Karzinom einhergehen. Die ACE hat das Potenzial, zu einer wertvollen Methode des Managements der AIN und der Prävention des Analkarzinoms bei HIV-Positiven zu werden.

ABSTRACT

Introduction Anal carcinoma represents an increasing problem in HIV-infected patients. Anal intraepithelial neoplasia (AIN), the precursor lesion, is currently diagnosed by high-resolution anoscopy (HRA) using optical magnification derived from gynecological colposcopy. This prospective study evaluates anal chromoendoscopy (ACE) using standard gastroenterological video-endoscopes in diagnosing AIN.
Methods After clinical examination, proctoscopy and surface staining with acetic acid followed by Lugol's solution, ACE was performed with a mucosectomy cap on the tip of the endoscope. Biopsy specimens

were collected from areas with a pathological staining pattern and from areas with normal appearance; combined results were considered as reference.

Results Two hundred eleven HIV-positive patients seen between 2007 and 2013 were evaluated. Of these, 95.7 % were males, and the median age was 45 years. In 86.7 %, the mode of HIV transmission was sex among males. Combination antiretroviral treatment was applied in 75.8 %. The sensitivity of ACE in diagnosing AIN was 0.85, the specificity was 0.55, the positive predictive value was 0.50, and the negative predictive value (NPV) was 0.87. Diagnostic performance increased in individuals with high-grade lesions (NPV: 0.99) and in the second study period from 2011 to 2013. Side effects were rare and of minor clinical relevance.

Conclusions Anal chromoendoscopy is safe and effective in diagnosing AIN in a population of HIV-infected patients. It is particularly useful for the exclusion of high-grade lesions that have the strongest risk of progression to anal carcinoma. Therefore, ACE may become a valuable new tool to manage AIN and to prevent anal malignancy in HIV-positive patients.

Introduction

In the majority of HIV-infected patients, combination antiretroviral therapy (cART) is likely to control the disease virologically and immunologically. However, malignant diseases are nowadays becoming a significant threat in the population of ageing HIV positives. In this field, anal carcinoma is a serious and increasing problem with an estimated incidence of 42 to 137 cases/100 000 person-years [1, 2]. Compared to the general population, HIV patients are more likely to suffer from anal carcinoma by a factor of up to 100 [2]. An even higher risk for anal malignancy was found in men who have sex with men (MSM), older patients, smokers, groups with a high number of sexual contacts and sexual partners, and individuals with a history of a low CD4 cell-count nadir [3 – 6]. A predisposing infection with human papillomavirus (HPV) was demonstrated in up to 98 % of individuals with anal carcinoma [4, 7 – 9]. Human papillomavirus types with a high risk for malignant progression are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, respectively [10, 11].

As in many other HPV-associated tumors, there is a sequence from persistent infection over low to high-grade anal intraepithelial neoplasia (AIN) and finally to invasive cancer [12]. Hence, these cancers may be prevented by diagnosing and treating their precursor lesions. These can be found in a large and continuously increasing number of HIV-infected patients [11, 13]. Depending on the HIV subgroup, high grade AIN (HGAIN) was described in 13 – 52 % in MSM, 15 % in heterosexual patients, 11 % in females, and in 23 – 27 % in unselected groups [3 – 5, 7]. An influence of cART could not be demonstrated. Notably, in one study the median time of progression to anal carcinoma in patients who refused treatment of HGAIN was estimated at 8.6 months [4]. A similar trial on patients examined cytologically showed a progression to cancer within a period of 57 – 64 months [14]. Severe anal dysplasia (HGAIN or AIN 3) is associated with the highest risk of short-term anal cancer, whereas lower grades of AIN may also develop spontaneous regression [4, 14, 15]. In summary, anal dysplasia is a common and increasing disorder in HIV positives and therefore has to be diagnosed and treated properly to prevent anal cancer.

High resolution anoscopy (HRA) has become the standard examination to diagnose or exclude AIN [16]. After staining the anal canal with 3 % acetic acid with or without subsequent Lugol's iodine solution, the anal canal and the transformation zone are visualized with an optical magnification device normally used for gynecological colposcopy. Site-directed biopsies are taken from areas with pathological staining pattern. Despite considerable experience with this method, the diagnostic value has not yet been defined given the fact that parameters to determine accuracy do not exist [17]. A number of studies used HRA as a reference to evaluate screening cytology with or without HPV determination, but only 1 study tried to apply an additional histological feature in order to better characterize the performance of HRA [18]. Moreover, the method is applied in only a limited number of medical sites and has a flat learning curve [2, 16]. Due to its complexity, HRA is not suitable for screening, which is usually performed using anal cytology [17]. The latter is routine in Germany according to current national guidelines [19].

From the gastroenterologist's point of view, visualization of the anus with a standard video-endoscope may be as useful as with a colposcopic setup because of good optical technology and similar extent of magnification. Therefore, we decided to use standard flexible gastroenterological endoscopes to perform HRA in HIV-infected patients. The study evaluates chromoendoscopy of the anorectum in a cohort of HIV-positive patients for diagnosis of anal dysplasia and cancer.

Methods

In a prospective cross-sectional study, HIV-infected patients underwent endoscopy of the anus and the rectum for detection of anal dysplasia or cancer. Inclusion criteria were HIV infection, age above 18 years, and willingness to participate after informed consent. All individuals were examined in left side position with propofol sedation; monitoring was performed with blood pressure measurement and pulse oxymetry. Before endoscopy, anal brush specimens were taken for epithelial cytology and HPV determination as well as genotyping [4, 8]. Subsequently, anal inspection and rectal-digital examination were added, followed by standard proctoscopy. In this step, acetic acid (3 %) and Lugol's solution were applied to the surface of the anal canal according to published routine [2]. After that, a video-endoscope for colonoscopy (Fujinon®, EC-590- and 600-series; Olympus®, CV-180-series) was used to visualize the rectum and the anal canal. The distension of the area was achieved by attaching a mucosectomy resection-cap to the tip of the endoscope. Biopsies were taken from areas with reduced uptake of iodine staining (suspected dysplasia—targeted or site-directed biopsy) and areas with normal appearance of the anal canal (random biopsy, at least 2 specimens). In cases of pathological findings, treatment as appropriate (e. g., loop resection or electrocautery) was carried out. The latter, however, was not subject of the presented study. In a subset of patients, the anal canal was additionally visualized using virtual

chromoendoscopy (FICE®: Fujinon intelligent chromoendoscopy) after staining with acetic acid and Lugol's solution.

Patients' characteristics, endoscopic findings, as well as results of cytology, histology, and virology were recorded as shown in the results section and in ▶ **Table 1, 2**. Diagnostic performance was estimated by comparing the optical impression of chromoendoscopy with the biopsy results. As targeted and random biopsies were used, areas with pathological appearance and areas with normal macroscopic impression were evaluated separately histologically. The combination of these histological findings was used as a reference to detect AIN and to exclude missed areas of dysplasia as much as possible. High resolution anoscopy has to be performed in a large number of cases so as to have enough experience for good diagnostic yield. Therefore, the dataset was divided into 2 periods (2007 – 2010 and 2011 – 2013) to find out if quality improves with time.

Endoscopies were carried out by 5 gastroenterological endoscopists in 2 centers; they were all trained by 1 clinician (MO). Each patient gave informed consent, and the 2 relevant institutional review boards rendered positive approval of the study design. The trial was supported by a national network for AIDS research (Klinische Arbeitsgemeinschaft AIDS Deutschland [KAAD]).

Data analysis was performed on individuals with complete histological results (targeted and random biopsies). For the statistical evaluation, SPSS, release 19, was used. Univariate comparisons were computed with Wilcoxon rank sum test or Fisher's exact test, where appropriate. A p-value of ≤ 0.05 was regarded as significant; adjustment for multiple testing was not applied. Multivariate analysis considering predictors for the diagnosis of anal dysplasia was done by logistical regression.

Results

Between 2007 and 2013, a total of 228 consecutive patients were screened for the study. Of these, complete histological data were available in 211 cases. This group was further evaluated for diagnostic output of video-chromoendoscopy. Two individuals declined participation.

Patients' characteristics are demonstrated in ▶ **Table 1**. In ▶ **Table 2**, optical and histological findings as well as results of cytology and virology are shown. 52.6 % of anal chromoendoscopies revealed a pathological staining pattern of the anus, whereas dysplasia was suspected in only 6.2 % of proctoscopical examinations (inspection before anal staining). Altogether, dysplasia was confirmed histologically in 34.6 % of all cases. ▶ **Fig. 1 – 6** display examples of endoscopic findings.

▶ **Table 3** describes the diagnostic outcome performance of anal chromoendoscopy (ACE) on the basis of combined histological workup of targeted and random biopsies. Approximately 60 % of the participants were analyzed in the second time period of the study. Diagnostic efficacy improved in this period, resulting in a higher sensitivity and negative predictive value (▶ **Table 3**). Overall accuracy was 0.65 in the whole study and 0.64 in period 2. Cor-

▶ **Table 1** Patients' characteristics.

parameter		results	
participants	whole study	n (%)	211 (100)
	period 2 (2011 – 2013)		132 (62.6)
gender	male	n (%)	202 (95.7)
	female		9 (4.3)
age	years	median (range)	45 (24 – 75)
duration of HIV infection	years	median (range)	8 (0 – 27)
transmission groups	MSM	n (%)	183 (86.7)
	heterosexual	n (%)	6 (2.8)
	endemic region	n (%)	5 (2.4)
	i. v. drug abuse	n (%)	5 (2.4)
	unknown	n (%)	12 (5.7)
CDC-stage	A	n (%)	85 (40.3)
	B	n (%)	46 (21.8)
	C	n (%)	77 (36.5)
ART at inclusion		n (%)	160 (75.8)
duration of ART	years	median (range)	2 (0 – 19)
CD4 cell count	/µL	median (range)	500 (20 – 1900)
HI-viral load	/mL	median (range)	19 (19 – 2 160 000)

MSM: Men who have sex with men, CDC: Centers for Disease Control, ART: Antiretroviral treatment.

▶ **Table 2** Diagnostic findings.

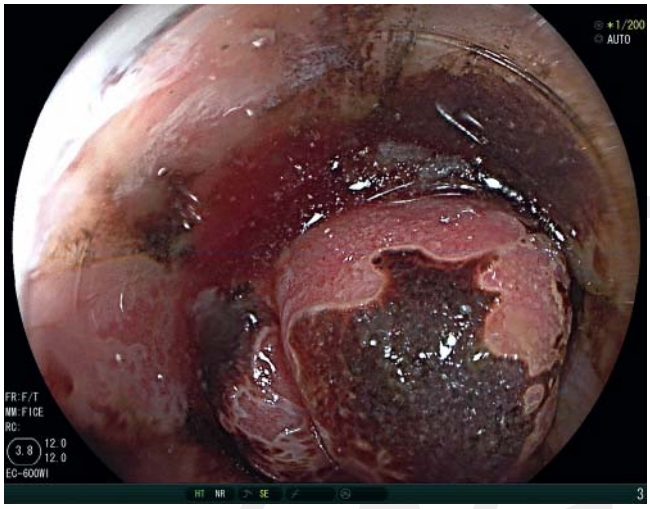
examination	finding	results [n (%)]
video-chromoendoscopy (histological results of areas with suspected dysplasia, targeted biopsies)	AIN 1	24 (11.4)
	AIN 2	30 (14.2)
	AIN 3	7 (3.3)
	inflammation	15 (7.1)
	hyper-/parakeratosis	14 (6.6)
	squamous cell hyperplasia, regenerate epithelium	12 (5.7)
	condyloma acuminata	8 (3.8)
	rectum mucosal islet	1 (0.5)
	normal appearance, no biopsy	100 (47.4)
video-chromoendoscopy (histological results of random biopsies)	AIN 1	15 (7.1)
	AIN 2	10 (4.7)
	AIN 3	1 (0.5)
	inflammation	36 (17.1)
	hyper-/parakeratosis	11 (5.2)
	squamous cell hyperplasia, regenerate epithelium	21 (10.0)
	condyloma acuminata	10 (4.7)
	squamous cell carcinoma	1 (0.5)
	normal	106 (50.2)
combined histological examination (targeted and random biopsies)	AIN 1	32 (15.2)
	AIN 2	34 (16.1)
	AIN 3	8 (3.8)
anal cytology	unspecific/normal	127 (60.2)
	ASCUS	3 (1.4)
	LSIL	51 (24.2)
	HSIL	23 (10.9)
	SCC	1 (0.5)
	others/missing	7 (3.3)
virology	HPV-PCR positive	173 (90.1)
	HPV-11	22 (11.5)
	HPV-16	71 (37.0)
	HPV-18	34 (17.7)
	HPV-45	22 (11.5)
	HPV-66	14 (7.3)
	other HPV-types (29)	< 10 (< 4.7)

AIN: Anal intraepithelial neoplasia, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma, HPV-PCR: Human papilloma-virus polymerase chain reaction.

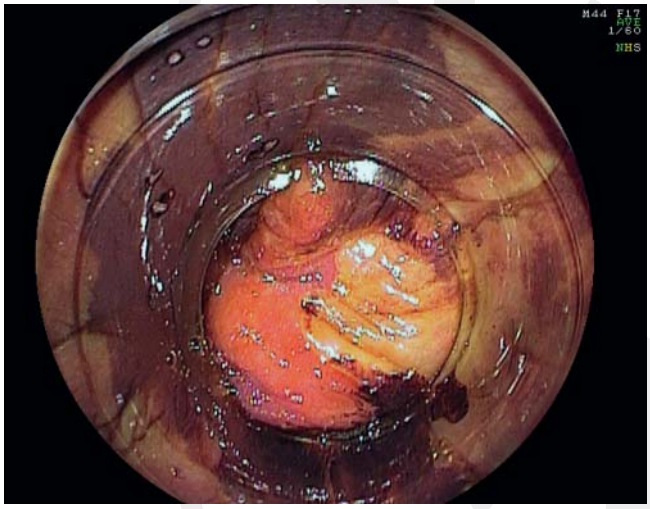
responding to these data, the values of anal cytology in the whole study group were as follows: sensitivity: 0.56; specificity: 0.75; positive predictive value: 0.55; negative predictive value: 0.76; accuracy: 0.69.

Notably, anal dysplasia was found in 15.1 % of the biopsies with normal endoscopical appearance of anoderma. This corresponds to 11 cases, 8 with AIN 1, 2 with AIN 2, and 1 with AIN 3. Moreover, an incipient squamous cell carcinoma was identified in one individual with normal impression of anoderma. In the second study period, AIN in normal epithelium was diagnosed in 7.1 %.

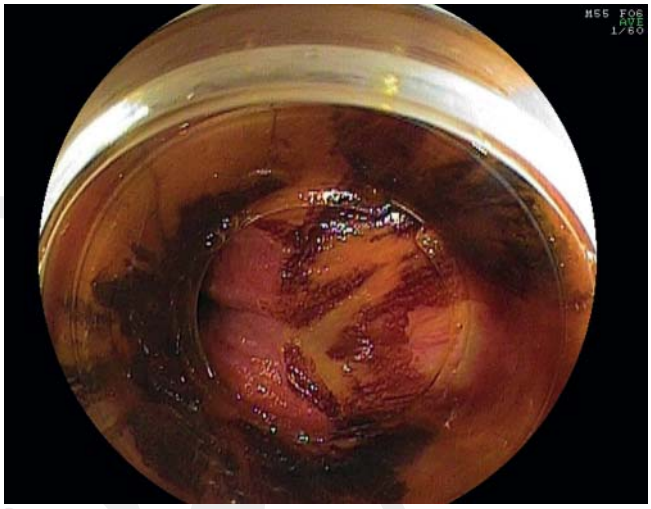
The detailed distribution of examination results in the subgroups with and without proven AIN is displayed in ▶ **Table 4**. In an attempt for better characterization of predictors for the presence of AIN, a model of a multivariate analysis was fitted to the



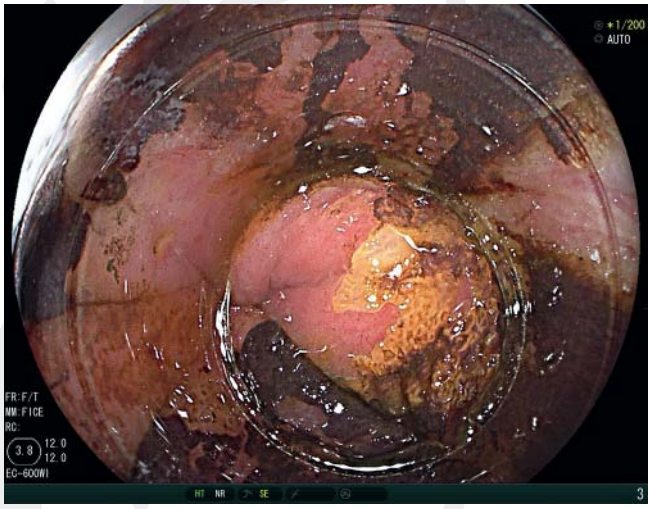
▶ **Fig. 1** Normal appearance of anal epithelium (brown) in comparison to rectum mucosa after application of Lugol's solution.



▶ **Fig. 2** Anal intraepithelial neoplasia (AIN 1, yellow-orange) after staining with acetate and Lugol's solution (pink: rectum mucosa; brown: normal anal epithelium).



▶ **Fig. 3** Anal intraepithelial neoplasia (AIN 1, yellow-orange) after staining with acetate and Lugol's solution (pink: rectum mucosa; brown: normal anal epithelium).



▶ **Fig. 4** Anal intraepithelial neoplasia (AIN 2, yellow) after staining with acetate and Lugol's solution (pink: rectum mucosa; brown: normal anal epithelium).

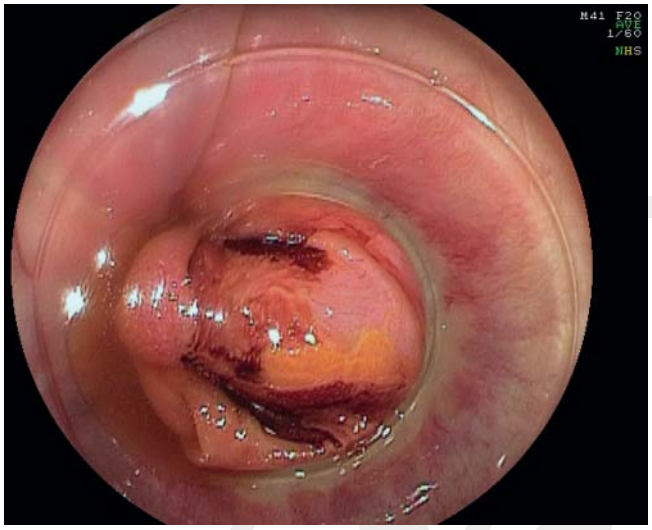
data. Parameters showing a clear association with anal carcinoma in the studies discussed in the introduction (age, HIV transmission group of MSM, low CD4 cell count, HPV positivity) were included into the model. Correspondingly, these items could be shown to be significantly associated with AIN in our univariate analyses (▶ **Table 4**). HI viral load was also added to the multivariate model because of its association with AIN (▶ **Table 4**). As a result, only HPV positivity (odds ratio [OR]: 8.48; 95 % confidence interval [95 % CI]: 1.07 – 66.95; p = 0.04) and a CD4 cell count of less than 500/µL (OR: 2.48; 95 % CI: 1.23 – 4.97; p = 0.01) were identified as risk factors for the later diagnosis of AIN.

Virtual chromoendoscopy (FICE®) was performed in 86 individuals. The method was able to discriminate pathological from normal anoderma (data not shown), but it did not identify any case with anal dysplasia that was not seen in white light endoscopy.

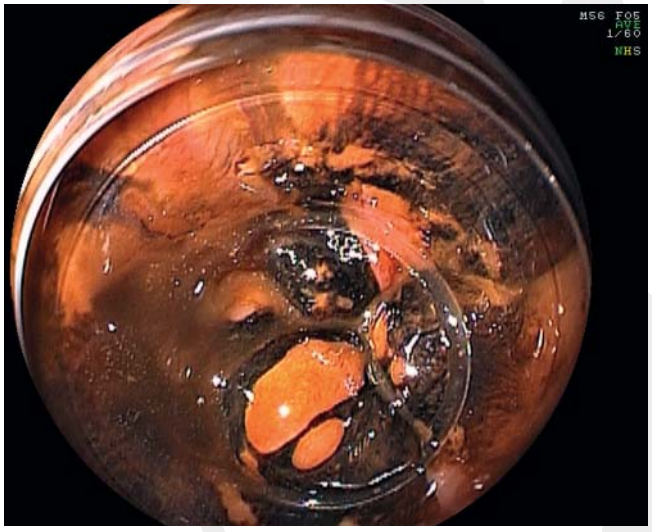
Side effects were bleeding from biopsy sites (n = 12) and extra-anal skin irritation from Lugol's solution (n = 1). However, bleeding stopped in all cases spontaneously, and the skin irritation resolved completely without treatment. No serious adverse event occurred.

Discussion

Nowadays, anal carcinoma is diagnosed far more often in HIV-infected patients than in the normal population. It represents a substantial and continuously growing burden of disease. Anal carcinoma may be prevented by early diagnosis and treatment of its precursor lesion: AIN. Besides anal cytology as a screening option, HRA is currently the standard method to confirm or exclude the presence of AIN. A magnification device used in gynecological colposcopy is used to perform the examination of HRA. Considering



▶ **Fig. 5** Anal intraepithelial neoplasia (AIN 3, yellow-orange) after staining with acetate and Lugol's solution (pink: rectum mucosa on the left side; light pink on the right side: perianal skin; brown: normal anal epithelium).



▶ **Fig. 6** Several rectum mucosa islets in stained anal epithelium (no AIN).

▶ **Table 3** Diagnostic performance of anal chromoendoscopy (ACE) in detecting anal dysplasia.

ACE	dysplasia (all stages)	AIN 1	AIN 2	AIN 3
	complete study (2007 – 2013)			
sensitivity	0.85	0.75	0.94	0.88
specificity	0.55	0.44	0.48	0.42
positive predictive value	0.50	0.19	0.26	0.06
negative predictive value	0.87	0.91	0.98	0.99
	cases of phase 2 (2011 – 2013)			
sensitivity	0.93	0.87	0.96	1.0
specificity	0.51	0.40	0.44	0.38
positive predictive value	0.47	0.16	0.27	0.05
negative predictive value	0.94	0.96	0.98	1.0

AIN: Anal intraepithelial neoplasia.

the excellent optical output of video-endoscopes, we modified HRA by applying endoscopical techniques in order to achieve a good diagnostic performance. This prospective study presents the evaluation of the new method of ACE.

The study recruited predominantly middle-aged males. The most common transmission mode of HIV infection was sex among males. The majority of patients were treated effectively with contemporary antiretroviral therapy; therefore, only a small number were at the stage of AIDS, and the median viral load was low. Anal dysplasia was diagnosed in 34.6 %, a result that is well in line with other studies (see introduction). The majority of dysplastic lesions were found to be AIN 1 and 2, and AIN 3 was seen in only 3.8 %. The diagnostic output of video-chromoendoscopy was good, as shown in ▶ **Table 3**. The general sensitivity (0.85) and negative predictive values (NPV: 0.87) of the method were excellent, especially for high-grade anal dysplasia (NPV: 0.99). Thus, ACE is effective in finding anal lesions and very effective in excluding HGAIN. However, the method has only a moderate specificity (0.55) and a suboptimal positive predictive value (0.50), showing that a suspicious lesion may be subject to a number of differential diagnoses (mostly inflammatory lesions and parakeratosis). Therefore, it is not suitable to perform the examination without histological confirmation of pathological staining patterns (also in case of HRA). On the other hand, a normal impression of the anal canal makes histological workup unnecessary. More efforts have to be undertaken to improve the prediction of pathology by optical impression.

The national German-Austrian guidelines on anal dysplasia and anal cancer in HIV positives recommend HRA or anal endoscopy in the case of suspicious anal cytology in screening [19]. On the one hand, anal visualization has to detect lesions that represent dysplasia or cancer in order to treat these. On the other hand, it has to exclude anal dysplasia given the fact that anal cytology has limited accuracy itself [17]. We could demonstrate that ACE is useful to manage the first task as it detects anal abnormalities effectively. However, it is not completely satisfactory because of the discussed limited specificity. Furthermore, ACE is very effective in handling the second task because of the good negative predictive value, especially in the case of high-grade lesions. Following a learning curve of all investigators, the NPV for AIN 3 reached 1.0 in the second period of the study. As discussed above, anal cancer predominantly proceeds from AIN 3. Consequently, a normal impression of the anal canal in ACE almost certainly excludes clinically relevant AIN.

Currently there is substantial evidence regarding HRA as a confirmatory examination after anal screening cytology, which may be abnormal in more than 40 % [5]. To our knowledge, only one

▶ **Table 4** Predictors of anal dysplasia, univariate analyses. Differences of distribution in subgroups with or without anal dysplasia, determined by combined targeted and random biopsies.

parameter			distribution in subgroups		p-value
			patients with proven AIN	patients without AIN	
gender	male	n (%)	72 (35.6)	130 (64.4)	0.17
	female		1 (11.1)	8 (88.9)	
age		years [median (range)]	44.5 (25 – 73)	44 (24 – 75)	0.61
duration of HIV infection		years [median (range)]	6 (0 – 27)	8 (0 – 27)	0.07
transmission groups	MSM	n (%)	66 (36.1)	117 (63.9)	0.29
	others		7 (25.0)	21 (75.0)	
CDC-stage	non-AIDS	n (%)	39 (29.8)	92 (70.2)	0.10
	AIDS		32 (41.6)	45 (58.4)	
ART at inclusion	yes	n (%)	56 (35.0)	104 (65.0)	0.86
	no		16 (32.7)	33 (67.3)	
duration of ART		years [median (range)]	1 (0 – 19)	2 (0 – 19)	0.17
CD4 cell count		/µL [median (range)]	419 (20 – 1630)	533 (59 – 1900)	< 0.001
HI-viral load		/mL [median (range)]	19 (19 – 740 000)	19 (19 – 2160 000)	0.035
anal chromoendoscopy (optical impression)	dysplasia suspected	n (%)	62 (50.0)	62 (50.0)	< 0.001
	normal appearance		11 (12.6)	76 (87.4)	
anal cytology	any dysplasia	n (%)	41 (54.7)	34 (45.3)	< 0.001
	normal cytology		32 (23.5)	104 (76.5)	
HPV determination	HPV-11 positive	n (%)	13 (59.1)	9 (40.9)	0.03
	HPV-11 negative		56 (32.9)	114 (67.1)	
	HPV-16 positive	n (%)	35 (49.3)	36 (50.7)	0.005
	HPV-16 negative		34 (28.1)	87 (71.9)	
	HPV-18 positive	n (%)	20 (58.8)	14 (41.2)	0.003
	HPV-18 negative		49 (31.0)	109 (69.0)	
	HPV-45 positive	n (%)	14 (63.6)	8 (36.4)	0.008
	HPV-45 negative		55 (32.4)	115 (67.6)	
	HPV-66 positive	n (%)	9 (64.3)	5 (35.7)	0.04
	HPV-66 negative		60 (33.7)	118 (66.3)	
	other genotypes	no significant difference			

AIN: Anal intraepithelial neoplasia, HPV: Human papilloma virus, PCR: Polymerase chain reaction.

study exists that evaluates HRA diagnostic performance itself in an HIV-infected patient population [18]. In this trial (n = 128), combined random and targeted biopsies of the anal epithelium were the reference standard for HRA. This approach is the same as in our study, and not surprisingly, the results are comparable with our findings. The sensitivity of HRA was 0.90, the specificity was 0.19, the positive predictive value was 0.42, and the negative predictive value was 0.75. Therefore, we conclude that the diagnostic efficacy of ACE is similar to HRA in the detection of AIN in HIV-positive patients. Given the fact that HRA is performed in only a small number of centers and flexible endoscopes are widely available, this is a strong indicator for the potential use of ACE to reach the target patient population everywhere.

Biopsies from normal epithelium identified AIN in 15.1 % of the complete study and 7.1 % of the second period. This may be considered high, as a substantial number of cases with a treatment option would be missed. However, another trial also found 9.8 % of HGAIN in random biopsies during HRA [20]. In only 1 patient, AIN 3 was detected by random biopsy. A potential benefit from random biopsies is therefore of limited use. In analogy to this, a low value of 4-quadrant biopsies versus targeted biopsies in the detection of metaplasia or dysplasia in Barrett's esophagus was described [21]. Meta-analyses of chromoendoscopy using methy-

lene-blue/acetic acid or virtual chromoendoscopy in this setting have shown contradictory results [22]. However, chromoendoscopy was shown to reduce the number of biopsies necessary for the detection of high-risk Barrett's esophagus [22], which is similar to the topic of the presented data.

The most prevalent HPV types were HPV 16 and 18 with 37.0 % and 17.7 %, respectively. This result is similar to another study that found these subtypes in 23 % and 10 % [23]. As discussed above, several HPV types may be typically characterized in cases with AIN. Corresponding to this, the types 16, 18, and 45 were also linked to AIN in our study. Additionally, we were able to show that the types 11 and 66 were significantly associated with this diagnosis. Combined with a low CD4 cell count, HPV infection was the most relevant predictor of AIN in multivariate analysis. However, routine HPV diagnostics seem to be of limited value, as more than 90 % of HIV patients show persistent infection. Anal endoscopy should be recommended especially to patients with immune deficiency, as low CD4 cells counts have an unfavorable effect on the development of malignancy.

Some limitations of the study have to be kept in mind. Firstly, it did not contain a pure screening population. This means that patients with anal symptoms were also accepted for inclusion. Consequently, other diagnoses like inflammation or condylomata acuminata were seen quite often. This may lead to broader differential diagnoses of anal abnormalities and consequently to an inferior diagnostic output of ACE. However, in a further analysis of this potential effect excluding cases showing inflammation, we did not see improved diagnostic performance (data not shown). A further study with an asymptomatic population seems to be reasonable. Secondly, the number of endoscopists may result in heterogeneous endoscopic quality. However, all investigators were trained under the same conditions and supervised by just one clinician. Thirdly, the true value of ACE may only be estimated properly by applying a randomized trial comparing the method with HRA. Anal cytology is not a reasonable comparator due to its function as a screening tool with broad availability, easy application, and acceptable costs. Anal chromoendoscopy and HRA are the more sophisticated examinations containing a substantial input of material, time, and costs with a flat learning curve. Finally, specificity of both methods has to be improved. Therefore, more data on the topic are needed. Despite the discussed aspects, we are convinced that ACE is a promising new application that adds to the gastroenterological diagnostic portfolio and should also be evaluated in HIV-negative individuals.

In summary, we could show that ACE is safe and effective in the management of precursor lesions of anal carcinoma in HIV-infected patients. In particular, it is a very valuable method to exclude anal dysplasia if it is applied by well-trained endoscopists. Further studies are needed to compare the method with the current standard of HRA.

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Conflict of Interest

No conflict of interest has been declared by the author(s).

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