Clinical spectrum and virologic characteristics of anal intraepithelial neoplasia in HIV infection

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Background: Anal intraepithelial neoplasia (AIN) represents a precursor lesion of invasive squamous cell carcinoma with a clear association to high-risk human papillomavirus (HPV) types. HIV infection is strongly associated with a higher prevalence of genital HPV infection, a higher incidence of AIN, and, consecutively, an increased risk for anal cancer.

Objective: The aim of this study was to determine the clinical spectrum of AIN and lesional HPV colonization in a cohort of homosexual men who were HIV positive and had a history of receptive anal intercourse.

Methods: In all, 103 men who were HIV-1 positive were screened by using clinical, proctologic, cytologic, histologic, and HPV DNA testing.

Results: Of all patients, 86% had anal HPV infection at their first visit. HPV-16 (53%), HPV-18 (27%), HPV-58 (22%), and HPV-83 (22%) were the most frequently found HPV types. AIN was diagnosed in 20 of the 103 patients (19.4%). High-risk HPV types were present in all AIN cases with up to 7 different high-risk and up to 5 different low-risk types per lesion. Histologically, 7 (35%), 7 (35%), and 6 (30%) of the patients had AIN grade I, II, or III, respectively. Four different types of clinical presentation could be distinguished in the 20 patients with AIN: bowenoid (1 case, 5%); erythroplakic (2 cases, 10%); verrucous (6 cases, 30%); and leukoplakic (11 cases, 55%). All verrucous lesions were graded as high-grade intraepithelial lesions in cytology, whereas 6 of the 11 leukoplakic lesions (55%) were low grade. All verrucous AIN carried at least 4 different HPV types, always including HPV-16, and the mean number of HPV types was higher in verrucous lesions than in leukoplakic lesions (5.5 vs 3.8, respectively).

Conclusion: These data confirm the high incidence and prevalence of AIN in patients who are HPV positive with HIV infection. Four different clinical types of AIN can be distinguised that might have prognostic implications. Standardized screening programs for anal cancer prevention and treatment protocols for AIN in patients infected with HIV must be implemented. (J Am Acad Dermatol 2005;52:603-8.)

Anal intraepithelial neoplasia (AIN) denotes a spectrum of histologic features ranging from mild dysplasia to squamous cell carcinoma in situ of the anus. It usually is a rare dermatologic condition of the elderly, but certain causative and pathogenetic factors have been detected during the last decades, indicating a growing high-risk population for AIN and anal carcinoma. Genetic or iatrogenic immunosuppression is known to play a decisive role in the initiation of a variety of cutaneous neoplasias. Compared with the general population, concomitant HIV infection drastically increases the relative risk for AIN and anal cancer (60.1 and 37.9, respectively). For homosexual men younger than 30 years, the relative risk is even higher (130.4 and 162.7, respectively).[1] Perianal human papillomavirus (HPV) infections have been detected in up to 93% of men who are HIV positve and a high incidence of high-grade AIN has been reported

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Table I. Patient characteristics

Patient	Age, y	Diagnosis of HIV, mo/y	Stage of HIV (CDC/WHO)	HIV RNA (copies/mL)	CD4/μL
1	40	10/92	C3	302	580
2	40	03/86	C2	<40	525
3	51	09/85	B2	<40	644
4	36	10/98	C2	<40	674
5	32	06/93	A3	<40	786
6	42	05/91	C3	2000	636
7	58	04/85	C3	16,100	254
8	34	07/01	C3	<40	462
9	42	08/02	C3	<40	503
10	41	07/01	A3	<40	311
11	35	09/02	C3	<40	104
12	65	12/96	C3	<40	526
13	43	10/94	C3	240	790
14	44	09/85	C3	<40	1015
15	60	06/89	C3	47,500	133
16	49	04/94	C3	40,700	30
17	42	06/93	C3	5960	67
18	33	06/03	A2	<40	417
19	37	03/99	B3	53	269
20	68	06/00	C3	<40	422

CDC, Centers for Disease Control and Prevention; *WHO*, World Health Organization.

in these patients.[2,3] To date, 96 HPV genotypes are completely described and a high number of sequences representing putative new types are known. Depending on their oncogenic risk, HPVs are divided into low- and high-risk types.[4,5] AIN is associated with high-risk oncogenic HPV types, predominantly HPV-16 and -18.[6] Highly active antiretroviral therapy, with its improvement in immune status and prolonged survival in patients infected with HIV, seems to have little impact on concomitant HPV infection and preliminary data suggest that high-grade AIN lesions most likely progress to invasive cancer.[7]

The histologic features of AIN are well characterized, including morphologic abnormalities such as atypical keratinocyte hyperplasia, nuclear pleomorphism or hyperchromatism, and koilocytosis. Depending on the localization of these changes, 3 grades of AIN have been implemented.[8] In AIN I, the histologic abnormalities are restricted to the lower third of the epidermis, whereas in higher grades, two thirds of (AIN II) or the complete (AIN III) epidermis are affected. However, there exists only limited data about the macroscopic appearances of AIN, as compared with other genital intraepithelial neoplasia (eg, penile intraepithelial neoplasia).[9] As early detection of these precursor lesions is the main goal to prevent invasive anal cancer, the knowledge of clinical features of AIN is of special importance for dermatologists seeing individuals with HIV infection. Therefore, the aim of this study was to specify the clinical characteristics of AIN and to further analyze the virologic features of these lesions in a cohort of homosexual men with HIV infection.

PATIENTS AND METHODS

Patient population

From December 2003 to July 2004, 103 homosexual men with laboratory-confirmed diagnosis of HIV were included in this screening program for AIN after giving informed consent for participation. The study protocol was approved by the ethics review board of the Ruhr University of Bochum, Germany. All patients underwent a standardized interview on medical history, sexually transmitted diseases, history of HPV-related diseases, and tobacco use before screening. Patients younger than 18 years, those with concomitant chronic or malignant disease, or those with active opportunistic infection were excluded. In all patients a complete blood cell count, CD4 T-lymphocyte count, HIV-1 RNA load, serum chemistry including glucose and electrolytes, and urine analysis were performed.

Examination and screening procedures

Clinical examination included the inspection of all mucous membranes (oral cavity, glans penis, corona, sulcus, frenulum, inner part of the foreskin). In case of the presence of any suggestive clinical lesions, biopsies were performed after local anaesthesia.

All participants underwent a standardized anal screening program, resembling those of the cervix,

Table II. Clinical, histologic, and virologic characteristics of patients with AIN

Patient	Clinical type	Histologic stage	Lesional smear (cytology)	High-risk HPV	Low-risk HPV
1	v	II	HSIL	16,45,59	42
2	l	II	HSIL	16,35	6
3	l	III	-	52,56	40,54
4	v	II	HSIL	16,18	11,34,83
5	v	II	HSIL	16,58,73	81
6	l	III	HSIL	16,18,33,52,58,66	40,44
7	e	III	HSIL	33,52,58,59,68,73	70,81,83,84,89
8	b	II	HSIL	16,18	11,42
9	l	II	LSIL	16,73	34
10	l	I	LSIL	16,18,33,58,59	89
11	l	I	LSIL	33,52,58	-
12	l	I	LSIL	16	-
13	v	I	HSIL	16,18,33,35,52,58,68	83,89
14	l	I	HSIL	16	6
15	l	I	LSIL	18,26,31	61,83
16	v	III	HSIL	16,18,52,73,82	11,72
17	e	II	HSIL	16,18,52	83
18	l	III	LSIL	16	6
19	v	III	HSIL	16,18,31	83
20	l	I	HSIL	33,52,58,59	89

b, Bowenoid; *e*, erythroplakic; HPV, human papillomavirus; *HSIL*, high-grade squamous intraepithelial lesion; *l*, leukoplakic; *LSIL*, low-grade squamous intraepithelial lesion; *v*, verrucous.

as described previously.[10] In brief, a visual and proctoscopic examination of the perianal skin, dentate line, and proximal anal canal was performed for the presence of AIN. In case of indistinct clinical presentation, application of 3% acetic acid solution was used to detect subclinical lesions. Categorization of clinical types of AIN was always performed before the application of acetic acid solution. Cytologic specimens were collected from all patients from the perianal area and the anal canal. In case of abnormal macroscopic or cytologic findings, biopsy specimens were obtained.

Cytologic analysis and HPV DNA sample collection

Cytologic specimens were obtained using a dry conventional Dacron-tipped swab (Noba, Wetter, Germany), which was rolled on the surface of the perianal or anal lesional area, placed on a glass slide, fixed with 95% ethanol, and stained with a Papanicolaou's stain. Anal cytologic results were classified as normal, atypical squamous cell of undetermined significance, low-grade squamous intraepithelial lesion, or high-grade intraepithelial lesion by using the Bethesda system criteria for evaluation of cervical cytologic results.[11] Smears were classified as unsatisfactory if no epithelial cells were present.

A second specimen for detection of HPV DNA was obtained in the same manner, placed in transport phosphate-buffered saline containing 0.05% merthiolate (Thimerosal; Sigma, Taufkirchen, Germany), and stored at −20°C until processing.

DNA isolation, polymerase chain reactions, and HPV typing

DNA isolation was performed (QIAamp DNA Mini-Kit, Qiagen, Hilden, Germany). Total cellular DNA was eluted with 250 uL of AE-buffer (Qiagen) and 5 uL were used in each polymerase chain reaction (PCR) analysis. Negative controls with water and human placental DNA instead of patient samples were included in each amplification series. ß-Globin gene PCR was performed with all scrapes to demonstrate that the samples contained adequate DNA and were free of substances inhibitory to PCR (268 bp PCO4/GH20 PCR product).[12] HPV DNA screening was performed with a highly sensitive group-specific nested PCR with degenerate primers A5/A10 and A6/A8 as previously described.[13] PCR products (5 μL) were separated on 2% agarose gels and visualized by ethidium bromide staining. For HPV typing internal biotinylated A6/A8-PCR products (270 bp) were hybridized with 37 type-specific digoxigenin-labeled oligonucleotide probes in an enzyme-immunoassay as previously described.[14,15]

Histopathologic analysis

Punch biopsy specimens (3 mm) were taken from a representative affected skin area after local anesthesia was induced to confirm the clinical diagnosis

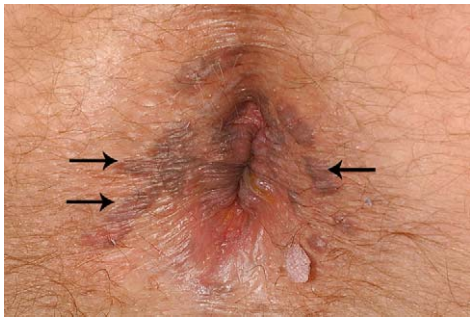

Fig 1. Bowenoid anal intraepithelial neoplasia (patient 8).

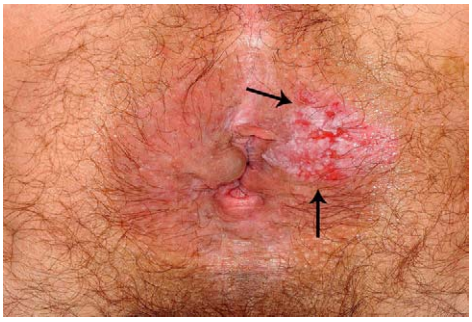

Fig 2. Erythroplakic anal intraepithelial neoplasia (patient 7).

of AIN (AIN grade I-III) or invasive cancer. Each biopsy specimen was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. Histologic results were evaluated independently by two histopathologists without knowledge of the clinical status. In case of discrepancies, all specimens were assessed by a third observer.

Statistical analysis

Statistical analyses were performed using software (SPSS 10.0.7, SPSS Inc, Chicago, Ill).

RESULTS

In all, 103 homosexual men were included in this anal screening program. All patients were treated with highly active antiretroviral therapy at the time of screening. In 86% of patients (89 of 103) anal HPV infection was present at their first visit. The most frequently found high-risk HPV types in this cohort were HPV-16 found in 53% of patients who were HPV positive, HPV-18 (27%), and HPV-58 (22%). The most prevalent low-risk types were HPV-83 (22%), HPV-11 (16%), and HPV-6 (14%). AIN was diagnosed in 20 of 103 men (19.4%). Characteristics of the 20 patients with AIN are summarized in Table I. The mean age was 36 years (range, 33-68 years). Mean duration of HIV infection was 9.5 years (range, 1-19 years), mean $CD4^+$ lymphocyte count was 457.4 cells/μL and mean HIV RNA was 5643 copies/mL. A total of 13 patients (65%) had a history of one or more opportunistic diseases and were classified as having AIDS (C3 Centers for Disease Control and Prevention/World Health Organization). Of the 20 patients with AIN, 15 (75%) were smokers and 48 of the 83 (58%) patients without AIN were smokers. The difference was not significant, however (P = .269; chi-square test). High-risk HPV was present in all cases of AIN. The number of high-risk types found per lesion ranged from 1 to 7 types and that of low-risk types from 0 to 5, with the maximum number of HPV types found in one lesion being 11 (Table II). At histologic examination, 7 (35%), 7 (35%), and 6 (30%) of the patients had AIN I, AIN II, and AIN III, respectively. Invasive anal cancer was not observed.

Anal lesions suggestive for AIN could be categorized in 4 different subtypes according to the clinical features of similar intraepithelial neoplasias of other localization (penis, vulva, oral cavity)[9,16,17]: (1) bowenoid AIN—single or multiple, well-demarcated, brown or red, slightly elevated papules and plaques resembling bowenoid papulosis (Fig 1); (2) erythroplakic AIN—one or more erythematous, slightly erosive or scaly macules in the mucosal surface resembling erythroplasia of Queyrat (Fig 2); (3) leukoplakic AIN—flat, well-demarcated, acetowhite lesions with punctuate vascular pattern (Fig 3); and (4) verrucous AIN—one or more white or grey, exophytic lesions with irregular and hyperkeratinized surface (Fig 4). The presence of other dermatologic conditions that can mimic these subtypes of AIN (eg, lichen sclerosus, lichen planus, condylomata acuminata, psoriasis, atopic eczema, or fixed drug eruption) were ruled out in all cases. According to the clinical criteria mentioned above, 1 bowenoid (5%), 2 erythroplakic (10%), 6 verrucous (30%), and 11 leukoplakic AIN (55%) could be distinguished. Interestingly, all verrucous AIN were high-grade intraepithelial lesion in cytology, whereas 6 of the 11 leukoplakic AIN (55%) were low grade (P = .035; chi-square test). Similarly, in histology, 5 of 6 (83%) verrucous lesions, but only 5 of 11 (46%) leukoplakic lesions were graded as AIN II or III. HPV-16 was detected in all cases of verrucous AIN (together with at least 3 other coinfecting HPV types) and in 64% of leukoplakic AIN. The mean number of HPV types was higher in verrucous than in leukoplakic lesions. However, this difference was not significant (5.5 vs 3.8; P = .127; t test).

DISCUSSION

HPV infections of the anogenital tract belong to the most common sexually transmitted diseases worldwide, causing a broad spectrum of clinical

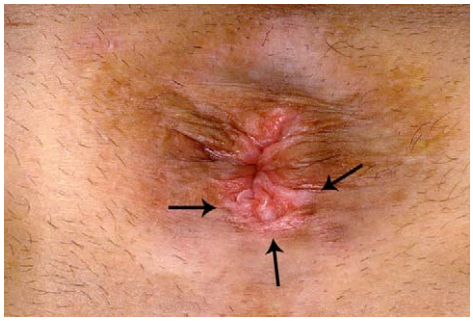

Fig 3. Leukoplakic anal intraepithelial neoplasia (patient 10).

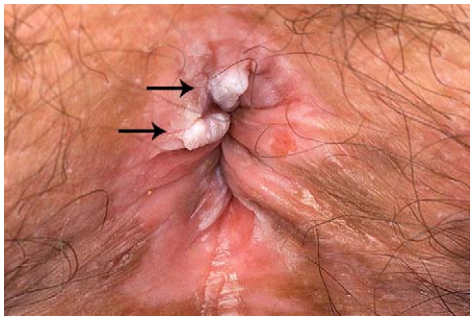

Fig 4. Verrucous anal intraepithelial neoplasia (patient 1).

symptoms ranging from genital warts to preinvasive or invasive cancer. The viral oncogenes E6 and E7 of high-risk HPV types such as HPV-16 or -18 are regularly expressed in anogenital precancerous lesions and cancers. They can deregulate normal cell growth by interfering with cell cycle checkpoints and lead to chromosomal instability.[18] HIV-1 tat seems to potentiate the expression of E6 and E7, and might be one reason for the high incidence of AIN in HIV infection.[19] More important is probably the HIV-induced immunosuppression leading to an insufficient control and increased replication of HPV.[20,21] Interestingly, highly active antiretroviral therapy seems to have little impact on the regression of AIN; there might even be an increased risk for progression and invasive disease because of prolonged survival.[22,23] We have found AIN in 19.4% and anal HPV in 86% of our patients, with HPV-16 being the most frequent type. These numbers are basically in line with other studies.[2,6,10] Palefsky et al[3] have demonstrated that 49% of 277 men with HIV infection developed high-grade intraepithelial lesion during a 4-year period. Thus, early detection and treatment of HPV-associated dysplasia in HIV infection will be of imminent importance.[10] As both cervical and anal cancer share distinct biologic similarities, an anal screening program has been recommended for the high-risk population, analogous to the cervical cancer screening established in the 1970s.[10,24]

Table III. Clinical subtypes of AIN

Subtype of AIN
Bowenoid
Erythroplakic
Leukoplakic
Verrucous

AIN, Anal intraepithelial neoplasia.

Because most of the research within the field focuses on cytology, histology, and molecular biology of AIN, only limited data exist on the clinical spectrum of these lesions. The macroscopic appearance remains rather imprecise and is usually described as eczematoid, papillomatous, papular, or plaquelike lesions.[7] For penile intraepithelial neoplasia, 3 clinical variants (ie, erythroplasia of Queyrat, penile Bowen's disease, bowenoid papulosis) are known and have been recently reported in 35 cases.[9] In addition, oral leukoplakia has been divided into different clinical subtypes (eg, a leukoplakic type including a verrucous variant and an erythroplakic type).[25] Based on observations and clinical similarities, we tried to categorize the clinical features of AIN and establish a classification parallel to other intraepithelial neoplasias. To our knowledge, this is the first report of a clinical classification for AIN. The 4 subtypes found in this study are summarized in Table III. Interestingly, all verrucous AIN were high-grade lesions, in contrast to only 55% of leukoplakic AIN. Furthermore, HPV-16 was present in all verrucous AIN, and the number of coinfecting HPV types was at least 3 and higher than in leukoplakic AIN. Although the number of patients with verrucous AIN is too small to draw any definite conclusions, one might speculate that HPV-16, together with coinfecting HPV types, induces the extensive epithelial proliferation of this clinical type. It remains to be clarified in larger studies whether this could be an indicator for accelerated progression toward invasive cancer and whether the clinical types of AIN have a different HPV spectrum and a different prognostic value.

To date, no standard of treatment for AIN exists and the literature on this subject is very limited.[10] Excision or laser ablation can be considered. However, smaller lesions might respond to local therapy (eg, 80% trichloracetic acid, podophyllotoxin, intralesional interferon, or interferon beta gel).[7] We recently reported our first experiences with imiquimod 5% cream in 10 patients with AIN of this cohort and observed a histologic regression of at least two grades in all of these cases.[26] Imiquimod

is approved for the treatment of genital warts and is increasingly used for the treatment of cutaneous neoplasias.[27,28] Follow-up studies are needed for all therapies of AIN to evaluate the long-term effects and recurrence rates.

In conclusion, this preliminary study demonstrates that AIN presents in different clinical appearances and might be classified in 4 subtypes. Future studies with larger patient numbers are now necessary to confirm our results, especially regarding HPV spectrum and clinical course.

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