Original article

Reduction of the risk of cervical intraepithelial neoplasia in HIV-infected women treated with highly active antiretroviral therapy

Emanuele Soncini[1], *Alessia Zoncada*[2], *Vittorio Condemi*[1], *Anna Degli Antoni*[2], *Elena Bocchialini*[1], *Paola Soregotti*[1]

[1] Department of Gynecology, Obstetrics and Neonatology, University of Parma, Parma, Italy; [2] Division of Infectious Diseases and Hepatology, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy

Abstract. *Background and aim of the work:* The purpose of our study was to determine whether highly active antiretroviral therapy (HAART) reduces the onset of cervical intraepithelial neoplasia (CIN) in HIV-positive women. *Methods:* The study was designed to assess CIN incidence in a cohort of 101 HIV-positive women and to evaluate its relationship with ongoing antiretroviral therapy. The patients were screened through a combined Pap smear and colposcopic examination on a yearly basis. If any abnormalities were reported, the patients underwent targeted biopsy with histological confirmation of the diagnosis. *Results:* During the follow-up period, 38 patients (37.6%) developed histologically verified CIN, including low-grade CIN in seven patients (6.9%) and high-grade CIN in 31 patients (30.4%). Over the study period, 43 patients (42.6%) were treated with HAART for at least 6 months, the average duration of treatment being 37 months. Analysis of HAART efficacy in preventing CIN onset, determined by the Cox regression model with a time-dependent covariate adjusted for the CD4 level at the first visit, showed that HAART significantly reduced the risk of developing CIN (hazard ratio, 0.3; p = 0.004). *Conclusion:* HIV-positive patients present a high incidence of CIN and of high-grade CIN in particular. HAART exhibits a protective action against the onset of cervical lesions. (www.actabiomedica.it)

Key words: Antiretroviral therapy, highly active; cervical intraepithelial neoplasia; cervix neoplasms; vaginal smear; colposcopy; cervix uteri/histopathology; cohort Studies

Introduction

Invasive cervical carcinoma and its precursors are the most significant gynecologic manifestation of HIV infection and in 1993 cervical cancer was even included among the AIDS-defining conditions (1). In HIV-positive women the annual incidence of squamous intraepithelial lesions (SILs) is estimated to be five times higher than in the general population (8.3/100 vs 1.8/100), with a three-fold risk of developing invasive cervical cancer (2). HIV-positive women also show an increased rate of concurrent HPV infection, especially with serotypes carrying a high risk of SILs (3). Thus, the hypothesis has been advanced that a synergic action of HIV-induced immunosuppression and HPV infection may actually favor the onset of precancerous cervical lesions (4). Highly active retroviral therapy (HAART) regimens have dramatically improved HIV prognosis. However, while systemic benefits are unquestionable, the beneficial actions that it may exhibit locally on the cervix are still unclear. The purpose of this study was to evaluate the effect of HAART on precancerous cervical disease in a population of HIV-positive women.

Materials and methods

Screening data from Pap smears and colposcopic examinations were collected for all HIV-positive women seen at the Colposcopy and Cervical Pathology Service of the Parma University Hospital, Obstetrics and Gynecology Unit, between January 1993 and December 2003. The patients were referred to this Unit from the Parma University Hospital Infectious and Liver Disease Unit within a year of their being admitted to a screening and early-diagnosis program for cervical cancer, either on an outpatient or day hospital basis. As part of the program, at each visit the women undergo a Pap smear and a colposcopic examination. Based on the protocol adopted at the Unit, if the Pap smear test and the colposcopic examination are negative, the women are requested to repeat them after a year; if the Pap smear and/or the colposcopic examination reveal any abnormalities, targeted biopsy is performed and treatment is given based on histological results.

Only patients without prior diagnosis of CIN at the first visit are enrolled in the study. For each patient we collected data from the Pap smear and colposcopic examination, as well as from any later biopsy. Pap smear and biopsy results were rated according to the 1991 Bethesda System classification (5). Cytological and histological samples were reviewed by the gynecologic pathologists of the Parma University Hospital Department of Pathology and Laboratory Medicine. Colposcopic examinations were performed by two expert colposcopists; the colposcopic findings were rated according to the International Federation for Cervical Pathology and Colposcopy (IFCPC) classification (6). For each patient we also reviewed clinical records and collected data on the HIV diagnosis date, the level of CD4+ lymphocytes/μl, and, when possible, the viral load (HIV RNA copies/ml) – both determined in the 6 months preceding the Pap smear and the colposcopic examination. HIV RNA plasma levels were measured by the Bayer HIV-1 RNA 3.0 Quantitative Assay (bDNA technology) with a sensitivity of 50-500,000 copies/ml. HAART efficacy was evaluated on the basis of the scheduled drug regimen administrated for at least 6 months after the examination: no therapy, single therapy or dual therapy with first-generation antiretrovirals – nucleoside analogue reverse transcriptase inhibitors (NRTIs) – and HAART based on a combination of three or more drugs. We also collected data from the patients' histories on the infection transmission route, drug abuse and cigarette smoking. Statistical analysis was performed using SPSS 11.5. For statistical analysis, we checked the CD4 and HIV RNA levels versus the stage of cervical disease progression at the last available visit using Student's t-test and the Mann-Whitney U test, respectively. We then assessed the effect of risk factors with the log-rank test and the effect of HAART on the SIL incidence rate with the Cox regression model taking HAART as a time-dependent covariate adjusted for the CD4 level at the first visit.

Results

A total of 120 HIV-positive women were studied in the period between January 1993 and December 2003. Of these, 101 did not present a prior diagnosis of SILs at the first visit and had been referred from the Parma University Hospital Infectious and Liver Disease Unit.

The baseline epidemiological and clinical features of the 101 patients, including age at HIV diagnosis, age at the first visit, geographic area of origin, infection transmission route, the presence of SIL risk factors, and CD4 lymphocyte count are summarized in Table 1.

For these patients we collected data from 340 visits, including 315 with combined Pap smear and colposcopic examination, and 25 with colposcopic examination only. In the data collection process we did not include the visits taken after histological confirmation of the SIL diagnosis, amounting to a total of 236 visits (218 with Pap smear and colposcopic examination, and 18 with colposcopic examination only).

Based on the results of the 236 visits considered in the study, 53 biopsies were performed, with positive histological evidence of SILs in 38 cases (71.7%) and negative histological findings in 15 cases (28.3%). During the observation period, 38 patients (37.6%) developed histologically verified SILs, low-grade

Table 1. Demographic characteristics and risk factors for cervical intraepithelial neoplasia at enrollment

	No. (%)
Geographic area of origin	
Italy	82 (81.2%)
Sub-Saharan Africa	14 (13.8%)
South America	3 (3%)
North Africa	1 (1%)
Eastern Europe	1 (1%)
Risk factors	
Drug abuse	36 (35.6%)
Prostitution	3 (3%)
Cigarette smoking	54 (53.5%)
Age (years) at HIV diagnosis mean (SD)	29 (7)
Transmission route	
Heterosexual intercourse	61 (60.4%)
IV drug abuse	36 (35.6%)
Blood transfusion	2 (2%)
Unknown 2 (2%)	
CD4+ cell counts/μL	
≥500	39 (38.6%)
200-499	50 (49.5%)
<200	12 (11.9%)

SD = Standard Deviation

(lSIL – CIN1) in seven patients (6.9%) and high-grade (hSIL - CIN2-3) in 31 patients (30.4%). At the time of SIL diagnosis (for the patients who developed the disease) or at the time of the last available visit (for the patients who did not develop the disease), mean CD4 levels appeared significantly lower in SIL patients (303/mm^3 vs 554/mm^3, p<0.001) and in patients with high-grade SILs compared with low-grade SIL patients (278/mm^3 vs 413/mm^3, p = 0.04) (Figure 1). The mean viral load was significantly higher in SIL patients than in non-SIL patients: 1.9 vs. 3.2 Log10 (HIV RNA copies)/mL; p = 0.004 (Figure 2). The

Table 2. Cervical biopsy results in the observation period

Biopsy diagnosis	No (%)
LSIL (CIN1)	7 (6,9%)
HSIL (CIN2-3)	31 (30,7%)
Total SIL	38 (37,6%)

LSIL = Low-Grade Squamous Intraepithelial Lesions
HSIL = High-Grade Squamous Intraepithelial Lesions
CIN = Cervical Intraepithelial Neoplasia

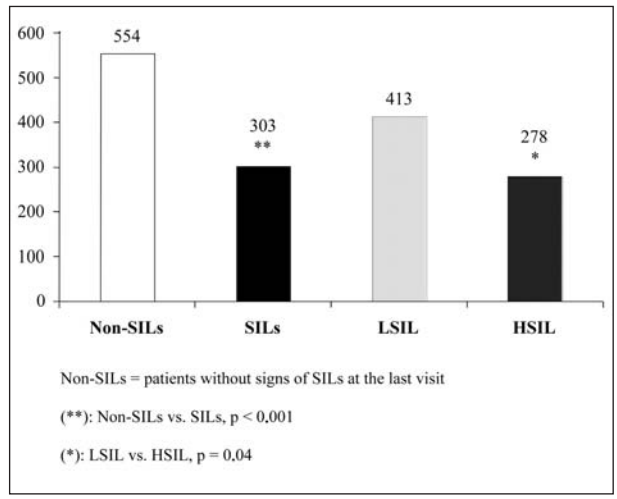

Figure 1. Mean CD4 levels at the end of the study and cervical status

mean observation period from HIV diagnosis to the last visit was 42 months (range, 6-72 months). During the study, 31 patients (30.7%) did not recieve antiretroviral therapy, 27 patients (26.7%) were given only one or two NRTIs for at least 6 months, 13 patients (12.9%) were given both first-generation antiretrovirals and then HAART, and 30 patients (29.7%) were given only HAART for a period of at least 6 months. Overall, 43 patients (42.6%) received HAART for at least 6 months, the average duration of treatment being 37 months (range, 7-60 months). The HAART

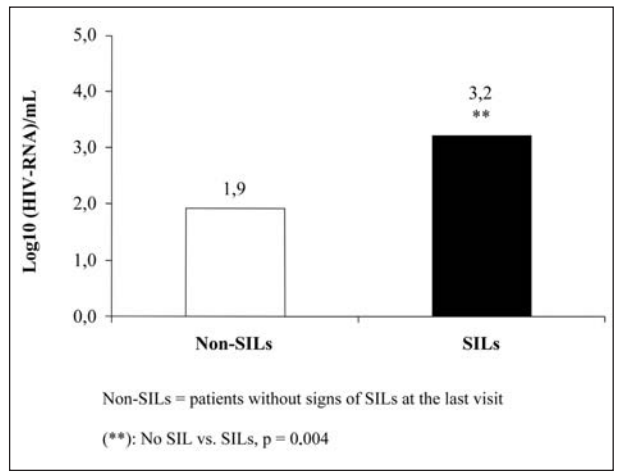

Figure 2. Mean viral load at the end of the study and cervical status

Table 3. Univariate and multivariate analysis of risk factors for cervical intraepithelial neoplasia

Univariate analysis	HR	CI 95%	p
Drug abuse	1.61	(0.85-3.07)	0.14
Cigarette smoking	0.91	(0.47-1.73)	0.77
CD4 count	2.26	(1.39-3.68)	0.001
HAART	0.3	(0.14-0.68)	0.004
Multivariate analysis	Adjusted HR	CI 95%	p
CD4 count	2.38	(1.44-3.96)	0.001
HAART	0.3	(0.13-0.68)	0.004

HR = Hazard Ratio; CI = Confidence Interval

regimen included two NRTIs + one protease inhibitor (PI) in 20 patients (46.5%), two NRTIs + one non-nucleoside reverse transcriptase inhibitor (NNRTI) in 18 patients (41.9%), and three NRTIs in 5 patients (11.6%). Univariate analysis of risk factors through survival curves and the log-rank test showed that low CD4 levels were associated with an increased SIL risk (hazard ratio = 2.26), but were not correlated with cigarette smoking and drug abuse. Multivariate analysis of HAART efficacy in preventing SIL onset was performed using the Cox regression model with a time-dependent covariate adjusted for the CD4 level at the first visit. It showed that HAART significantly reduced the risk of developing SILs (hazard ratio, 0.3; p = 0.004) (Table 3).

Conclusions

HAART has dramatically changed the natural history of HIV, strongly increasing CD4+ lymphocyte levels, reducing the incidence of AIDS-related diseases, and prolonging survival. Immunosuppression, characterized by a progressive decline of CD4 lymphocyte levels, is correlated with the onset of CIN and of high-grade CIN in particular (2). Our study results confirm this close correlation. Recent studies have indicated that HAART may show a beneficial effect on cervical diseases, but evidence is still too scant. In a study on 96 patients treated with antiretroviral drugs, Heard observed a positive correlation between regression of cervical lesions and improvement of the immunitary system (7). In a subsequent study, Heard found that CD4 cell counts <200/mm^3 and a positive margin were predictors of recurrence, whereas HAART had a strong protective effect (8). Minkoff found a reduced progression and an increased regression of lesions (9). Finally, Robinson demonstrated that patients treated with HAART have a lower risk of CIN relapse, persistence and progression (10).

In our study, we observed the onset of CIN in a large number of patients (37.3%) with a large proportion of high-grade cases (30.4%). The higher the CIN grade, the worse the viral immunological state of the patient. As we took only HAART as a time-dependent covariate in our comparison, we could not investigate the efficacy of antiretroviral therapy with NRTIs alone. The fact that HAART has been proved effective in reducing the risk in the treated group versus the control group of patients not treated with HAART, regardless of whether they were given or not NRTIs, indirectly supports evidence of the inefficacy or lower efficacy of non-HAART regimens in preventing CIN onset. This finding is in agreement with a pre-HAART study by Ellerbrock (2), who did not find any differences in the incidence of cervical disease between patients given antiretroviral-therapy compared to those who were not treated. A limiting factor of our study is the lack of information on the HPV state of the patients. Very few studies have considered the association between HIV, HPV correlated cervical lesions and HAART therapy. To date, the available data suggest that the widespread use of HAART has not led to reduced prevalence of genital HPV infection in HIV-positive women (7, 11). A recent report has shown that antiretroviral therapy does not prevent the development of HPV-associated lesions and does not eliminate HPV infection (12), however in the same study regression of low-grade lesions was more prevalent among patients receiving highly active antiretroviral therapy than among those receiving other regimens. The conflicting findings of literature suggest that further studies on larger case series are needed to evaluate whether HAART-induced reduction of the risk of developing SILs will be sufficient to change the aggressive approach to cervical disease screening currently recommended by several authors and authoritative clinical guidelines (2, 7, 13).

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Correspondence: Emanuele Soncini, MD, PhD.
Department of Gynecology, Obstetrics and Neonatology,
University of Parma,
Viale A. Gramsci, 14
43100 Parma, Italy
Tel. +390521290500
Fax +390521290508
E-mail: soncio@tin.it, www.actabiomedica.it