Human Papillomavirus Infection and Associated Cervical Disease in Human Immunodeficiency Virus–Infected Women: Effect of Highly Active Antiretroviral Therapy

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To determine the effect of highly active antiretroviral therapy (HAART) on high-risk human papillomavirus (HR-HPV) infections and related cervical lesions, the virologic and cytologic markers of HPV infection were prospectively studied in 163 human immunodeficiency virus (HIV)–infected women, including 27 untreated, 62 treated with reverse transcriptase inhibitors, and 74 treated with HAART. A high prevalence of both infections with HR-HPV types (68%) and squamous intraepithelial lesions (SILs; low grade, 20.2%; high grade, 6.2%) was observed. The risks of infection and disease were inversely correlated with CD4 cell counts (P = .015 and P = .022, respectively). During the observation period (mean, 15.4 months; range, 6–24 months), CD4 cell counts increased significantly only in subjects receiving HAART (P < .001). Persistence of HR-HPV infection and progression of SILs were comparable in the 3 groups. These results indicate that, even in the era of HAART, HIV-infected women should be monitored carefully for the emergence of high-grade SILs and cervical cancer.

The role of some human papillomaviruses (HPVs) in the etiology of cervical cancer has been clearly established [1–3]. Prospective studies have supported the concept that cervical intraepithelial neoplasia is consistently preceded by the detection of high titer of oncogenic HPV DNA in healthy women [4–6]. The persistence and progression of HPV infection is enhanced in immunosuppressive conditions [7–9]. Thus, human immunodeficiency virus (HIV)–infected subjects are at higher risk for developing HPV-related anogenital neoplasms [10–12]. In the era before highly active antiretroviral therapy (HAART), HPV-associated cervical neoplasia was ~5 times more likely to occur in women infected with HIV type 1 (HIV-1) than in uninfected women [13]. The use of HAART has greatly improved patient survival [14], and this longer life expectancy is generally associated with a significant decrease of HIV-1 replication, increase in CD4 cell count, and reduction of most opportunistic diseases. As for AIDS-associated malignancies, studies in Europe and the United States show a decline in Kaposi’s sarcoma but not in lymphoma [15, 16].

Limited, conflicting data are available on the evolution of HPV infection and associated lesions in HIV-positive subjects treated with different antiretroviral regimens. Before the introduction of combination therapy in 1993–1996, 0.4% of all Italian AIDS cases had invasive cervical carcinoma as the AIDS-defining condition. A slight increase (0.6%) was seen in the HAART era (1997–2000) [17], which suggests that immune reconstitution resulting from HAART might not be sufficient to prevent the development of cervical cancer once the process has been triggered. Moreover, in Italy, among AIDS-defining illnesses characterized by a high survival rate before the introduction of HAART (>50% of patients alive 2 years after diagnosis), women with invasive cervical carcinoma had only a low and not significant increase in survival [18]. In one study, there was a regression of cervical lesions from 69% to 53% in women treated with HAART [19]. Others reported that patients receiving antiretroviral therapy who had baseline normal Pap test results tended to have a lower incidence of squamous intraepithelial lesions (SILs) than untreated women (26.1% and 42.3%, respectively; P = .44) [20]. Conversely, in another study of anal SILs in HIV-1–positive homosexual men, a condition that shares biologic and histopathologic features of cervical SILs, the lesions of >75% of men with high-grade SILs (HSILs) who received HAART did not regress [21].

Here we report the immunologic, gynecologic, and virologic markers of HPV infection in HIV-positive women attending the infectious diseases and gynecological departments at San Raffaele Hospital, Milan. Our purpose is to contribute to the...
understanding of the long-term effect of HAART on persistent high-risk HPV (HR-HPV) infection and related cervical lesions and to compare these findings with those among untreated or reverse transcriptase inhibitor (RTI)-treated patients.

**Patients and Methods**

**Study population.** Between May 1995 and December 1997, 163 HIV-seropositive women were recruited from a patient care program that included gynecologic monitoring. Standardized interviews obtained information pertaining to the patients’ risk factors for HIV-1 infection, sexually transmitted diseases, and gynecologic history. HIV infection was classified according to the 1993 CDC criteria [22]. The subjects were seen at 6-month intervals. At each visit, CD4 cell count and HIV-1 viremia (NASBA; Organon-Teknika; lower detection limit, 1.9 log copies/mL of plasma) were determined, and each subject had a Pap smear; samples collected for detection of HPV genomic sequences, and colposcopy with biopsy, if necessary. Patients with HSILs underwent loop electrosurgical excision procedures.

**HPV status definition.** Women were defined as having persistent HPV infection when the same HPV type(s) was detected both at the time of enrolment and at follow-up visits; transitory infection was defined as having a discordant result (negative to positive or positive to negative) during the observation period. HPV-negative women were defined as those who were consistently negative by 2 HPV detection methods (hybrid capture [HC] and polymerase chain reaction [PCR]).

**HPV detection tests.** Screening for viral sequences was done by the nonamplified hybridization test (HC system II; Digene), in accordance with the manufacturer’s instructions.

**Molecular amplification procedures.** Samples collected by brushing of the squamocolumnar junction of the cervical epithelium were lysed for amplification. We added 100 μL of the cell preparations to an equal volume of buffer containing 50 mM Tris-HCl (pH 8.5), 1 mM EDTA, 10% Tween 20, and 200 g/mL of proteinase K, which was then incubated for 1 h at 55°C and for 15 min at 95°C.

**PCR.** HPV PCR was done by using degenerated consensus primers (MY09–MY11), as described elsewhere [23], to amplify a 450-bp region in the L1 gene encoding for the major capsid protein and a 240-bp fragment in the E6 region (WD72/76 WD66/67/154) [24]. One primer of each set was modified by means of biotination at the 5′ end, to allow subsequent detection and typing.

**Viral typing.** Viral typing was obtained for both the L1 and E6 regions after the hybridization of 2.5 μL of the PCR products in 100 μL of buffer containing specific oligomeric probes for HPV-6, -11, -16, -18, -31, -33, -35, and -45, modified by incorporating a fluorescein molecule at the 5′ end. As described elsewhere [25], we used a specific oligomeric probe (PDR′), modified by the incorporation of a fluorescein molecule at the 5′ end, to detect the PCR product. The results were related to an external standard curve obtained in a peripheral blood mononuclear cell dilution series.

**Cytology and biopsy.** We classified Pap smears according to the Bethesda system [26]. A modified version of this system was used for the current histologic classification. Slides with abnormal smears were regularly seen by 2 cytopathologists and by 2 physicians.

**Statistical methods.** HIV RNA levels (copies per milliliter) were log transformed before analysis. Categorical variables were compared by χ² test, χ² for trend, or Fisher’s exact test, when appropriate. Within-group variations of CD4 cell counts and HIV RNA levels were tested by paired samples t test. The odds ratios (ORs) for new or persisting HR-HPV infection and for cytologic lesion progression were estimated by logistic regression after adjusting for CD4 cell count, HIV RNA levels, and gynecologic treatment. P < .05 was considered statistically significant.

**Results**

**Study population characteristics.** The mean age of the study subjects was 33.6 years (range, 20–68 years). HIV-1 infection was acquired via sexual intercourse in 77 cases (47.3%), by injection drug use in 76 (46.6%), and by other means in 10 (6.1%). In all, 27 patients (16.5%) did not receive any antiretroviral therapy during the observation period (mean CD4 cell count at enrollment, 627 ± 37.7 cells/μL; log10 HIV RNA level, 3.03 ± 1.9 copies/mL), 62 (38%) were treated with 1 or 2 RTIs (mean CD4 cell count at enrollment, 336 ± 25.3 cells/μL; log10 HIV RNA level, 2.46 ± 2.1 copies/mL), and 74 (45.5%) received HAART (mean CD4 cell count at enrollment, 260 ± 22.9 cells/μL; log10 HIV RNA level, 3.07 ± 2.1 copies/mL). Baseline values for viremia were available only for patients enrolled after the introduction of the test (late 1996; n = 67).

**HPV DNA detection and typing at baseline.** HPV infection was investigated by the HC II assay and was typed by PCR amplification of the L1 and E6 regions. At baseline, by HC II assay, HPV was detected in 106 (65%) of the 163 subjects. PCR typing of the positive samples showed only HPV-16 infection in 26 subjects (24.5%) and only HPV-18 in 2 (1.8%). Infections involving only HPV-31, -33, -35, or -45 were detected in 35 positive subjects (33%); 26 subjects (24.6%) had baseline samples that hybridized with multiple probes (mixed infection); 17 subjects (16%) had infection with undefined HPV types. Infection with HR-HPV types occurred more often in women with lower CD4 cell counts (<350 cells/μL), but this trend was not statistically significant (P = .823).

**Baseline cytologic and histologic evaluations.** Baseline Pap...
Table 1. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for persistent high-risk human papillomavirus cervical infection in women treated with highly active antiretroviral therapy (HAART) or reverse-transcriptase inhibitor (RTI).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAART treated vs. all</td>
<td>1.244 (0.654–2.369)</td>
<td>1.479 (0.633–3.458)</td>
<td>.37</td>
</tr>
<tr>
<td>HAART treated vs. untreated</td>
<td>1.075 (0.446–2.590)</td>
<td>1.184 (0.373–3.772)</td>
<td>.77</td>
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<tr>
<td>RTI treated vs. all</td>
<td>0.800 (0.319–2.010)</td>
<td>0.666 (0.303–1.464)</td>
<td>.31</td>
</tr>
<tr>
<td>RTI treated vs. untreated</td>
<td>0.760 (0.388–1.492)</td>
<td>0.741 (0.253–2.161)</td>
<td>.58</td>
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</table>

NOTE. P values were calculated by logistic regression.

test results were normal in 120 women (73.4%); a low-grade SIL (LSIL) was detected in 33 subjects (20.2%), and an HSIL in 10 (6.2%). The prevalence of cervical cytologic lesions increased with decreasing CD4 cell counts (P = .022, χ² for trend). Women with >500 CD4 cells/µL (n = 35) had LSILs in 5 cases (14.2%) and an HSIL in 1 (2.8%), whereas subjects with 200–500 CD4 cells/µL (n = 80) or <200 cells/µL (n = 48) had LSILs in 18 (22.5%) and 17 cases (35.4%) and HSILs in 4 (5%) and 3 cases (6.3%), respectively. The prevalence of HR-HPV types increased in parallel with lesion grade and were the only types detected in HSILs (P < .001, χ² for trend). In total, 53 women underwent colposcopically driven biopsy. In 10 cases (18.8%), the histologic findings were negative, and in 36 (67.9%) and 7 (13.3%) cases, LSILs or HSILs were detected, respectively.

Follow-up. Study women were observed a median of 15.4 months (range, 6–24 months; 25th percentile, 12.1 months; 75th percentile, 20.1 months). Over the follow-up period, the mean CD4 cell count and HIV viremia were essentially unchanged, compared with baseline values, among untreated subjects (CD4 cell count variation, −5.1 ± 29.2 cells/µL, P = .87; log₁₀ HIV RNA level, −0.59 ± 1.97 copies/mL, P = .36) and subjects receiving RTIs only (CD4 cell count variation, +14 ± 19.8 cells/µL, P = .47; log₁₀ HIV RNA level, −0.27 ± 1.85 copies/mL, P = .50). Women receiving HAART showed a significant increase in CD4 cell counts relative to baseline values (+88 ± 17.9 cells/µL, P < .001), whereas the modification of the log₁₀ HIV RNA level was −0.55 ± 2.0 copies/mL (P = .15).

Our goal for data analysis was to learn the relationship between antiretroviral therapies of different potencies and the effect of the related improvement in CD4 cell counts and HIV viremia on HPV infection and cervical lesions. Table 1 shows the ORs of having a persistent HR-HPV infection in treated groups before and after adjustment for CD4 cell count, HIV RNA level, and gynecologic therapy. Neither RTI treatment nor HAART was associated with significant risk reduction. Only a small, nonsignificant reduction was observed in the RTI-treated group.

The only variable with a strong association with persistence of HR-HPV infection, after adjustment for gynecologic therapy, was a CD4 cell count <350 cells/µL at the end of the follow-up period (OR, 2.4; 95% confidence interval [CI], 1.3–4.5; P = .013), whereas a baseline CD4 cell count <350 cells/µL resulted in a slight and not significant increase of risk of HR-HPV persistence (OR, 1.3; 95% CI, 0.7–2.6; P = .36). The CD4 cell count increase during follow-up showed a negative association with the risk of persistence (OR, 0.92; 95% CI, 0.83–1.03; P = .015 for an increase of 50 cells/µL).

Neither baseline nor final viremia was significantly associated with HR-HPV persistence, although a nondetectable viremia at the final visit was nonsignificantly associated with a lower proportion of persistence (29.7% vs. 40.2% of women with positive viremia, P = .26). A minimal and not significant protection from acquiring new HPV infections was detected in the HAART-treated patients when all HPV types were considered (relative risk, 0.79; 95% CI, 0.28–2.13; P = .63).

Twenty-one incident HPV-16 or -18 infections were detected: 5 (18.5%) among untreated women, 12 (18.5%) in the RTI group, and 4 (5.4%) in those treated with HAART. When compared with the other 2 groups together, the HAART-treated patients appeared to be significantly protected from incident HPV-16 and -18 infections (OR, 0.276; 95% CI, 0.089–0.857; P = .020, Fisher’s exact test).

The effect of antiretroviral therapy on evolution of the dysplastic lesions of the uterine cervix, detected by Pap smear, was not conclusive. With use of the Cox model, the results were virtually identical to those by logistic regression.

Only 50 patients underwent histologic examination at baseline and at the follow-up visit; 7 (14%) showed improvement, whereas 9 (18%) had a worse diagnosis at follow-up. No significant difference was observed after stratification for anti-HIV therapy, although women receiving HAART had a slightly lower progression rate as a result of the HAART or RTI regimen, but we found an increased risk for progression (table 2), although the small number of women with progression (n = 17) was not conclusive. With use of the Cox model, the results were virtually identical to those by logistic regression.

Table 2. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for progression of cytologically detected cervical squamous intraepithelial lesions in women treated with highly active antiretroviral therapy (HAART) or reverse-transcriptase inhibitor (RTI).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAART treated vs. all</td>
<td>1.819 (0.690–4.791)</td>
<td>3.504 (1.013–12.120)</td>
<td>.047</td>
</tr>
<tr>
<td>HAART treated vs. untreated</td>
<td>1.153 (0.336–3.956)</td>
<td>2.012 (0.440–9.201)</td>
<td>.36</td>
</tr>
<tr>
<td>RTI treated vs. all</td>
<td>0.419 (0.132–1.327)</td>
<td>0.268 (0.070–1.018)</td>
<td>.053</td>
</tr>
<tr>
<td>RTI treated vs. untreated</td>
<td>0.464 (0.108–2.003)</td>
<td>0.396 (0.078–1.994)</td>
<td>.26</td>
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</tbody>
</table>

NOTE. P values were calculated by logistic regression.
higher rate of worsening (7 [29%] of 24). In a multivariate model, the only parameter associated with a worse diagnosis was the gynecologic treatment (OR, 0.142; 95% CI, 0.03–0.74; \( P = .02 \)), whereas no significant effect of the anti-HIV therapy was observed after adjustment for the final CD4 cell count.

**Discussion**

The wide use of HAART for treatment of HIV-1 infection has deeply changed the scenario of the opportunistic pathologies once used to define the evolution of the disease. However, in some cases, as with HPV-related lesions and cervical cancer, few and conflicting data are available, and the impact of treatment-induced immune recovery is unclear. In this study, we evaluated the persistence of HR-HPV infection and the evolution of SIL among untreated HIV-positive women and women receiving either RTIs or HAART. A significant increase in CD4 cell counts was induced by HAART; however, the not significant variation of HIV viremia (–0.55 ± 2.0 copies/mL; \( P = .15 \)) was due to the study design, which included women already receiving treatment. Our data show that neither the prevalence and persistence of HR-HPV infection nor the natural history of the related cervical lesions differed in treated versus untreated women.

One of the earliest studies published on this topic [19] described the early regression of cervical lesions in women receiving protease inhibitor therapy. Those findings differed from ours, perhaps because of the composition of the comparison/control groups in the 2 studies (treated group at baseline in [19] vs. women untreated or undergoing different therapeutic regimens in our study) and because of the mean duration of the follow-up, 5 versus 15.4 months.

In a study on the prevalence and persistence of high-grade anal lesions in HIV-infected homosexual men, the lesions did not regress in 75% of men undergoing HAART [21]. Others reported that the cumulative rate of regression of cervical LSILs in women with CD4 cell counts <500 cells/\( \mu L \) at baseline did not differ significantly between women receiving antiretroviral therapy (20.5%) and untreated women (31.4%; \( P = .30 \)) [20]. However, compared with women with a preserved immune function (CD4 cell counts >500 cells/\( \mu L \)), immunodeficient women who were not receiving antiretroviral therapy had a significantly increased risk of developing SILs (no significant increase was observed in treated women), and the authors suggested that HAART could have a protective effect against the occurrence of SILs. Moreover, they showed a lower incidence of SILs 1 year after a normal Pap test result in 68 women with CD4 cell counts <500 cells/\( \mu L \) (41 treated with RTI and 27 treated with a drug combination) than in untreated HIV-positive women. These numbers were too small to allow evaluation of incidence of SILs by type of antiretroviral therapy.

In our study, to avoid selection bias due to the lower CD4 cell counts generally typical of patients treated with HAART, all statistical analyses were done after adjusting for CD4 cell count and HIV viremia at baseline and at end-point visit and for variation during follow-up. The only variable strongly associated with the persistence of HPV infection and, to a lesser extent, with the evolution of related lesions was CD4 cell count at the end of follow-up, independent of antiretroviral therapy, which underscores the relevance of immunosuppressive conditions in the HPV-related oncogenic process.

Of interest, we found a significant reduction in the incidence of new HPV-16 and -18 infections in the HAART-treated women, compared with the other groups (\( P = .043 \)). This may suggest that the immune recovery induced by HAART can (in patients with higher CD4 cell counts) duplicate that found in the general population (i.e., early treatment results in regression of acute HPV infections at higher risk for evolution but in less effect on well-established or advanced HPV disease). These findings parallel those described by Delmas et al. [20]: a lower incidence of SILs 1 year after a normal Pap test result in women treated with anti-HIV drugs versus untreated subjects.

The tendency to treat HIV disease with HAART may lessen the impact of immune suppression as a major cofactor for the increased frequency of HR-HPV infection and SILs in HIV-seropositive subjects and may make the course of such HPV infections more similar to that among women who are not infected with HIV. In healthy women, Pap and/or HPV testing and typing have been important in prevention of cervical cancer [27, 28]. Similarly, in our study of HIV-infected women, combined gynecologic and virologic monitoring allowed earlier identification and treatment of lesions that may progress to carcinoma. However, the high prevalence of HR-HPV infection in this population requires further evaluation, to assess the appropriateness of HPV testing in clinical practice. In our rigorously monitored population, we have not yet detected any case of invasive carcinoma. During the same period, there were 2 cases among HAART-treated patients attending our infectious diseases department (not enrolled in the present study). Both had a negative HIV-1 load and an increase in CD4 cell counts. This anecdotal observation supports the importance of routine monitoring for HR-HPV–related cervical disease for all HIV-infected women, including those receiving HAART.

Our results indicate that HPV cervical infection and related disease persist in a high proportion of women receiving HAART, particularly those with the longest history of HIV infection, and suggest that, regardless of antiretroviral regimen and its effect on HIV replication, the number (and probably competence) of CD4 cells is crucial. Because HAART has little effect on the natural history of HR-HPV–related cervical disease, all HIV-positive women should be routinely monitored, to reduce the risk of evolution of HSILs.

**References**


