Abstract. Background and Aim: HIV-infected patients show a high rate of anal dysplasia and anal carcinoma but there is no gold standard for early detection. Therefore, the objectives of this prospective study were: a) evaluation of an anal screening using anal/perianal cytology; b) in case of a positive result to investigate its relation to immune status, clinical symptoms of HIV infection and antiretroviral therapy. Patients and Methods: In every HIV-infected woman visiting our gynaecological outpatient clinic, an anal and perianal swab for anal cytology was taken. One experienced cytologist examined all specimens. Relevant details of the HIV-related history such as CDC classification, CD4 count, viral load, actual antiretroviral therapy etc. were documented. Results: Altogether, 104 HIV-infected women were enrolled on this study. The results of 13 (13.5%) anal cytologies were classified as suspicious for low-grade or high-grade anal dysplasia and 6 of these were confirmed in an anal biopsy. A total of 9 out of 13 also had a cervical dysplasia and 12 were positive for high-risk HPV at the cervix. Ten of these women had already experienced clinical symptoms of their HIV infection and 8 showed a nadir of the CD4 count below 200 cells/μl. All but one took a highly active antiretroviral therapy. Conclusion: In this pilot study, anal screening using anal cytology showed 13.5% suspected anal dysplasia in HIV-infected women. All performed biopsies revealed the presence of a high-grade anal lesion. The majority of these women already had an advanced disease and/or immune defect related to their HIV infection. In summary, we found anal cytology to be a useful tool to early detect anal dysplasia of high-risk patients such as HIV-infected women. How far this screening method contributes to the prevention of anal cancer has to be evaluated in further investigations.

Even if the Center of Disease Control (CDC) did not change the CDC classification concerning AIDS defining diseases, it is proven that HIV-infected patients suffer significantly more frequently from anal cancer than does the general population (1). Proven risk factors for anal cancer include persistent anal human papilloma virus infection (HPV), receptive anal intercourse, history or present anal condyloma, smoking and reduced CD4 count (2, 3). Therefore, HIV-infected men who have sex with men (MSM) were thought to be the main population at risk for anal cancer but then it was shown that HIV-infected women also suffer frequently from this problem (3). More and more data show that the administration of highly active antiretroviral therapy (HAART) in fact extends the life expectancy of HIV-infected people (4) but does not substantially decrease the incidence of anogenital dysplasia or anal cancer (5, 6). However, similarly to cervical cancer, anal cancer has precursor lesions (7, 8), so called anal intraepithelial neoplasia (AIN), which can often be treated successfully if diagnosed early enough (9). Despite this knowledge, no routine anal cancer screening for HIV-infected individuals has yet been established.

Therefore, the objectives of this study were to evaluate an anal screening for women based on anal cytology and to find correlations between detected anal lesions and the HIV-related disease of affected patients.

Patients and Methods

This prospective study was performed at a specialized gynaecological outpatient clinic for HIV-infected women at the University of Munich. Consecutive women with a known HIV infection who came for a gynaecological examination for different reasons between September 2007 and September 2008 were included in the analysis. Corresponding cervical samples anal and perianal cytology samples were taken with a moistened cotton swab from the anal canal, fixated and stained according to the Papanicolaou protocol. Specimen evaluation was carried out by an
experienced cytologist using Munich nomenclature II (10) and Bethesda nomenclature (11) (Figure 1, Figure 2). If the cytology was suspicious of a low-grade or high-grade lesion, high resolution anoscopy (HRA) and anal biopsy of visible lesions were undertaken (Figure 3). The anal histology represented the basis for the treatment decision. If the cytology found a low-grade anal lesion and the HRA was inconspicuous, a close follow-up was arranged. Baseline characteristics such as age, country of origin and reason for this gynaecological visit were documented. The history of previous or current cervical dysplasia was assessed and every result of a cervical cytology, cervical HPV detection (low-risk, high-risk), cervical biopsy or surgical treatment with the corresponding histology was registered. Cervical smears were performed in-house using a cytobrush and a conventional cytology. Cervical HPV detection was conducted using Hybrid Capture® 2 from Digene (Hilden, Germany) according to the recommendations of the manufacturer. Relevant details of the HIV-related history including CDC classification, time since first diagnosis, HIV-related diseases, current CD4 count and nadir, viral load and actual antiretroviral therapy were collected and compared to the assessed anal dysplasia. Statistical analysis was performed using SPSS Statistics software version 15.0 (IBM, Armonk, NY, USA). The threshold of significance was set at \( p=0.05 \). Groups were compared using Mann-Whitney U-test for quantitative and Chi-square test for qualitative data.

**Results**

The baseline characteristics of the examined cohort (n=104 HIV-infected women) are presented in Table I in comparison with a subgroup of HIV-infected (n=13) women with anal cytology suspicious of an anal lesion. No significant difference between this subgroup (n=13) and the rest of the cohort (n=91) was detected (the respective \( p \)-value is given in Table I). The CDC classification of the cohort and the above mentioned subgroup is shown in Figures 4 and 5. The distribution of CDC status was not significant different between the subgroup and the rest of the cohort (\( p \)-value: 0.13).
Of the 104 screened HIV-infected women, 57 (54.8%) had a previous history of a suspicious cytology of the cervix or proven cervical dysplasia. 35 had already had an operation because of cervical dysplasia (25 of them with a histological result of carcinoma in situ) or cervical carcinoma (n=3). Twenty-nine patients (27.9%) of the whole group had positive prior examination for high-risk HPV performed by cervical swab. There were 10 women already suffering from multisite dysplastic lesions (e.g. of the cervix and vulva). The current reason for the gynaecological visit for 29 (27.9%) of the women was a previous history of genital dysplasia or due to a suspicious cervical cytology at the last visit.

In 13.5% patients of the whole cohort (13 out of 104) the anal cytology was suspicious of a low-grade (n=11) or high-grade anal dysplasia (n=2). HRA-guided anal biopsies confirmed a histological anal dysplasia in 6 patients. In four of those women a carcinoma in situ of the anus was found. The other 7 patients are under close follow-up. From this subgroup (n=13), 9 women had already experienced a surgical procedure because of a cervical dysplasia. The result of the histology revealed a carcinoma in situ of the cervix in seven cases. A total of 5 out of 13 had already a prior surgery because of an anal dysplasia. The current cervical swab in 9 of these women again revealed a suspicious cytology (two of a high-grade lesion) and 12 women were also positive for high-grade HPV of the cervix. A swab for anal HPV was not performed in this study. Regarding their HIV-related disease, it was recorded that 10 women of the mentioned subgroup (n=13) had already experienced clinical symptoms related to HIV and 8 patients already showed a nadir of the CD4 count under 200 cells/μl (Table I). As a consequence of their immune status and/or clinical symptoms all but one has to take an antiretroviral therapy.

Discussion

The increasing number of anal cancer of HIV-positive men and women is possibly a consequence of longer survival due to the modern antiretroviral combination therapy (HAART or cART) (1, 5) and therefore a longer persistence of high-risk HPV-infection as precondition for anogenital cancer (12). It is not proven, although very likely, that the screening and treatment of high-grade anal lesions prevent the development of anal cancer (9). However, the similarity of cervical cancer in comparison to anal cancer leads to the assumption that therapy of detected precursor lesions will reduce the incidence of the corresponding cancer. Currently, there are no recommendations for routine anal screening in HIV-positive individuals.

In our study, we found an abnormal anal cytology suspicious of dysplasia in 13.5% of screened HIV-positive women. In another study performing an anal screening of HIV-infected women, the authors detected a similar rate, with 14% abnormal anal cytology (13). Of course, the population we screened were at high-risk for anal disease in addition to their immunodeficiency because approximately half of them (54.8%) had already experienced genital dysplasia (three of them a cervical carcinoma) or suspicious cervical cytology. Previous history of HPV-associated genital
cancer and/or dysplasia represents one of the risk factors for developing AIN and, accordingly, anal cancer (14).

After HRA-guided biopsies of six patients in this investigation, the histology proved an anal dysplasia (high-grade in four patients). However, only in two of these patients did the cytology indicate a high-grade lesion, but all showed a dysplasia. In an MSM cohort (whole cohort: n=125, HIV-positive: n=35), the investigators found a sensitivity of 87% and a specificity of 47% for anal cytology in detecting high-grade anal neoplasia in HIV-positive men but the sensitivity was only 55% (with a specificity of 76%) in HIV-negative men (15). Furthermore, they observed only a slight benefit of an added HPV-test for HIV-positive patients (in contrast to HIV-negative MSM), explained by the given high prevalence of anal HPV. Of course, the authors also defined HRA-guided biopsies as the gold standard for detecting high-grade lesions. However, the problem of HRA lies in the limited resources of trained specialists to perform all needed inspections of HIV-infected patients or other high-risk people such as MSM. Anal cytology and anal HPV-testing can therefore be a useful and universally applicable tool to identify patients who should undergo HRA.

The effect of immune restoration caused by cART or HAART on the development and regression of anogenital dysplastic lesions remains unclear (e.g. 16, 17). In 2003, Palefsky reviewed (18) the literature regarding the influence of HAART on cervical lesions and found overall no great benefit thereby. Another review reported no reduction in the incidence of cervical and anal cancer of patients with HIV in the era of HAART (19). In a cross-sectional study of HIV-infected men (20), immune recovery due to HAART showed no influence on the prevalence of AIN or anal HPV infection. The subgroup of HIV-infected women with an abnormal anal cytology in this investigation showed no statistical significant difference regarding CDC stage, CD4 cell count, viral load, current ART and clinical symptoms experienced related to HIV disease compared to the rest of the group with a normal cytology. However, the women (in this subgroup with an abnormal cytology) with a nadir of CD4 count <200 cells/μl, at 8 out of 13 was relatively high, as well as the rate of women (10 out of 13) who had already experienced clinical symptoms due to their HIV infection.

In conclusion, with regard to the high rate of AIN and increasing incidence of anal cancer, there is an urgent need for a more accessible method to screen for high-grade lesions. The use of anal cytology and anal HPV-testing could be a valuable tool in this regard.
to implement a routine anal screening for HIV-infected women as well as for men, independently of their immune status. Due to the fact that HRA-guided biopsies are not often available, regular anal cytology possibly combined with anal HPV testing, represents an effective screening tool for this population at high-risk for anal cancer.

References


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