Cervical squamous intraepithelial lesions in HIV-infected women: prevalence, incidence and regression

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Objectives: To assess the impact of HIV-related immunodeficiency and antiretroviral treatment on the occurrence and evolution of abnormal Papanicolaou tests.

Study design: Cohort of 485 HIV-infected women with a known date of infection, enrolled during May 1993–April 1998 in 23 centres (gynaecology, infectious disease or STD clinics, or drug treatment centres) in 12 European countries; in 21 centres, follow-up was performed every 6 months (median follow-up: 2 years).

Methods: Human papillomavirus (HPV) was detected at inclusion by Southern blot and PCR. The prevalence of squamous intraepithelial lesions (SIL), the incidence of SIL and regression from low-grade SIL were studied according to CD4 count after controlling for HPV detection results.

Results: Compared with women with CD4 cell counts $> 500 \times 10^6$/l, women with CD4 cell counts $< 200 \times 10^6$/l had a twofold increase in both prevalence and incidence of SIL and in non-regression from untreated low-grade SIL; in addition, these women had a lower response rate to treatment of high-grade cervical intraepithelial neoplasia. The increase in SIL incidence associated with a low CD4 cell count was significant in women not receiving antiretroviral treatment (relative risk, CD4 cell count $200–499 \times 10^6$/l, 1.9; CD4 cell count $< 200 \times 10^6$/l, 2.9; CD4 cell count $> 500 \times 10^6$/l, reference), whereas it was less marked and not statistically significant in treated women.

Conclusions: Severe HIV-related immunodeficiency strongly increases the risk of occurrence of SIL; antiretroviral treatment may reduce this risk, probably by restoring or at least preserving immune function.

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Introduction

The relationship between human papillomavirus (HPV) infection and both cervical cancer and its precursor, cervical intraepithelial neoplasia (CIN), in women is now well established [1]. The association between HIV infection and HPV-related lesions, initially suggested by a higher prevalence of cervical lesions in HIV-infected than in uninfected women [2–5], was confirmed in other studies in which confounding factors (e.g. sexual behaviour and HPV infection) were controlled for [6–8]. The prevalence and the severity of cervical lesions have been reported to increase with increasing HIV-induced immunodeficiency [4,6–11]. A few prospective studies have also shown that cervical lesions are more likely to occur, progress, and recur after treatment in HIV-infected than in HIV-negative women [8,12–14]. Specific guidelines for the management of gynaecological diseases among HIV-infected women are needed. Whereas there appears to be a general consensus that HIV-infected women with a still-preserved immune function should be treated in the same way as HIV-uninfected women, the management of cervical lesions in severely immunocompromised HIV-infected women remains controversial.

Improving the diagnosis and clinical management of HPV-related cervical lesions in HIV-infected women requires that the natural history of HPV infection in these women be better understood. To assess the impact of immunodeficiency and of antiretroviral therapy on the occurrence and evolution of cervical lesions, data from a European cohort of HIV-infected women were analysed.

Methods

Study population

The European cohort on natural history of HIV infection in women is a prospective cohort of HIV-infected women with a known date of infection. Enrolment required that the date of HIV infection could be estimated within at most a 2-year time frame, either by a negative followed by a positive HIV test, or by a limited period of HIV risk behaviour, irrespective of the delay between infection and enrolment. Between May 1993 and April 1998, 485 women who gave informed consent were enrolled from 23 centres in 12 countries: Belgium, Denmark, Finland, France, Greece, Italy, Netherlands, Norway, Portugal, Spain, Sweden and Switzerland. In 21 centres, follow-up was performed every 6 months and is still ongoing.

Data collected

At each visit, women were interviewed about their socio-demographic characteristics, sexual behaviour and gynaecological and obstetric history using standardized questionnaires; HIV-related data (CD4 cell count, antiretroviral treatment, clinical stage [15]) were collected and a gynaecological examination including Papanicolaou (Pap) test was performed. No guidelines were given to the participating physicians regarding the management of abnormal Pap tests. All Pap smear slides were read in a single laboratory (Pasteur-Cerba, Cergy-Pontoise, France). Using the Bethesda classification, cervical cell abnormalities were classified either as atypical squamous or glandular cells of undetermined significance (ASCUS or AGCUS), or as low- or high-grade squamous intraepithelial lesions (SIL) [16]. Data on colposcopic examination and on biopsy results (if any) were collected. Histological lesions were classified as CIN I, II or III according to the Richart classification [17].

At enrolment, cervical samples for HPV detection were collected by brushing and placed in cryotubes containing Minimum Eagle's Medium and antibiotics at −80°C. DNA was extracted and quantified. HPV was detected using the Southern blot (SB) technique with hybridization using three different cocktails, each containing three radioactive probes to identify mildly oncogenic (HPV 6, 11 and 42), moderately oncogenic (HPV 31, 35 and 39) and highly oncogenic (HPV 16, 18 and 33) types [18]. The analysis of the restriction patterns obtained after washing the blots at low and high stringency allowed detection of all HPV types with high-level homology to the nine probes used as well as those with partial homology. As SB is not very sensitive in detecting HPV [19], a second test was performed using PCR. HPV DNA was amplified using the 'consensus' primers of Manos [20]. Hybridization of the PCR product with a cocktail of probes was used to specifically identify HPV 16, 18, and 33, and another cocktail of 'consensus' probes (GP1, GP2 [20]) was used to detect all other HPV types.

Data analysis

The prevalence of SIL on the first interpretable Pap test (initial Pap test) was calculated and studied according to HPV detection, adequacy of the Pap tests for diagnostic evaluation, clinical and biological stage of HIV infection, antiretroviral therapy, gynaecological and obstetrical history and socio-demographic characteristics. The incidence of SIL was estimated in women with normal initial Pap tests using the Kaplan–Meier method. For both analyses, Pap smears with ASCUS or AGCUS evoking a reactive process were considered as normal; those with ASCUS evoking SIL were counted as SIL.

Progression and regression rates from untreated low-grade SIL were estimated using the Kaplan–Meier method in women with low-grade SIL detected at enrolment or during follow-up. Progression was as-

sumed when high-grade SIL was detected on a subsequent Pap test. Regression was defined by a normal Pap smear, if the following smear was also normal. For women for whom no further Pap test was available after a single normal smear, follow-up was censored at the visit preceding the single normal smear. Women treated for cervical lesions were censored at the time of treatment.

For all analyses, Pap tests for which no cervical squamous cell could be observed, for whatever reason (e.g. obscuring inflammation), were considered as uninterpretable. Results of HPV detection by SB were grouped into: (i) 16, 18, 33 (HPV types '16, 18, 33' and related types); (ii) '31, 35, 39' (types 31, 35, 39 and related types); or (iii) '6, 11, 42' (types 6, 11, 42, related and undetermined types). When several types were detected by SB, results were classified according to the most oncogenic type. A woman was considered as receiving antiretroviral treatment at a given visit if the treatment had been prescribed at least 1 month prior to the visit. Adequacy of the Pap tests was defined by the presence of an endocervical/transformation zone component.

Differences in proportions, means and medians were tested using respectively the Pearson χ^2 (or Fisher's exact test where appropriate), Student's t and Kruskal–Wallis tests. Differences in incidence or regression rates were tested using the log-rank test. Stepwise multiple logistic regression models were used to identify factors independently associated with prevalent SIL. Multivariate analyses of factors associated with incident SIL or with regression from low-grade SIL were performed using Cox regression models. The Kaplan–Meier and the multivariate analyses were performed using BMDP software (University of California Press, Berkeley, California, USA). A significance level of 0.05 was chosen and adjusted odds ratios (aOR) or relative risks (aRR) were computed with 95% confidence intervals (CI).

Table 1. Characteristics of the study population at enrolment, 467 HIV-infected women with at least one interpretable cervical Papanicolaou (Pap) test[a].

Age (years) [median (range)]	31 (17–68)
Region of birth [n (%)]	
Europe	417 (89.3)
Sub-Saharan Africa	22 (4.7)
Other	28 (6.0)
European region of enrolment[b] [n (%)]	
Southern	235 (50.3)
Middle	139 (29.8)
Nordic	93 (19.9)
Recruitment site [n (%)]	
Infectious disease clinic	275 (58.9)
Gynaecology clinic	116 (24.8)
STD clinic	28 (6.0)
Drug treatment centre	48 (10.3)
HIV transmission group [n (%)]	
Injecting drug user	108 (23.1)
Heterosexual contact	331 (70.9)
Transfusion recipient	19 (4.1)
Other or unknown	9 (1.9)
Time since HIV infection (years) [median (range)]	4.3 (0.2–15)
HIV infection [n (%)]	
HIV-1	464 (99.4)
HIV-2	3 (0.6)
CD4 cell count ($\times 10^6$/l) [median (range)]	378 (0–2180)
Clinical stage [n (%)]	
A	282 (60.4)
B	145 (31.0)
C	40 (8.6)
HPV detection[c] [n (%)]	
SB (−) PCR (−)	174 (43.8)
SB (+) PCR (−)	10
SB (−) PCR (+)	125 } (56.2)
SB (+) PCR (+)	88

[a]For four women with uninterpretable Pap test at enrolment, the first visit with interpretable Pap test result was considered to be the enrolment visit. [b]Southern: Greece, Italy, Portugal, Spain; Middle: Belgium, France, Netherlands, Switzerland; Nordic: Denmark, Finland, Norway, Sweden. [c]Results of human papillomavirus (HPV) detection were available for 397 women. SB, Southern blot.

Results

Study population

The study population comprised 467 women with at least one interpretable Pap test. Population characteristics are presented in Table 1. In the 21 centres participating in follow-up, 423 women were included, among whom 310 were followed-up at least once and contributed to the follow-up analysis with a median time of 2 years (25th percentile, 1 year; 75th percentile, 2.5 years).

The proportion of women followed-up was 66.4% (310/467) and differed significantly according to the type of recruitment site (53.6% in STD clinics, 61.5% in infectious disease clinics, 72.9% in drug treatment centres, 78.4% in gynaecological clinics; $P < 0.01$) and according to the region of enrolment (51.1% in southern Europe, 82.7% in middle Europe, 80.6% in Nordic countries; $P < 0.001$). No difference in the proportion of women followed-up was observed according to age at enrolment, region of birth, HIV-related data (CD4 cell count, clinical stage, HIV transmission group, time between infection and enrolment) or results of the Pap test and of HPV detection performed at enrolment.

Prevalence of and risk factors for SIL

SIL was detected on the initial Pap test in 24.2% (113/467) of the women (low-grade, 98; high-grade, 13; ASCUS evoking SIL, two). SIL detection differed significantly according to HPV detection, CD4 cell count, age, presence of genital warts, past history of genital warts, previous pregnancy, history of SIL and

type of recruitment site (Table 2). Among women with a past history of SIL, the prevalence of SIL at enrolment was lower if past SIL had occurred prior to or within the year of HIV infection (9.1%; 1/11) than if it occurred later (47.9%; 35/73) ($P = 0.02$). The difference remained statistically significant when excluding the 29 women who had a recent history of untreated SIL (within 1 year preceding enrolment). No significant difference was found according to other factors studied: year of enrolment, HIV transmission group, time since HIV infection, antiretroviral therapy, past STD (genital herpes, gonorrhoea or trichomoniasis), sexual behaviour, contraceptive history, frequency of previous Pap test screening and Pap test adequacy (data not shown).

In multivariate analysis, six factors were found to be independently associated with a higher prevalence of

Table 2. Factors associated with detection of squamous intraepithelial lesions (SIL) at initial Papanicolaou (Pap) smear, among 467 HIV-infected women with at least one interpretable cervical Pap test, univariate and mutivariate analyses[a].

	No. women	(%) with SIL	aOR	95% CI
HPV detection and typing[b]				
SB (−) PCR (−)	174	10.9[c]	1	
SB (+) '16, 18, 33'	47	59.6	12.7	5.4–29.9
SB (+) '31, 35, 39'	28	53.6	6.0	2.6–13.7
SB (+) '6, 11, 42'	23	47.8	2.4	0.6–9.7
SB (−) PCR (+) 16, 18, 33	16	25.0		
SB (−) PCR (+) ≠ 16, 18, 33	109	18.3	2.2	1.1–4.6
CD4 cell count ($\times 10^6$/l)				
⩾ 500	155	19.4[c]	1	
200–499	211	23.7	1.6	0.9–3.0
< 200	101	32.7	2.4	1.2–4.7
Age (years)				
⩽ 27	144	30.6[c]	0.7[d]	0.5–1.0
28–33	173	26.0		
⩾ 34	150	16.0		
Presence of genital warts				
No	418	20.1[c]	1	
Yes	49	59.2	4.5	2.1–9.8
History of genital warts				
No	368	22.0[c]		
Yes	99	32.3		
History of SIL[e]				
No SIL[f]	373	20.6[g]	1	
SIL > 1 year before enrolment	37	27.0		
SIL < 1 year before enrolment, treated	18	22.2	1.0	0.5–2.2
SIL < 1 year before enrolment, untreated	29	75.9	8.1	2.9–23.0
Pregnancy history				
Never	127	33.9[c]		
Ever	340	20.6		
Recruitment site				
Infectious disease clinic	275	21.1[c]		
Gynaecology clinic	116	32.8		
STD clinic or drug treatment centre	76	22.4		
European region of enrolment[h]				
Southern	235	20.0	1	
Middle	139	27.3	1.7	0.9–3.1
Nordic	93	30.1	3.0	1.6–5.9

[a]For four women with uninterpretable Pap test at enrolment, the first visit with interpretable Pap test result was considered to be the enrolment visit. [b]Results of human papillomavirus (HPV) detection were available for 397 women. [c]P value (Pearson χ^2 test) < 0.05. [d]For a 10-year increase in age. [e]Information available for 457 women. [f]Including 70 women with no past Pap test screening. [g]P value (Pearson χ^2 test) < 0.05, after regrouping women with SIL > 1 year before enrolment and with treated SIL < 1 year before enrolment. [h]Southern: Greece, Italy, Portugal, Spain; Middle: Belgium, France, Netherlands, Switzerland; Nordic: Denmark, Finland, Norway, Sweden. aOR, logistic regression odds ratio adjusted for HPV detection, CD4 count, SIL history, age, genital warts, and region of enrolment; CI, confidence interval; SB, Southern blot.

SIL: positive HPV detection, low CD4 cell count, young age, history of recent untreated SIL, presence of genital warts and enrolment in Nordic countries (Table 2). Similar results were found when the analysis was restricted to the 310 women followed-up at least once (data not shown).

The magnitude of the association between HPV detection by SB and prevalent SIL increased with decreasing CD4 cell count, although the interaction was not statistically significant: the aOR associated with a positive detection by SB ranged from 5.6 (95% CI, 1.4–21.7) for CD4 cell counts $> 500 \times 10^6$/l to 10.6 (3.7–30.2) for CD4 counts of $200–499 \times 10^6$/l and 17.9 (4.0–79.7) for CD4 counts $< 200 \times 10^6$/l. The aOR associated with a positive detection by PCR only remained unchanged regardless of the immunodeficiency level.

Incidence of and risk factors for SIL

Among the 317 women who were enrolled in the 21 centres with follow-up and whose initial Pap test was normal, 229 were followed-up for a median time of 2 years (range, 6 months to 4 years). SIL was diagnosed in 70 women (low-grade, 61; high-grade, six; ASCUS evoking SIL, three). The cumulative incidence of SIL was estimated to be 23.6% at 1 year and 29.5% at 18 months (27.7% and 2.5% at 18 months for low- and high-grade SIL respectively). As the incidence of SIL in the 101 women with no history of SIL and whose last normal Pap test had been performed within the year preceding enrolment did not differ from that in other women (27.0% versus 31.8% at 18 months; $P = 0.36$) data from both groups were analysed together.

The incidence of SIL was significantly higher in HPV-positive than in HPV-negative women ($P < 0.001$) (Table 3). It tended to increase with decreasing CD4 cell count at the time of the initial normal Pap test ($P = 0.16$). It was significantly higher in women who had genital warts at the initial visit than in other women ($P = 0.01$). Higher incidences were observed in women with past SIL, young women and women recruited in Nordic countries than in other women, but none of the differences was statistically significant (respectively $P = 0.12$, 0.22 and 0.78).

Using a Cox regression model, HPV detection and CD4 cell count were independently associated with incident SIL. Compared with HPV-negative women, the risk of developing SIL was increased in SB-positive women and in women positive for HPV types 16, 18,

Table 3. Incidence of squamous intraepithelial lesions (SIL) among 229 HIV-infected women followed-up after initial normal Papanicolaou (Pap) test, univariate and multivariate analyses.

	No. women	% with SIL at 18 months	aRR	95% CI
HPV detection and typing[a]				
SB (−) PCR (−)	105	22.6[b]	1	
SB (+) '16, 18, 33'	13	79.5	5.5	2.5–12.4
SB (+) ≠ '16, 18, 33'	18	47.0	2.8	1.3–6.1
SB (−) PCR (+) 16, 18, 33	10	53.7	3.5	1.4–8.7
SB (−) PCR (+) ≠ 16, 18, 33	60	27.2	1.6	0.9–3.0
CD4 cell count ($\times 10^6$/l)[c]				
⩾ 500	87	22.0	1	
200–499	99	30.5	1.6	0.9–2.8
< 200	43	43.5	1.9	1.0–3.7
Age[c]				
⩽ 27	66	36.4		
28–33	85	25.7		
⩾ 34	78	28.7		
Presence of genital warts[c]				
No	211	28.1[b]		
Yes	18	45.7		
History of SIL[d]				
No[e]	187	27.4		
Yes	34	43.4		
European region of enrolment[f]				
Southern	94	24.3		
Middle	83	31.9		
Nordic	52	34.5		

[a]Results of human papillomavirus (HPV) detection were available for 206 women. [b]*P* value (Log-rank test) < 0.05. [c]At initial normal Pap test. [d]Information available for 221 women. [e]Including 27 women with no past Pap test screening. [f]Southern: Greece, Italy, Portugal, Spain; Middle: Belgium, France, Netherlands, Switzerland; Nordic: Denmark, Finland, Norway, Sweden. aRR, relative risk adjusted for HPV detection and CD4 count. CI, Confidence interval; SB, Southern blot.

33 by PCR only. It increased with decreasing CD4 cell count and was almost twice as high for CD4 cell counts $< 200 \times 10^6$/l as for CD4 cell counts $> 500 \times 10^6$/l.

Stratified by CD4 count, women on antiretroviral therapy at initial normal Pap test tended to have a lower incidence of SIL (17.5% and 28.0% at 1 year for CD4 cell counts of $200-499 \times 10^6$/l and $< 200 \times 10^6$/l respectively) than untreated women (26.1% and 42.3% respectively) ($P = 0.44$). Among the 68 treated women with CD4 counts $<500 \times 10^6$/l, 41 were treated with a single nucleoside analogue (mainly zidovudine) and 27 with a drug combination (including a protease inhibitor for seven women). These numbers were too small to study the incidence of SIL according to the type of antiretroviral therapy. All but one of the 68 women were still receiving antiretroviral therapy at the end of follow-up. After adjustment for HPV detection (Cox regression model), the risk of developing SIL was higher in women not receiving than in women receiving antiretroviral therapy: the increase in SIL incidence associated with CD4 cell counts $< 500 \times 10^6$/l versus $> 500 \times 10^6$/l was significant in untreated women [aRR, 1.9 (95% CI, 1.0–3.6) and 2.9 (1.1–8.0) for CD4 counts of $200-499 \times 10^6$/l and $< 200 \times 10^6$/l respectively], whereas it was less marked and not statistically significant in treated women [aRR, 1.3 (0.6–2.8) and 1.7 (0.8–3.5) respectively].

Evolution of low-grade SIL

Among the 165 women diagnosed with low-grade SIL, 40 had no subsequent Pap test and 10 were immediately treated for SIL/CIN. For the remaining 115 women, the median time of follow-up in absence of treatment for SIL/CIN was 18 months (range, 6 months to 3.5 years).

Only 11 women progressed to high-grade SIL and regression was observed in 33 women. The cumulative rates of progression and of regression at 1 year were 8.1% and 29.5% respectively. Both rates remained unchanged (8.3% and 30.9%) when women who underwent a biopsy were censored at the time of biopsy. Regression rates were higher in HPV-negative than in other women ($P = 0.18$) and decreased with increasing immunodeficiency ($P = 0.12$) (Table 4). They ranged from 77.8% at 1 year in eight HPV-negative women with CD4 cell counts $> 500 \times 10^6$/l to 0% at 1 year in 11 women SB-positive for HPV '16, 18, 33' with CD4 cell counts $< 200 \times 10^6$/l. Among women with CD4 cell counts $< 500 \times 10^6$/l, the cumulative rates of regression at 1 year were 20.5% in those receiving antiretroviral therapy at low-grade SIL diagnosis and 31.4% in untreated women ($P = 0.30$), but the median CD4 cell count was lower in treated (218×10^6/l) than in untreated women (348×10^6/l) ($P < 0.01$).

Table 4. Rates of regression from untreated low-grade squamous intraepithelial lesions (SIL) according to CD4 cell count and human papillomavirus (HPV) detection, among 115 HIV-infected women followed-up after a diagnosis of low-grade SIL.

	No. women	% Regression at 1 year
HPV detection and typing[a]		
SB (−) PCR (−)	32	49.2
SB (+) '16, 18, 33'	20	10.0
SB (+) ≠ '16, 18, 33'	21	31.4
SB (−) PCR (+)	35	18.4
CD4 cell count ($\times 10^6$/l)[b]		
⩾ 500	29	46.1
200–499	55	26.8
< 200	31	19.5

[a]At enrolment; results available for 108 women. [b]At diagnosis of low-grade SIL. SB, Southern blot.

After adjustment for HPV detection (Cox regression model), a decreasing trend in the probability of regression was found with decreasing CD4 cell count but was not statistically significant: compared with CD4 cell counts $> 500 \times 10^6$/l, the aRR associated with CD4 cell counts $< 200 \times 10^6$/l was 0.5 (95% CI, 0.2–1.4).

Biopsy results and treatment for CIN

A cervical biopsy was performed in 22 of the 30 women diagnosed with high-grade SIL. Biopsy confirmed high-grade CIN in 15 women (68%) (CIN II, nine; CIN III, six), revealed CIN I in four and was CIN-negative in three. Treatment for CIN was performed in all women with high-grade CIN and in three women with CIN I or CIN-negative biopsy. In two of these three women, the colposcopy showed acetowhite lesions; in the third woman, the squamous-columnar junction was not visible. Among the four women with CIN I or CIN-negative biopsy who were not treated, two had a normal colposcopic examination, one had signs of chronic cervicitis, and one had acetowhite lesions. Among the 18 women treated for CIN, one (with CIN I at biopsy) was treated by cryotherapy, 15 by excisional procedures (loop electrosurgical excision or conization) and two by hysterectomy. The histological results of surgical specimens were available for 14 women: high-grade CIN was confirmed in 13 (CIN II, six; CIN III, seven) and CIN I was diagnosed in one woman (who had CIN II at biopsy).

A biopsy was performed in 58 of the 165 women with low-grade SIL. Normal results were found in 20 women, CIN I was diagnosed in 21 and high-grade

CIN in 17 (CIN II, 10; CIN III, seven). Among the 15 biopsies performed after a single smear with low-grade SIL, 33.3% showed high-grade CIN and 40.0% were CIN-negative; these proportions were respectively 27.9% and 32.6% among the 43 biopsies performed for persistent low-grade SIL. Eleven women with high-grade CIN were treated: eight by excisional procedures and three by destructive treatments (cryotherapy or laser vaporization).

Recurrence or persistence of SIL after treatment for high-grade CIN was assessed in 21 women in whom a subsequent Pap test was performed (within 2–15 months of treatment). The proportion of persistent/recurrent SIL was 50% in 12 women treated for CIN II and 67% in nine treated for CIN III. None of the four women with more than 500×10^6 CD4 cells/l at the time of CIN treatment, six of the 11 with $200–499 \times 10^6$ CD4 cells/l and all six with $< 200 \times 10^6$ CD4 cells/l had persistent/recurrent SIL. The median CD4 cell count was lower in women with persistent/recurrent SIL than in others (196×10^6/l and 411×10^6/l respectively, $P = 0.02$).

Discussion

This study is one of the largest to evaluate the incidence of SIL in HIV-infected women. Our findings, obtained through a 2-year median follow-up of 229 women, clearly support an increase in SIL incidence with increasing HIV-related immunodeficiency. In the only published prospective study in which SIL incidence could be estimated according to the severity of HIV-related immunodeficiency, the cumulative rates in 160 women followed-up for 13 months ranged from 17% in both women receiving antiretroviral therapy and untreated women with CD4 cell counts $> 500 \times 10^6$/l to 27% in untreated women with CD4 cell counts $< 500 \times 10^6$/l [8]. In our study, although the difference was not statistically significant, incidence rates were lower in women receiving antiretroviral therapy at the time of initial normal Pap test than in untreated women with the same levels of immunodeficiency. However, compared with women with a still-preserved immune function, a significant increase in the risk of developing SIL was found in immunodeficient women who were not receiving antiretroviral therapy, whereas no significant increase was observed in treated women. This result may suggest a protective effect of antiretroviral therapy against the occurrence of SIL in HIV-infected women. However, the specific effect of antiretroviral therapy cannot be disentangled from a more general effect of regular medical care prior to enrolment (which was not measured in our study). Furthermore, in our study, few women have been followed-up beyond mid-1996, when highly active antiretroviral therapy (HAART) and HIV viral load measurement became available. The influence of viral load lowering due to antiretroviral therapy on the occurrence of SIL could not therefore be assessed.

The association between prevalent SIL and HIV-related immunodeficiency has been reported previously [6–9]. The protective role of the integrity of the immune system against HPV-related lesions was first suggested by studies showing an increased risk of such lesions in women receiving immunosuppressive treatments [21,22]. However, the mechanism through which HIV-related immunodeficiency increases the risk of SIL/CIN is not well understood. In the present study, the association between HIV-related immunodeficiency and prevalent or incident SIL remained statistically significant after controlling for HPV detection. This association may be interpreted as a promoting effect of immunodeficiency on the carcinogenic effect of HPV. By decreasing the immune control of HPV infection, immunodeficiency may increase the duration of HPV infection or may allow HPV to replicate to higher levels, thus increasing the risk of oncogenic progression. As SB is less sensitive than PCR in detecting HPV [19], detection by SB should correspond to a higher HPV load in the cervix than detection by PCR only. Because HPV detection by SB and by PCR only was controlled for, differences in HPV load were partly taken into account. The increase in the risk of SIL associated with a positive SB detection according to the severity of immunodeficiency may therefore be explained by a higher HPV load in highly immunocompromised women.

Regression at 1 year from untreated low-grade SIL was observed in only 30% of the women. A similar regression rate was found when women who underwent a biopsy were censored at the time of biopsy, suggesting that the spontaneous evolution of low-grade SIL was not altered by biopsies. In order to reduce the false-negative rate, regression was defined by two consecutive normal smears. The rate of regression may therefore have been underestimated. However, the estimated rate is similar to those reported among HIV-infected women in two studies [8,23]. In another study in which immunocompromised HIV-infected women were examined prior to and after initiation of HAART, a higher regression rate (43%) was observed [24]. Although much greater than in these three studies, the relatively small size of the present study sample (115 women) makes it difficult to identify factors associated with regression from low-grade SIL. Nevertheless, a decreasing trend in regression rates was observed with decreasing CD4 cell count. The rate estimated in women with CD4 cell counts $> 500 \times 10^6$/l (46% at 1 year) is consistent with that of 62% observed in 555 HIV-negative immunocompetent women followed-up for 4 years [25].

The response to treatment for high-grade CIN was associated with the level of immunocompetence, with rates consistent with other studies. In women with severe HIV-related immunodeficiency, persistence or recurrence of CIN is very common and high recurrence/persistence rates ($> 60\%$) were reported in all previously published studies [13,14]. In contrast, in immunocompetent HIV-negative women, treatment for CIN is usually successful, with cure rates $> 90\%$ [26]. Compared with HIV-negative women, more extensive CIN which are more likely to involve the endocervical canal were reported in HIV-infected women [27]. As the study questionnaire did not include specific data on histology of surgical specimen margins, persistent CIN due to residual CIN after treatment could not be distinguished from recurrent CIN.

Although in HIV-infected women, the sensitivity of Pap smear, compared with cervical biopsy, was questioned in one study [28], subsequent reports have shown high sensitivity (ranging from 63% to 94%) and positive predictive value (from 64% to 94%), similar to those observed in HIV-uninfected women [6,29,30]. Biopsy is recommended only in cases of persistent low-grade SIL; for evident ethical reasons, no further guidelines regarding the diagnostic confirmation of CIN were given for the purpose of this study. Among women with low-grade SIL, although very few had a biopsy, the proportion with high-grade CIN was high (29%), whether the biopsy was done following one or more than one smear showing low-grade SIL. Moreover, the proportion of CIN-negative biopsies remained as high (34%) among biopsies performed for persistent low-grade SIL as among those done after a single smear with low-grade SIL. This suggests that the proportion of CIN-negative biopsies among smears with low-grade SIL was overestimated in our study, probably because some lesions were missed at biopsy. For high-grade SIL, concordance with results of biopsy or of surgical specimen examination was quite satisfactory. It is therefore unlikely that the true rate of high-grade CIN was overestimated with Pap tests.

Whereas the risk of prevalent SIL was increased in women with untreated SIL within the year preceding enrolment in the cohort, no significant association was found with treated SIL within the year of enrolment or with earlier SIL. However, the history of abnormal Pap tests was not estimated accurately as the data were collected retrospectively. The decreasing trend in SIL prevalence with increasing age is consistent with that observed in the general population in the USA [31]. As genital warts are related to HPV types which are rarely associated with genital cancer [18], the association between SIL and genital warts may result from infections with multiple HPV types (including multiple oncogenic types). The association with the region of enrolment is difficult to interpret and may be due to confounding factors not taken into account in our study.

The detection of SIL was not associated with sexual behaviour or with contraceptive history, although these factors have been shown to be associated with cervical cancer in HIV-uninfected women [32]. However, our study population comprised only HIV-infected women, most of them infected through heterosexual intercourse or through injecting drug use, and its relative homogeneity regarding risk factors for cervical cancer may have precluded the ability to detect associations of these factors with SIL.

Despite high incidence of CIN and progression to higher grade CIN in immunocompromised women, no invasive cervical cancer was diagnosed. The follow-up may not have been long enough for cancer to occur. Furthermore, the incidence of cervical cancer may have been underestimated as treatment for CIN was not performed systematically using excisional procedures and as, for obvious ethical reasons, biopsy was not performed at each visit. In addition, timely treatment of SIL/CIN may have prevented the progression of these lesions to cancer.

In HIV-infected women, severe immunodeficiency is associated with a high prevalence of SIL, a high incidence of SIL, a low regression rate from low-grade SIL, and a high rate of recurrence or persistence after treatment for high-grade CIN. In women with severe immunodeficiency, preventing CIN from progressing without repeated excisional treatment, which could result in cervical stenosis, may require an optimization of the immune function which should be obtainable by HAART.

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Appendix

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References

1. Muñoz N, Bosch FX. **HPV and cervical neoplasia: review of case-control and cohort studies.** In: *The Epidemiology of Human Papillomavirus and Cervical Cancer*. Edited by Muñoz N, Bosch FX, Shah KV, Meheus A. Lyon: IARC Scientific Publications; 1992:251–261.
2. Bradbeer C. **Is infection with HIV a risk for cervical intraepithelial neoplasia? [Letter].** *Lancet*. 1987, **2**:1277–1278.
3. Schrager LK, Friedland GH, Maude D, *et al*. **Cervical and vaginal squamous cell abnormalities in women infected with human immunodeficiency virus.** *J Acquir Immune Defic Syndr*. 1989, **2**:570–575.
4. Schäfer A, Friedman W, Mielke M, Schwartländer B, Koch MA. **The increased frequency of cervical neoplasia in HIV-infected women is related to the degree of immunosuppression.** *Am J Obstet Gynecol*. 1991, **164**:593–599.
5. Mandelblatt J, Fahs M, Garibaldi K, Senie R, Peterson H. **Association between HIV infection and cervical neoplasia: implications for clinical care of women at risk for both conditions.** *AIDS* 1992, **6**:173–178.
6. Wright TC, Ellerbrock TV, Chiasson MA, Van Devanter N, Sun XW, and the New York Cervical Disease Study. **Cervical intraepithelial neoplasia in women infected with human immunodeficiency virus: prevalence, risk factors, and validity of Papanicolaou smears.** *Obstet Gynecol*. 1994, **84**:591–597.
7. Klein RS, Ho GY, Vermund SH, Fleming I, Burk RD. **Risk factors for squamous intraepithelial lesions on Pap smear in women at risk for HIV infection.** *J Infect Dis* 1994, **170**:1404–1409.
8. Six C, Heard I, Bergeron C, *et al*. **Comparative prevalence, incidence and short-term prognosis of cervical squamous intraepithelial lesions amongst HIV-positive and HIV-negative women.** *AIDS* 1998, **12**:1047–1056.
9. Conti M, Agarossi A, Parazzini F, *et al*. **HPV, HIV infection and risk of cervical intraepithelial lesions in former injecting drug abusers.** *Gynecol Oncol* 1993, **49**:344–348.
10. Williams A, Darragh T, Vranizan K, Ochia C, Moss A, Palefsky J. **Anal and cervical HPV infection and risk of anal and cervical epithelial abnormalities in HIV-infected women.** *Obstet Gynecol* 1994, **83**:205–211.
11. Langley CL, Benga-De E, Critchlow CW, *et al*. **HIV-1, HIV-2, human papillomavirus infection and cervical neoplasia in high-risk African women.** *AIDS* 1996, **10**:413–417.
12. Petry KU, Scheffel D, Bode U, *et al*. **Cellular immunodeficiency enhances the progression of HPV-associated cervical lesions.** *Int J Cancer* 1994, **57**:836–840.
13. Wright TC, Koulos J, Schnoll F, *et al*. **Cervical intraepithelial neoplasia in women infected with the human immunodeficiency virus: outcome after loop electrosurgical excision.** *Gynecol Oncol* 1994, **55**:253–258.
14. Fruchter RG, Maiman M, Sedlis A, Bartley L, Camilien L, Arrastia CD. **Multiple recurrences of cervical intraepithelial neoplasia in women infected with the human immunodeficiency virus.** *Obstet Gynecol* 1996, **87**:338–344.
15. Centers for Disease Control and Prevention. **1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults.** *MMWR* 1992, **41**:1–19.
16. National Cancer Institute Workshop. **The 1988 Bethesda system for reporting cervical/vaginal cytologic diagnoses.** *JAMA* 1989, **262**:931–934.
17. Richart RM. **Cervical intraepithelial neoplasia: a review.** In: *Pathology Annual*. Edited by Sommers SC. East Norwalk: Appleton-Century-Crofts; 1973:301–328.
18. Barrasso R, de Brux J, Croissant O, Orth G. **High prevalence of papillomavirus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia.** *N Engl J Med* 1987, **317**:916–923.
19. Shiffman MH. **Validation of HPV hybridization assays: correlation of filter *in situ*, dot blot and PCR with Southern blot.** In: *The Epidemiology of Human Papillomavirus and Cervical Cancer*. Edited by Muñoz N, Bosch FX, Shah KV, Meheus A. Lyon: IARC Scientific Publications; 1992:169–179.
20. Manos MM, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. **The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses.** In: *Molecular Diagnostics of Human Cancer*. Edited by Furth M, Greaves M. New York: Cold Spring Harbor Laboratory Press; 1989:209–214.
21. Schneider V, Kay S, Lee HM. **Immunosuppression as a high-risk factor in the development of condyloma acuminatum and squamous neoplasia of the cervix.** *Acta Cytol* 1983, **27**: 220–224.
22. Halpert R, Fruchter RG, Sedlis A, Butt K, Boyce JG, Sillman FH. **Human papillomavirus and lower genital neoplasia in renal transplant patients.** *Obstet Gynecol* 1986, **68**:251–258.
23. Belafsky P, Clark RA, Kissinger P, Torres J. **Natural history of low-grade squamous intraepithelial lesions in women infected with human immunodeficiency virus [Letter].** *J Acquir Immune Defic Syndr* 1996, **11**:511–512.
24. Heard I, Schmitz V, Costagliola D, Orth G, Kazatchkine MD. **Early regression of cervical lesions in HIV-seropositive women receiving highly active antiretroviral therapy.** *AIDS* 1998, **12**:1459–1464.
25. Nasiell K, Roger V, Nasiell M. **Behavior of mild cervical dysplasia during long-term follow-up.** *Obstet Gynecol* 1986, **67**:665–669.
26. Follen Mitchell M, Tortolero-Luna G, Cook E, Whittaker L, Rhodes-Morris H, Silva E. **A randomized clinical trial of cryotherapy, laser vaporization and loop electrosurgical excision for treatment of squamous intraepithelial lesions of the cervix.** *Obstet Gynecol* 1998, **5**:737–744.
27. Fruchter RG, Maiman A, Sillman FH, Camilien L, Webber CA, Kim DS. **Characteristics of cervical intraepithelial neoplasia in**

women infected with the human immunodeficiency virus. *Am J Obstet Gynecol* 1994, **171**:531–537.

28. Maiman M, Tarricone N, Vieira J, Suarez J, Serur E, Boyce JD. **Colposcopic evaluation of human immunodeficiency virus-seropositive women.** *Obstet Gynecol* 1991, **78**:84–88.
29. Korn AP, Autry M, Deremer PA, Tan W. **Sensitivity of the Papanicolaou smear in human immunodeficiency virus-infected women.** *Obstet Gynecol* 1994, **83**:401–404.
30. Adachi A, Fleming I, Burk RD, Ho GYF, Klein RS. **Women with human immunodeficiency virus infection and abnormal Papanicolaou smears: a prospective study of colposcopy and clinical outcome.** *Obstet Gynecol* 1993, **81**:372–377.
31. Sadeghi SB, Sadeghi A, Cosby M, Olincy A, Robboy SJ. **Human papillomavirus infection. Frequency and association with cervical neoplasia in a young population.** *Acta Cytol* 1989, **33**: 319–323.
32. Brinton LA. **Epidemiology of cervical cancer – Overview.** In: *The Epidemiology of Human Papillomavirus and Cervical Cancer.* Edited by Muñoz N, Bosch FX, Shah KV, Meheus A. Lyon: IARC Scientific Publications; 1992:3–23.