

Persistence of High Percentage of Peripheral Activated CD8+ T Cells Predict Cytologic HPV-Related Dysplasia in cART-Treated, HIV-Positive Subjects

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Background. People with HIV are at increased risk of human papillomavirus (HPV) disease progression, given the persistence of immune activation and residual inflammation despite effective combination antiretroviral therapy (cART). Whether a low CD4:CD8 T-cell ratio, known to mirror peripheral immune dysfunction, is associated with squamous intraepithelial lesions (SILs) is unknown.

Methods. This was a retrospective cohort study on cART-treated HIV-positive subjects undergoing screening for HPV-related dysplasia (anal/cervical cytology and HPV genotyping). SIL was defined as the presence of either atypical squamous cells of undetermined significance (ASCUS), low-grade SILs, or high-grade SILs. Demographic and viro-immunological parameters (T-cell count, CD4:CD8 T-cell ratio, CD8+ CD38+ T-cell percentage) at the time of screening were analyzed by the chi-square test, Mann-Whitney test, and multivariate logistic regression analysis.

Results. A total of 419 cART-treated subjects were included. Half of the patients had cervical/anal SIL. Individuals with SIL were more commonly males, were men who have sex with men, were coinfecting with *Treponema pallidum*, had been treated with integrase inhibitor (INSTI)-based cART regimens, and had a shorter time since HIV diagnosis and cART initiation than subjects with normal cytology. CD38+ CD8+ T-cell percentage, but not the CD4:CD8 T-cell ratio, correlated with SILs. HPV infection, especially with multiple and high-risk genotypes, was confirmed to be associated with SIL. In multivariate analysis, the only factors independently associated with cervical/anal dysplasia were HPV infection and harboring higher percentages of peripheral activated CD38+ CD8+ T cells.

Conclusions. HPV infection is the major driver of dysplasia in the setting of HIV infection. In this study, CD8+ CD38+ T cells were an independent predictor of dysplasia in cART-treated subjects, while CD4:CD8 T-cell ratio was not. In the setting of HIV-HPV coinfection, CD4:CD8 T-cell ratio may not fully capture the alterations of HPV-specific immunity.

Keywords. activated CD8+ CD38+ T cells; CD4:CD8 ratio; cervical–anal dysplasia; HIV infection; HPV infection.

With the advent of combination antiretroviral therapy (cART), cancers are the leading cause of death in people with HIV (PWH) [1]. Worldwide, human papillomavirus (HPV) is the causative agent of all cervical cancers and almost all anal cancers, as well as the majority of other genital male and female neoplasms [2]; when compared with their uninfected counterparts, women and men who have sex with men (MSM) with

HIV experience a 2–40 times greater risk of cervical cancer [3] and a 4.5–9.2 times higher anal cancer incidence [4, 5], respectively. This phenomenon is the consequence of the negative impact of HIV-associated disruption of T-cell homeostasis on the natural history of HPV infection with a higher prevalence [4, 6, 7] and lower clearance [6, 7] of multiple and high-risk (HR) HPV genotypes [7, 8] in PWH than in general population.

All these findings translate into a greater risk of precancerous lesions as squamous intraepithelial lesions (SILs) [9, 10] and faster progression to cancer [9, 11] in the setting of HIV-HPV coinfection compared with HPV alone. The reasons for this scenario are several: Next to a higher behavioral exposure of PWH to HPV [12] and a greater prevalence of cancer co-factors (eg, smoking) [13, 14], a central role is attributable to HIV-related mucosal and systemic immune alterations, which are both key factors in hindering HPV infection and its progression. At a local level, HIV-induced mucosal damage is coupled

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with a significant density reduction of immune cells—CD4+ T lymphocytes [15], especially naïve CD4+ and memory CD8+ CD45R0+ T-cell pools [16], macrophages, neutrophils, and natural killer (NK) cells—an important downrepresentation of both pro- and anti-inflammatory cytokines [15], as well as increased but dysfunctional CD8+ T infiltrates [16–18]. As CD4+ T-cell count is a marker of HIV-induced immunosuppression and viral replication, it inversely correlates with HPV infection risk (especially by HR-HPV genotypes), SIL development, and progression to cancer [6]. Even if cART effectively reduces the rates of acquisition and persistence of HPV and the incidence of dysplastic lesions [19, 20], literature has shown conflicting data on the potential impact of antiretroviral therapy on lowering the risk of HPV-related cervical and anal cancer development in PWH [4, 21, 22].

In the setting of HIV infection, CD4+ T-cell count and HIV viremia are generally used to monitor the viro-immunological response to cART. More recently, CD4:CD8 ratio emerged as an interesting parameter of immune system competence. Indeed, a low CD4:CD8 ratio is linked to a T-cell phenotype skewed to terminally differentiated CD8+ T cells, increased CD8+ activation, and senescence [23]. These features are also well described in the course of physiological non-HIV aging [24], so, in the context of HIV infection, the CD4:CD8 ratio is also a hallmark of non-AIDS-defining events [25] and predicts cardiovascular, muscle, and renal age-associated diseases [26], and therefore non-AIDS morbidity and mortality [25]. All these characteristics make the CD4:CD8 ratio an ideal tool for monitoring PWH, aiming to identify those at higher risk for the development non-AIDS comorbidities and for whom a stricter follow-up may be advocated [25].

The role of CD4:CD8 ratio as a marker of immune system dysregulation and a predictor of HPV disease has yet to be assessed; thus, the aim of our work was to investigate whether the CD4:CD8 ratio value correlates with HPV-dependent SILs.

METHODS

Study Population

We conducted a cross-sectional retrospective study enrolling all HIV-positive patients of the San Paolo Infectious Diseases (SPID) cohort, San Paolo Hospital, University of Milan, Italy, who underwent anal or cervical HPV screening from January 2016 to February 2019. Inclusion criteria were being on combination antiretroviral therapy with at least 2 consecutive determinations of HIV-RNA <50 copies/mL.

HPV-Related Dysplasia Screening

All patients were screened for HPV-related dysplasia and underwent anal (males) or cervical (females) cytology and HPV genotyping during a surgical/gynecological examination. Anal and cervical specimens were collected using a disposable plastic

cytological brush (Ningbo HLS Medical Products Co.) to perform a cervical/anal cytology. SIL was defined according to the 2001 Bethesda System and included atypical squamous cells of undetermined significance (ASCUS), low-grade SILs (LSILs), high-grade SILs (HSILs), or carcinoma. Cytological samples were considered invalid, and thus excluded from the analyses, if the result was insufficient cellularity.

Specimen Collection

Anal/cervical samples were collected in a liquid-based cytology medium (PreservCyt-Hologic). Total DNA was extracted using a commercial kit (QIAamp DNA Blood Mini KIT, Qiagen).

Flow Cytometry

T-cell surface phenotypes were evaluated using fresh peripheral blood (FACSCanto II; Becton Dickinson Italia Spa, Milan, Italy), CD4-PE-cy7, CD8-PE-cy5, and CD38-FITC (Becton Dickinson).

HPV Genotyping

HPV-DNA was detected with polymerase chain reaction using both the L1 consensus primers MY09/MY11 and the E6/E7 consensus primers PU-1M/PU-2R [27, 28]. Viral genotyping was performed using a direct sequencing kit (BD Terminator Kit, version 1.1; Applied Biosystems, Life Technologies) on an automated capillary electrophoresis sequencer (ABI3130; Applied Biosystems, Life Technologies). The sequences obtained were blasted against the NCBI nucleotide DNA database using the Basic Local Alignment Search Tool (BLAST: <http://blast.ncbi.nlm.nih.gov/>) to identify the viral genotype. The different viral genotypes were classified according to the oncogenic risk in low- and high-risk genotypes [29].

Demographic and Viro-immunological Parameters

Demographic, HIV-related, and viro-immunological parameters (CD4+ and CD8+ T-cell count, CD4:CD8 ratio, activated CD8+ CD38+ T-cell percentage in peripheral blood) at the time of the cytological evaluation were collected in an electronic data set.

Statistical Analysis

Chi-square and Mann-Whitney tests were used to compare patients with and without anal/cervical HPV-related dysplasia. Spearman's correlation coefficient was used to investigate the correlation between immune activation (CD8+ CD38+ T cells) and CD4:CD8 ratio. We investigated the association between immunosuppression (measured by CD4:CD8 ratio) and HPV-related dysplasia by fitting a multivariable logistic regression analysis, adjusting for possible confounders (HPV infection, time since HIV diagnosis, cART duration, age, CD4+ nadir, and smoking). A second model of logistic regression analysis was also performed to explore the

association between peripheral immune activation (measured by percentages of CD8+ CD38+ T cells) and HPV-related dysplasia. Statistical analyses were performed by SPSS software (version 19.0).

RESULTS

A total of 419 patients with HIV on effective cART (HIV-RNA <50 copies/mL) undergoing anal or cervical HPV screening were analyzed. The median age of the study population (interquartile range [IQR]) was 42 (36–48) years, 72.3%

of subjects were males, and most of the subjects were MSM (49.8%). Demographic, HIV-related, and HPV-related characteristics of the study population, according to anal/cervical dysplasia, are shown in [Table 1](#).

Demographic Characteristics Associated With SIL

Half of the patients (214/419, 51%) were SIL positive ([Table 1](#)). The presence of SILs was more commonly associated with male gender (171/214, 79.9%, vs 132/205, 64.4%; $P < .0001$), men who have sex with men (MSM) as risk category for HIV transmission (126/214, 60.3%, vs 83/205, 40.5%; $P = .001$), and

Table 1. Demographic and Clinical Characteristics of Study Population According to Cervical/Anal Dysplasia

Characteristics	Population (n = 419)	SIL+ (n = 214, 51%)	SIL- (n = 205, 51%)	PValues
Males, No. (%)	303 (72.3)	171 (79.9)	132 (64.4)	<.0001
Age, median (IQR), y	42 (36–48)	42 (36–48)	43 (36–48)	.373
Mode of HIV transmission, No. (%)				.001
Homosexual contact	209 (49.8)	126 (60.3)	83 (40.5)	
Heterosexual contact	124 (29.6)	55 (25.9)	69 (33.7)	
IDU	48 (11.5)	15 (7.1)	33 (16.1)	
Other/unknown	38 (9.1)	18 (7.5)	20 (9.8)	
Syphilis coinfection, No. (%)				.001
TPPA negative	251 (59.9)	111 (51.9)	140 (68.6)	
TPPA positive	131 (31.3)	85 (39.7)	46 (22.5)	
Unknown	37 (8.8)	18 (8.4)	19 (8.8)	
HCV coinfection, No. (%)				.198
Negative	340 (81.1)	175 (81.8)	165 (80.5)	
HCV-Ab positive, RNA negative	48 (11.5)	21 (9.8)	27 (13.2)	
HCV-Ab positive, RNA positive	22 (5.3)	15 (7)	7 (3.4)	
Unknown	9 (2.1)	3 (1.4)	6 (2.9)	
HBV coinfection, No. (%)				.914
Negative	291 (69.4)	150 (70.1)	141 (68.8)	
HBsAg positive	11 (2.6)	6 (2.8)	5 (2.4)	
HBcAb positive	105 (25.1)	52 (24.3)	53 (25.9)	
Unknown	12 (2.9)	6 (2.8)	6 (2.9)	
AIDS-defining conditions, No. (%)	86 (20)	40 (21.4)	46 (25.1)	.394
Time since HIV diagnosis, median (IQR), mo	88 (30–165)	71 (20–151)	95 (37–189)	.019
Nadir CD4 + T-cell count, median (IQR), cells/mm ³	250 (144–358)	260 (148–358)	234 (128–355)	.583
Pre-cART CD4/CD8 ratio	n = 250 0.35 (0.21–0.54)	n = 138 0.36 (0.21–0.54)	n = 112 0.34 (0.21–0.54)	.913
cART duration, median (IQR), mo	43 (12–99.7)	33 (9–92)	49 (17–108)	.055
cART regimen, No. (%)				.021
PI-based	133 (31.8)	68 (31.8)	65 (31.7)	
NNRTI-based	167 (39.8)	76 (35.5)	91 (44.4)	
INSTI-based	74 (17.8)	49 (22.9)	25 (12.2)	
Other	41 (9.7)	18 (8.4)	23 (11.2)	
Unknown	4 (0.9)	3 (1.4)	1 (0.5)	
Smoking history, No. (%)				.099
Never smokers	163 (38.9)	81 (37.9)	82 (40)	
Active smokers	209 (49.9)	114 (53.2)	95 (46.3)	
Ex-smokers, quit >1 y ago	39 (9.3)	18 (8.4)	21 (10.3)	
Unknown	8 (1.9)	1 (0.5)	7 (3.4)	

Quantitative variables are presented as median (interquartile range), categorical variables as absolute number (percentage); P values were calculated by chi-square test or nonparametric Mann-Whitney test, as appropriate.

Abbreviations: Ab, antibody; cART, combination antiretroviral therapy; HBcAb, anti-HBV core antigen antibodies; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HPV, human papillomavirus; IDU, intravenous drug users; INSTI, integrase strand transfer inhibitor; IQR, interquartile ranges; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SIL, squamous intraepithelial lesion; TPPA, *Treponema pallidum* particle agglutination test.

syphilis coinfection (85/214, 39.7%, TPPA positive vs 46/205, 22.5%; $P = .001$), compared with patients presenting a normal cytology. No correlation between SIL status and HCV and/or HBV coinfection was found.

SIL-positive patients also showed a more recent median (IQR) HIV diagnosis (71 [20–151] months vs 95 [37–189] months; $P = .019$), a shorter time from cART introduction (33 [9–92] months vs 49 [17–108] months; $P = .055$), and were more frequently treated with integrase strand transfer inhibitor (INSTI)-based cART (49/211, 22.9%, vs 25/204, 12.2%; $P = .021$) compared with patients without SIL on anal/cervical cytology.

We also assessed demographic characteristics associated with SIL separately in males and females (Supplementary Table 1). In males, SIL was associated with syphilis coinfection, shorter cART duration, and active or former smoking; in females, no association was seen between SIL and the demographic or HIV-related parameters described above.

HPV-Related Characteristics Associated With SIL

As expected, SIL-positive patients were more commonly infected with HPV than SIL-negative individuals (95/201, 47.3%, vs 30/207, 14.5%; $P < .0001$).

Furthermore, SIL-positive patients more frequently harbored multiple (93/214, 43.5%, vs 32/205, 15.6%; $P = .002$) (Figure 1A)

and high-risk (151/207, 72.9%, vs 76/201, 37.8%; $P < .0001$) (Figure 1B) HPV genotypes, compared with subjects with negative cytology. The HR genotypes most frequently detected were 16, 18, 33, 58, 53, 31.

HIV-Related Characteristics Associated With SIL

In terms of immunological parameters associated with cervical/anal SIL, no difference in median current CD4:CD8 ratio (IQR) between SIL-positive and SIL-negative patients (0.64 [0.44–0.89] vs 0.66 [0.47–0.9]; $P = .404$) was reported. Likewise, median (IQR) nadir CD4+ T-cell count (260 [148–358] cells/mm³ vs 234 [128–355] cells/mm³; $P = .583$), current CD4+ T-cell count (565 [409–725] cells/mm³ vs 539 [414–712] cells/mm³; $P = .76$), and CD8+ T-cell count (881 [692–1119] cells/mm³ vs 840 [640–1049] cells/mm³; $P = .107$) were similar between the 2 groups of patients (Table 1, Figure 2).

Interestingly, we found a higher proportion of peripheral activated CD8+ CD38+ T-cell percentages (IQR) in patients diagnosed with SILs (3% [1%–5%] vs 2% [1%–4%]; $P = .018$) (Figure 2).

CD8+ CD38+ T-cell percentages negatively correlated with CD4:CD8 ratio in the entire population ($r = -0.202$; $P = .005$) and in males ($r = -0.152$; $P = .004$), yet not reaching statistical significance in females ($r = -0.19$; $P = .161$).

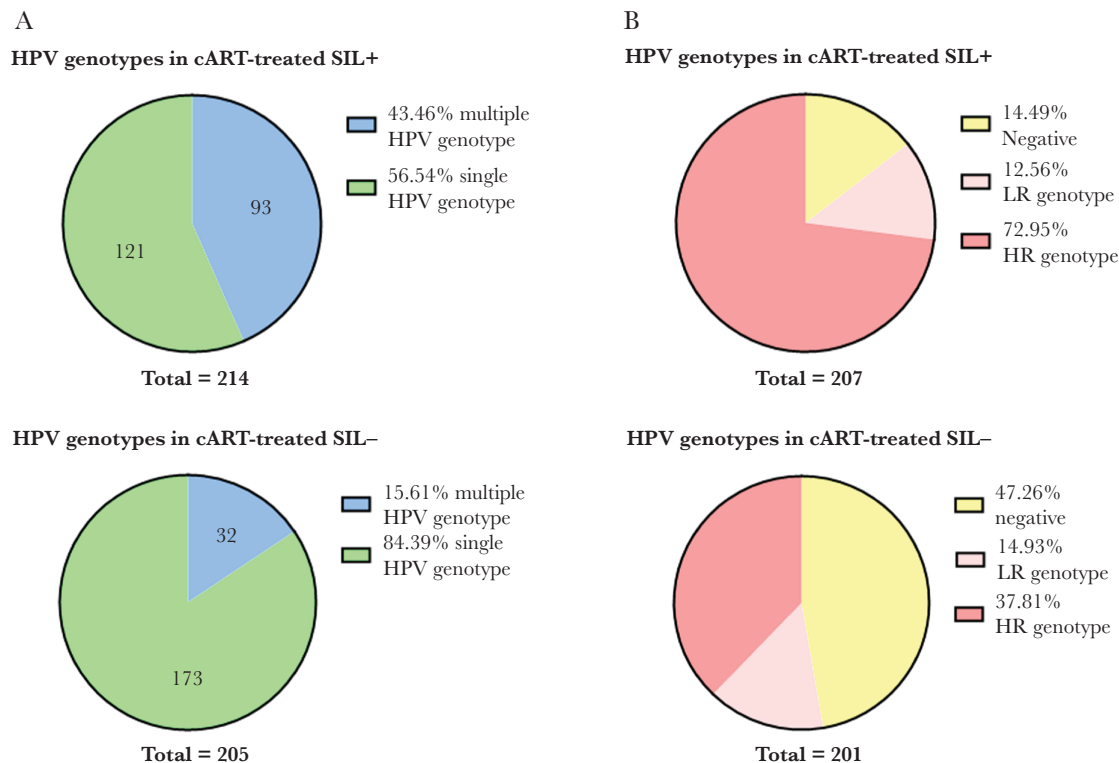


Figure 1. Multiple-HPV genotype infection and low/high-risk HPV genotypes according to anal/cervical dysplasia. Abbreviations: cART, combination antiretroviral therapy; HPV, human papillomavirus; HR, high-risk; LR, low-risk; SIL, squamous intraepithelial lesion.

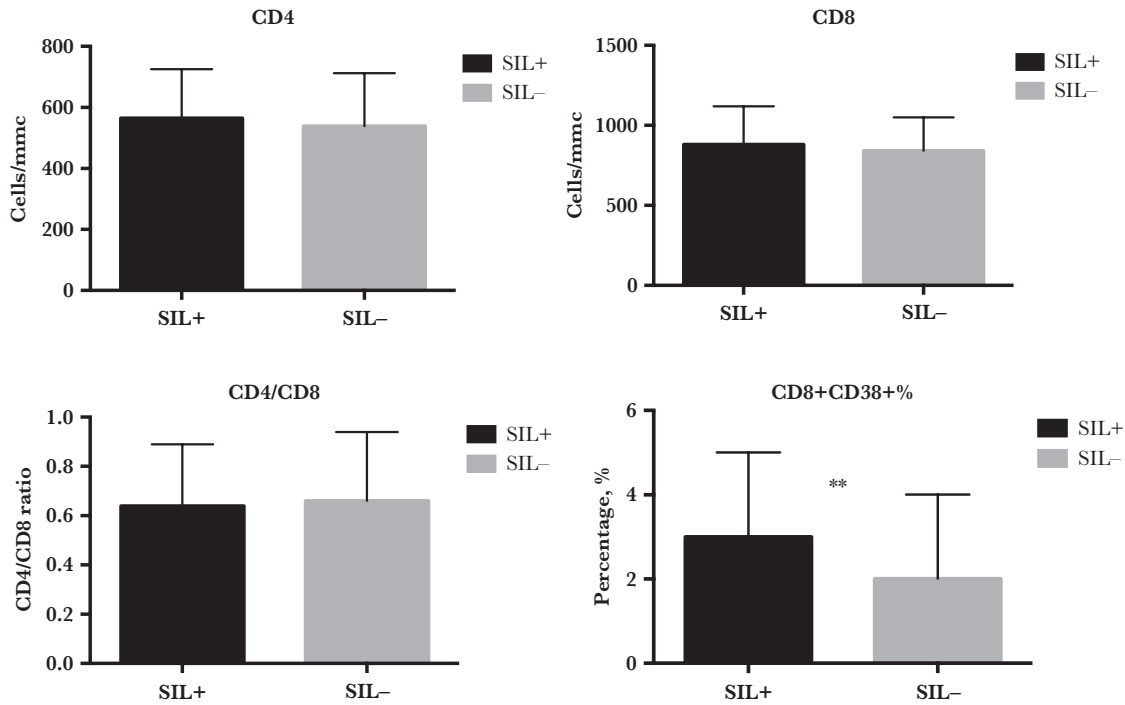


Figure 2. CD4+ T-cell count, CD8+ T-cell count, CD4/CD8 ratio and activated CD8+ CD38+ T-cell percentage according to anal/cervical dysplasia. CD8+ CD38+ T-cell percentage correlates with SILs. *P* values were calculated by Mann-Whitney test. **P* < .05. Abbreviation: SIL, squamous intraepithelial lesion.

Multivariate Logistic Regression Analysis

The association between percentage of CD38+ CD8+ T-cell and HPV-related anal/cervical dysplasia was explored by a multivariable logistic regression model, adjusting for HPV infection, age, time since HIV diagnosis, cART duration, CD4+ T-cell nadir, and smoking (model 1, Table 2A). We also investigated the possible association between CD4:CD8 ratio and SILs by fitting a second model of logistic regression (model 2, Table 2B).

A higher proportion of activated CD8+ CD38+ T cells (adjusted odds ratio [aOR], 3.253; 95% CI, 1.602–6.605; *P* = .001), but not CD4:CD8 ratio (aOR, 0.998; 95% CI, 0.242–4.109; *P* = .997), was independently associated with anal/cervical dysplasia (Table 2). The association between immune activation/immune senescence and cytologic HPV-related dysplasia was

also investigated, stratifying for anal and cervical dysplasia (Supplementary Table 2). Immune activation was confirmed to be independently associated with anal dysplasia (aOR, 2.625; 95% CI, 1.142–6.033; *P* = .023); conversely, no association was reported between CD8+ CD38+ T cells or CD4:CD8 ratio and cervical dysplasia, probably due also to the small sample size of females.

DISCUSSION

With the present study, we aimed to evaluate if, in the setting of HIV infection controlled by effective cART, HPV-related SILs are associated with peripheral T-cell immune activation and immune senescence. For this purpose, we used a well-recognized

Table 2. Parameters Independently Associated With Cytologic HPV-Related Dysplasia by Fitting 2 Models of Multivariable Logistic Regression Analyses

A, Model 1 of logistic regression analysis: association between CD8+ CD38+ T-cell percentages and HPV-related anal/cervical dysplasia			
Parameter	aOR	95% CI	<i>P</i> Value
Log ₁₀ CD8+ CD38+ T cells, percentages, each additional unit	3.253	1.602–6.605	.001
B, Model 2 of logistic regression analysis: association between CD4/CD8 ratio and HPV-related anal/cervical dysplasia			
Parameter	aOR	95% CI	<i>P</i> Value
CD4/CD8 ratio, each unit more	0.998	0.242–4.109	.997

Adjusted for HPV infection, age, time since HIV diagnosis, cART duration, CD4+ nadir T cells, and smoking.

Time since HIV diagnosis is the time from HIV diagnosis to surgical/gynecological evaluation for detection of HPV-related dysplasia; cART duration is time from cART introduction to surgical/gynecological evaluation for detection of HPV-related dysplasia.

Abbreviations: aOR, adjusted odds ratio; cART, combination antiretroviral therapy; HPV, human papillomavirus.

marker of poor immunological rescue, CD4:CD8 ratio, and an index of peripheral immune T-cell activation, CD8+ CD38+ T-cell percentage [23].

In our study population, anal/cervical SILs were associated with HPV detection in both univariate and multivariate analyses, but this result was expected and not surprising, considering that HPV is the causal agent of virtually all cervical dysplastic lesions and cancers and is responsible for 88% of anal cancer cases [2]. Since 2007, HPV vaccination programs have been introduced in many countries worldwide, and very recent evidence has proven the efficacy of vaccines in reducing HR-HPV genotype infections and SIL incidence in women, as well as the occurrence of genital warts in men [30]. This highlights the importance of HPV vaccination for both males and females as a unique instrument of primary prevention, which is potentially able to eradicate all cervical cancers and most anal cancer cases.

In our study cohort, SIL detection was higher in men, especially MSM. This is in line with previous data from the literature [31, 32] and is probably linked to a high number of sexual partners. The same epidemiological interpretation could justify our findings of the association between dysplasia and a history of syphilis infection or multiple-HPV genotype detection. As already described, MSM are more likely to harbor multiple HPV genotypes or to be syphilis coinfecting than men who have sex with women [33].

As a relevant result of our analysis, we found that SIL-positive patients experienced higher levels of peripheral activated CD8+ CD38+ T cells than their negative counterparts and, interestingly, CD8+ CD38+ T-cell percentage was an independent predictor of SILs even in multivariate analysis. This finding is in line with the well-documented phenomenon of residual immune activation under successful cART, which specifically affects the compartment of CD8+ T cells, which present altered cytotoxic functions and a more activated, exhausted, and terminally differentiated phenotype [34, 35]. This skewed and dysfunctional arm of cellular immunity sustains and is sustained by high levels of systemic inflammation and is associated with an increased risk of non-AIDS-related events [35].

This scenario calls attention to the problem of the inability of cART to restore broad immune competence despite stable HIV replication inhibition [24], thus creating a permissive environment for HPV persistence, replication, and SIL onset.

In the present work, we were not able to test for HPV antigen-specific CD8+ T-cell responses. Given the possible link between T-cell activation and impaired HPV-specific immunity in HIV infection, future studies are needed to assess this correlation and provide insights into the pathogenesis of HPV-related dysplasia in this context.

The more activated immune profile of patients with SILs than that of those without dysplasia may be well explained by our finding that the former had a more recent HIV diagnosis

as well as shorter time from cART introduction, given that the introduction of antiretroviral therapy is the *primum movens* to immunological recovery [36]. This concept is sustained by several authors who have demonstrated that starting cART affects a reduction in prevalence of high-risk HPV genotypes and SILs, as well as dysplasia progression [21, 22]. Moreover, as the beneficial effect of antiretroviral therapy on the risk of HR-HPV acquisition and dysplasia development seems to compound with time [21, 37], we can speculate that, in our cohort study, the association between SILs and high-risk HPV genotypes might mirror a shorter time from cART introduction. Analogously, the finding of a correlation between SIL detection and INSTI-based cART could be justified by the fact that, in our cohort of patients, people with a more recent diagnosis of HIV were more likely to be treated with relatively new drugs—such as INSTIs—than patients with an older diagnosis. Both of these findings, that is, time from cART introduction and INSTI-based cART, relate to younger age, which, in turn, is associated more frequently with risky sexual behavior.

As regards the possible capability of CD4:CD8 ratio to predict SILs, our analysis failed to demonstrate such an association. As previously described, several non-AIDS-defining conditions correlated with an inverted CD4:CD8 ratio [26], so, given the noninvasive nature of this marker, we postulated its possible predictive role in detecting HPV-related dysplasia. Even though previous reports have shown the validity of our hypothesis in the setting of HIV infection and in both female and male patients [38, 39], our data concluded for a lack of correlation between CD4:CD8 ratio and dysplastic lesions. There are several reasons for this result: First, our study is retrospective in nature, and our cohort is relatively small and composed of subjects with heterogeneous courses of HIV infection (different times since HIV diagnosis and cART initiation, different types and numbers of antiretroviral regimens experienced). This could translate into deep immunological modifications, only in small part attributable to HPV infection, so CD4:CD8 ratio may not fully capture those relative to HPV disease. In this respect, an elegant work by Tong et al. demonstrated that HIV infection is an independent variable associated with systemic HPV-specific CD8+ T-cell responses and that HIV-infected subjects with AIDS had decreased HPV-specific CD4+ T-cell immunity, which the authors found to be linked to HSIL clearance [40]. These literature findings, together with our results on the lack of association between CD4:CD8 ratio and dysplasia, indeed demonstrate that inferring HPV antigen-specific T-cell responses from crude measures of T-cell function is problematic and highlight the need for subtle assessments of HPV-specific immunity in large-cohort studies.

Otherwise, we may have investigated the wrong “side of the coin”: in fact, the CD4:CD8 ratio is calculated using peripheral T cells and may therefore not be able to completely mirror the features of a process—as the anti-HPV immune defense—that

is mostly local and mucosal [41]. However, as the assessment of the possible correlation between CD4:CD8 ratio and SILs derives mainly from small, monocentric, retrospective studies [38, 39], future investigation with prospective studies and larger cohorts of patients are needed to ascertain the accuracy of this association. Finally, a further limitation of the study is that we analyzed only cytologic HPV-related dysplasia without a histological validation.

In conclusion, in our cohort of men and women with HIV on suppressive cART, CD4:CD8 ratio did not associate with SILs, despite its already recognized capacity to predict non-AIDS-defining conditions. This was probably due to the limits of our work and advocates for further studies to better define its role in this setting, especially in the cART era, during which age-related and non-AIDS comorbidities represent the most important cause of mortality in this population. Otherwise, our analysis revealed a trend toward a correlation between the activated CD8+ T-cell immunophenotype and HPV-related anal/cervical dysplasia. This result underlines the importance of residual immune dysfunction, which is not fully captured by conventional parameters of immunological evaluation—such as CD4+ T-cell count and CD4:CD8 ratio—even under effective antiretroviral therapy. Stemming from these observations is the need for additional methods to identify patients with a higher risk of SIL development as well as adjuvant strategies to correct residual immune dysfunction.

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Patient consent. All patients provided written informed consent, and the local ethical committees approved the study.

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