Follow-up of High-grade Squamous Intra-epithelial Lesions (H-SILs) in Human Immunodeficiency Virus (HIV)-positive and Human Papillomavirus (HPV)-positive Women. Analysis of Risk Factors

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Abstract. *Background: Human immunodeficiency virus (HIV)-positive women are at high risk of co-infection from human papillomavirus (HPV) and of developing squamous intra-epithelial lesions of the cervix. Materials and Methods: From April 1997 to March 1999, 86 women, affected by high-grade squamous intra-epithelial lesions (H-SILs), were enrolled: 41 were HIV+ (CD4+ count >500/ml) and 45 were HIV–. The diagnosis of high-grade squamous intra-epithelial lesion (H-SIL) was established for each patient by Pap test , colposcopy and guided biopsy. For all samples, the HPV/DNA test was also performed by PCR. The patients' lesions and recurrence were treated by cone biopsy or large loop excision (LEEP). Annual controls were performed for 5 years. Results: A high rate of alcohol and drug use (60.7% vs. 31.4%; p=0.004; 80% vs. 27.5%; p<0.001, respectively) and number of male partners (4.5 vs. 3.0; p<0.001) were found in the HIV+ patients, compared to the HIV– patients. Both groups were HPV+ for high-risk types. No difference was found in the percentage of patients who had received a second LEEP. Conclusion: Our findings suggest the treatment of H-SIL in HIV-positive women, for a longer disease-free survival, or a lower risk of developing cervical cancer.*

Human immunodeficiency virus (HIV)-positive women are at high risk of co-infection from human papillomavirus (HPV) and, consequently, of developing squamous intra-epithelial lesions (SILs) of the uterine cervix (1, 2). Moreover, HIV seropositive status is also considered to be a risk factor for invasive cervical cancer (2-4).

The reason why cervical cancer rates have not reached more worrying epidemiological proportions in HIV-infected women could be related, not to the rather short lifespan of untreated HIV-positive women, but to the average 10-year period required for cervical intra-epithelial neoplasia (CIN) to progress to invasive cervical cancer (3, 5, 6).

The life expectancy of HIV-positive women is still increasing, mostly as a consequence of highly-active antiretroviral therapy (HAART). The number of HIV-seropositive patients who will approach the age at which cervical cancer is more common is, therefore, certain to grow, and thus monitoring the long-term impact of HIV/HPV co-infection is very important. The effectiveness of the strategies carried out to prevent and/or to treat HPV-related precancer cervical lesions takes into account the fairly contained incidence of cervical cancer in women infected with HIV.

More than 40 different HPV types are known to infect the lower genital tract, the high-risk types (HPV 16, 18, 31, 33, 56) being involved in persistent disease and related to progression *versus* invasive cervical cancer, especially in HIV-positive subjects (7). These data support the hypothesis that the *tat*-HIV gene could activate the early HPV E6 and E7 oncogenes involved in neoplastic progression (8, 9). To avoid this risk, these patients should be treated with excisional therapy (3).

In the present study, the management and rate of persistence and/or recurrence of H-SIL, in a group of HIV-seropositive women, was evaluated after surgical therapy.

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Table I. *Risk factors between HIV-positive vs. HIV-negative women with H-SIL.*

	HIV-positive	HIV-negative	*P* value
Age	34.5±5.15	37.33±9.07	0.095
Alcohol consumption (%)	60.7	31.4	0.004
Drug use (%)	80	27.5	<0.001
Smoking (%)	60.7	42.22	0.052
Number of partners	5.5±2.68	3.46±1.68	<0.001
Condom use (%)	50	22.2	0.028
EP contraception (%)	16.6	45.4	0.057

HIV=human immunodeficiency virus.
H-SIL=high-grade squamous intra-epithelial lesions.
EP=estroprogestinic.

Table II. *Follow-up for cervical lesion between HIV-positive vs. HIV-negative women with H-SIL.*

	HIV-positive	HIV-negative	*P* value
% of positive at 1-year follow-up	8.69 (n.2)	5.12 (n.2)	NS
% of positive at 2-year follow-up	4.34 (n.1)	2.56 (n.1)	NS

HIV=human immunodeficiency virus.
H-SIL=high-grade squamous intra-epithelial lesions.
NS=not significant.

Materials and Methods

From April 1997 to March 1999, 41 HIV-seropositive women, affected by H-SIL lesions, were enrolled. Information regarding personal history (age, smoking, oral contraceptive use, parity) was available for each selected patient. Written informed consent for scientific use of the clinical data and biological specimens in follow-up was obtained from all patients. The women were followed in the Institute of Infectious Diseases, University of Rome "La Sapienza", Italy, and this cohort presented a settled cell CD4+ count > 500 μl. As a control group, 45 HIV-negative patients with H-SIL lesions were selected. The diagnosis of H-SIL was established for each patient by Pap test, colposcopy and guided biopsy. An HPV/DNA test by PCR (10) was also performed for all samples.

Cytological analysis. Samples were evaluated for the grade of SIL, on the basis of the revised 2001 Bethesda System (11).

Colposcopic examination. The localization, width of the lesion and the involvement of the squamous-columnar junction were evaluated, following international guidelines (IFCPC) for describing the colposcopic samples.

Histological diagnosis. Low-grade lesions were defined as CIN 1, and high-grade dysplastic lesions as CIN 2 and CIN 3, according to the Richart classification.

The patients were treated by cone biopsy or colposcopic-guided large loop excision (LEEP) of the transformation zone. On evidence of a positive cone apex border, the LEEP excision was repeated. After treatment, all the patients were followed-up at 3-month intervals during the first year, and at 6-month intervals in the second year. Annual controls were performed for 5 years. Pap tests and colposcopy examinations were carried out at the time of follow-up. In every additional positive biopsy for H-SIL, another LEEP treatment was performed.

Statistical analysis. For comparison between numerical variables, a Student's *t*-test was performed when suitable. For the analysis of nominal data, a Chi-square (with previous Yates corrections) or a Z-test for percentage was performed. The Microsoft Excel for XP was used as a worksheet program. The statistical analysis was performed by means of the Sigma Stat 2.03 statistical package. The conventional probability value $p<0.05$ was considered to be significant. The total number of samples satisfied the statistical power (0.8 at $\alpha<0.05$).

Results

There were no positive statistical differences regarding age between the HIV+ and HIV– groups: (34.5 *vs.* 37.0, $p=0.095$). Regarding behavioral habits, a statistically significant high rate of alcohol and drug use was identified in the HIV+ patients compared to the controls (60.7% *vs.* 31.4%; $p=0.004$; 80% *vs.* 27.5%; $p<0.001$, respectively). No significant difference was found in the number of cigarettes consumed (10% *vs.* 0%; $p=0.052$).

Regarding sexual habits, a higher number of male partners were recorded in the HIV+ group than in the controls (4.5 *vs.* 3.0; $p<0.001$). An increase in the use of barrier contraceptive methods (50% *vs.* 22.2%; $p<0.001$) and a lower use of estroprogestinic hormones were also recorded for the HIV+ group, although these data were not statistically reliable (16.6% *vs.* 45.4%; $p=0.057$) (Table I).

Both patient groups were HPV-positive for the high-risk types. No difference was found in the percentage of patients who received a second LEEP due to preneoplastic lesion involvement of the surgical margins.

During follow-up, a low-grade CIN was identified in two HIV-positive and two HIV-negative patients in the first year, and one in both groups during the second year. The patients were treated with LEEP (Table II).

Discussion

Women infected with HIV have an increased risk of developing CIN as a cervical cancer precursor. The risk of developing CIN has been associated with HPV infection, which was detected in more than 60% of HIV-infected women, and HIV-induced immunodeficiency.

The high prevalence of HPV in HIV+ women is determined by the increased incidence of new infections rather than by the persistence of viral HPV disease (virus host) in the lower genital tract. However, evidence that "new"

infections can unexpectedly occur, even in the absence of recent sexual activity, leads to the hypothesis that activation of latent HPV infection is also responsible for the high rates of HPV infections usually detected in HIV-seropositive subjects (2). Co-infection with HPV in HIV+ women seems attributable to the loss of immunocompetence (2, 3, 12). The repeatedly observed connection between severe immunosuppression, defined as a CD4+ cell count below 200/mm^3 and/or HIV RNA level of more than 100,000 copies/ml, a detected risk of both infections and the development and/or progression of HPV-associated lesions is certainly not accidental. It has been, however, suggested that HIV proteins co-activate HPV (13, 14).

The analysis performed in our study emphasized the high incidence of SIL related to high HPV-risk types in a cohort of behaviorally similar HIV-positive women. In our study, the number of partners was higher in the HIV+ patients, and this is also a major risk factor for cervical cancer due to an increase in the probability of acquiring the HPV infection (2, 15, 16). Davis *et al.* (17) reported that 81% of patients with HIV+ status had a previous history of, or were currently infected with, other sexually transmitted diseases. Moscicki *et al.* (18), in their study of a population of adolescent women, also reported that women with HIV infection had a greater sexual behavior risk at baseline, including young age at first vaginal intercourse and a large number of sexual partners.

In contrast to these observations, in a large study that included 871 HIV-seropositive patients, Shuman *et al.* (19), reported that the incidence rate of H-SIL among HIV+ women was 1.6 cases/100 persons/year, compared with 0.3 cases/100 persons/year observed among HIV– women ($p=0.002$), and was not related to increased sexual activity, number of partners or history of prostitution with regard to the risk of developing SIL. We found a high rate of barrier contraceptive method use in the HIV+ women; probably due to recent information campaigns regarding the spread of the disease.

The data for alcohol and drug use are probably confounding factors, related to the behavior, environment and lifestyle of these women. Drugs and alcohol do not play a direct role in the development of cervical SILs, even if these are index factors for the frequency of sexual encounters and, thus, the chances of HPV infection. Overall, HPV infection seems to be the major cause of cervical cancer (16), also in HIV+ sexually-active women (2, 15). Moreover, Cohn *et al.* (20) showed that the high viral load of HPV/DNA 16/18 types was associated with the presence of H-SIL ($p=0.0006$). The immune function is currently one of the most important factors in cervical dysplasia, and the CD4+ count, viral load and cytokine concentration are considered to be risk factors by many authors (2, 17-19, 21).

Persistent and recurrent disease after treatment, or rapid progression, if untreated, has been reported at particularly high rates in HIV-infected and immunosuppressed women (18, 22). Nappi *et al.* (4) reported an increasing number of recurrences in women with HIV+ status compared with HIV– status (36.2% *vs.* 11.1%; $p=0.0067$). Recurrences and progression of invasive cervical cancer play an important role during the follow-up of these patients. Tate and Anderson calculated the point relative risk of recurrence after standard therapy with negative margins, related to an immunosuppressive state at 17.5. Patients with recurrent dysplasia were more likely to present a low CD4+ count and a higher viral load (23, 24).

In our study, no differences after one or two LEEP treatments during the 2-year follow-up were observed in either the HIV-positive or control groups. On the contrary, Holcomb *et al.* (25) studied 151 cases with the same single surgical technique, and did not indicate cervical conization as an effective means of eradicating CIN in HIV+ women.

Our study, in considering the state of disease for HIV+ patients by CD4+ count and viral load, was not different from others, and our results emphasize the use of drugs and a large number of male sexual partners (15) as major risk factors for H-SIL in patients with HIV+ status. We demonstrated that the prevention, continuous follow-up (26), state of surgical margins during electrosurgical cone and the treatment of recurrences can improve the prognosis of HIV+ patients with H-SIL, in the same way as for HIV– patients with H-SILs.

With regard to HIV-positive women, considering the increased prevalence of HPV-related lesions, gynecologists must find a more appropriate approach for cervical cancer screening, and must determine strategies for the management of pre-invasive lesions of the uterine cervix.

The most recent cervical cancer screening guidelines established that the time-interval for screening can be safely extended to 3 years for a population of healthy women with normal cytology results and negative oncogenic HPV testing (12). It was previously shown (12) that HIV+ women with normal cervical cytology, negative HPV-DNA test and a CD4+ count greater than 500 cells/μL presented a low risk of SIL development (4%), a value which overlaps with that of HIV-seronegative women (3%).

If we take into account that, in our cohorts, no HPV-negative participant developed H-SIL within 3 years, it is appropriate to evaluate the possibility, at least under certain circumstances, of applying similar cervical cancer screening practices for both HIV+ and HIV– women. The recurrence and progression of invasive cervical cancer is an important question during the follow-up of these patients. As previously seen, the relative risk of recurrence after standard therapy is related to a higher immunosuppressive state (23, 24).

The management of low-grade cervical SIL in HIV-positive women should indeed be more aggressive, especially in cases with CD4+ <200 cells/mm^3 and a

positive HPV-DNA test, considering the poor compliance to gynecological follow-up of the HIV+ population (4).

In conclusion, our findings suggest the treatment of H-SIL in HIV-positive women, in order to ensure a longer disease-free survival, or a lower risk of developing cervical cancer.

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