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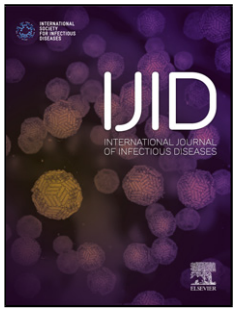

$CD4^+$ and $CD8^+$ cell populations in HIV-positive women with cervical squamous intra-epithelial lesions and squamous cell carcinoma

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$CD4^+$ and $CD8^+$ cell populations in HIV-positive women with cervical squamous intra-epithelial lesions and squamous cell carcinoma

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Highlights

- ▸ Significant differences in HPV subtypes between HIV+ and HIV- were observed
- ▸ The density of $CD8^+$ T cell infiltrate correlated with the severity of lesions
- ▸ In HIV- patients, stromal $CD8^+$ T cell scores increased from LSIL to SCC

- In HIV+ patients, stromal and epithelial $CD8^+$ T cell scores increased from LSIL to SCC

Abstract

Introduction: This study aimed to analyse cervix lymphocytic populations in HIV+ and HIV- patients and correlate different cervical lesions with HIV viral load and presence of high-risk HPV types.

Material and methods: A total of 132 histological specimens from 40 HIV+ and 72 HIV- patients were evaluated for $CD4^+$ and $CD8^+$ T cell distribution, presence of high-risk HPV types, peripheral blood HIV viral load and $CD4^+/CD8^+$ ratio.

Results: High-grade squamous intraepithelial lesions (HSIL) and squamous cell carcinoma (SCC) from HIV+ patients had lower $CD4^+$ T cell scores compared with HIV- patients. In all lesion groups, HIV+ patients presented higher epithelial and stromal $CD8^+$ T cell scores. HIV viral load was more often detectable in patients with SCC than in those with low-grade squamous intraepithelial lesion (LSIL) ($p=0.0409$). HSIL HIV+ patients had lower circulating $CD4^+$ T cell counts ($p=0.0434$) and $CD4^+/CD8^+$ ratio ($p=0.0378$) compared with LSIL HIV+ patients. High-risk HPV types other than 16 and 18/45 were more prevalent in the HIV+ group.

Discussion: These results support an imbalance between cervical $CD4^+$ and $CD8^+$ T lymphocytes of HIV+ patients with SIL and SCC, with increased CD8+ infiltrate density with lesion severity, even in patients with immune system recovery under cART.

Keywords: carcinoma, $CD4^+$ T cell, $CD8^+$ T cell, cervix, HIV, viral load

Introduction

Human immunodeficiency virus (HIV)-infected (HIV+) women have a higher incidence of human papilloma virus (HPV)-associated cervical lesions, reduced HPV infection clearance, and higher risk of progression to cervical invasive neoplasia.[1,2] High prevalence of high-grade cervical lesions associated with high-risk HPV is observed even in HIV+ women on antiretroviral therapy (ART), although early ART initiation and sustained therapy adherence is likely to reduce the incidence and progression of squamous intraepithelial lesions.[3]

Studies investigating the role of ART in cervical cancer have included different HIV regimens and patient monitoring coverage methodologies, what hampers data interpretation.[4,5] Current WHO guidelines recommend viral load testing instead of $CD4^+$ T cell count for ART response monitoring in HIV treatment and prevention.[6]

The more aggressive cervical lesion behavior in HIV+ compared with HIV- women may be attributed to systemic and local immunity alterations that weaken the immune system, promoting carcinogenesis.[7–9] Both incidence and progression rates of cervical intra-epithelial lesions in HIV+ women have been associated with lower $CD4^+$ T cell counts in blood and lower $CD4^+/CD8^+$ ratios in tumor infiltrating lymphocytes.[10–17]

HPV infection can interfere with local immune vigilance mechanisms, both at the induction and effector phases.[18] The $CD4^+$ T cell function impairment observed in HIV infection has also been reported as a promoter of HPV-associated cancer.[19]

Few studies have characterized the different T lymphocyte populations in intra-epithelial squamous cervical lesions in HIV+ patients.[20–22] Kobayashi *et al.* reported that $CD8^+$ T cell aggregates were predominant in HIV+ women with high-grade squamous intraepithelial lesions (HSIL) and were associated with worse clinical outcome.[21]

The main aim of this study was to characterize the local immune microenvironment of squamous intraepithelial lesions (LSIL, HSIL), and squamous cell carcinoma (SCC) regarding presence and quantification of cervical $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T lymphocytes in HIV+ women and compare it with HIV-negative (HIV-) counterparts. Secondary aims were to correlate different cervical lesions with HIV viral load, $CD4^+$ T cell counts and $CD4^+/CD8^+$ ratio, and HPV type.

Material and methods

A total of 112 patients were included in this study, 40 of which HIV+ and 72 HIV-. Patients included in the HIV+ group with SIL diagnosis were under hospital surveillance and cART treatment. Of the 16 SCC cases, 10 were also in long-term (>1 year) hospital surveillance. The HIV- control group consisted of women recruited at the Gynecology clinic of Hospital Garcia de Orta. When available and/or applicable, the following data was retrieved from patients' clinical records: age, HIV status, HIV infection duration, $CD4^+$ blood count, $CD4^+/CD8^+$ ratio, and HPV typing.

Written informed consent was retrieved from all patients and the study was approved by the ethics committee of Hospital Garcia de Orta (HGO-CGO 23/2014), Institute of Oncology Francisco Gentil de Lisboa (IPO-UIC/996), and Nova Medical School (NMS-03/2015/CEFCM).

Viral detection

HIV status was determined by fourth-generation serological testing (including p24 assessment) and subsequently confirmed by HIV viral load quantification through real-time PCR.

HPV typing was performed using molecular biology techniques implemented in recruitment centres, including HPV genotyping - CLART HPV4 (Genomica), an *in vitro* assay for detection and genotyping of 35 genotypes, including high, probably high and low risk; DNA high-risk HPV typing (Cobas-Roche Diagnostics), an automated qualitative *in vitro* test for detection of HPV DNA of 14 high-risk HPV types; mRNA high-risk HPV typing (Aptima-Hologic), an *in vitro* nucleic acid amplification test for qualitative detection of E6/E7 viral

messenger RNA (mRNA) from 14 high-risk HPV types in cervical specimens; and an in-house screening protocol developed at IPO – Lisboa, with positive samples confirmed with INNO-LIPA/Seegene HPV28.

Histological specimens

A total of 132 histological specimens were assessed in this study, 60 from 40 HIV+ patients and 72 from 72 HIV- patients.

Specimens were classified as LSIL, HSIL, or SCC according to morphological type (hematoxylin- and eosin-stained slides), supported by complementary immunohistochemical evaluation (p16 antibody – Ventana, Roche Diagnostics), when necessary.

Characterization of lymphocytic populations was performed by immunohistochemistry (IHC) using SP35 ($CD4^+$) and SP75 ($CD8^+$) clones (Ventana, Roche Diagnostics). These antibodies were applied to 3-µm sections, and a double staining detection kit (Optivision DAB; UltraView Universal AP Red) was used. Antigen retrieval was carried out with CC1 (Roche, Ventana) for 40 min at 97°C and stains were processed in a Roche Ventana BenchMark ULTRA equipment.

$CD4^+$ and $CD8^+$ T lymphocyte scoring of the obtained immunostaining was performed following a modified version of the protocol previously described by Park & Kim,[23] with immune stained-positive lymphocytes counted in five randomly selected high-power fields at 400X magnification and counts averaged. Scores in the epithelium and stroma were separately considered. $CD4^+$ and $CD8^+$ T cells were recorded as 1–25 cells (score 1), 26–50 cells (score 2), or ≥51 cells (score 3).

Peripheral blood evaluation

Peripheral blood $CD4^+$ cell absolute counts and $CD4^+/CD8^+$ cell ratios, determined by flow cytometry using a 4-color BD FACS Calibur (BD Biosciences), were retrieved from clinical records of 24 of the 26 HIV+ patients with LSIL and HSIL and 7 of the 16 HIV+ patients with SCC. HIV blood viral load, evaluated by Abbott Real-Time HIV-1 (M2000), was also retrieved from clinical records of the same patients.

For comparison purposes, $CD4^+$ absolute count, $CD4^+/CD8^+$ ratio, and HIV viral load data were retrieved from the same year as histological samples collected.

Statistical analysis

Statistical analysis was performed with Graph Pad Prism 6. Chi Square and Fisher's exact test were used for categorical variables, Mann Whitney test for continuous variables when comparing two groups, and Kruskal-Wallis test followed by Dunn's multiple comparisons test was used whenever three or more groups were compared. Level of significance was set at 0.05.

Results

Characterization of the study population

Clinicopathological characteristics of the HIV+ and HIV- patient populations according to lesion type are depicted in **Table 1**. No significant differences were found between the two groups regarding patients' age (p=0.1746), with HIV+ patients having a median of 40.5 years [IQR 34.0−47.5] and HIV- patients a median of 41.5 years [IQR 34.0−51.0]. In subgroup analysis, HIV- women with SCC were older than their HIV+ counterparts (p=0.0015).

No significant differences were found in HIV infection duration between LSIL (6.9 years) and HSIL (6.4 years) subgroups. In the subgroup of SCC patients, this information was limited to 6 of 16 cases. Despite this limitation, no significant differences were found in HIV infection duration between LSIL, HSIL, and SCC (**Table 1**).

Among the 60 cervical lesion samples (diagnosed in 46 cervical punch biopsies, 13 cervical cones, and 1 hysterectomy specimens) from 40 HIV+ patients, 21 corresponded to LSIL, 23 to HSIL, and 16 to SCC. In the HIV+ group, 10 out of 40 patients provided more than one specimen, obtained throughout annual follow-up visits (**Table 2**). All 16 invasive carcinoma samples belonged to 16 different HIV+ patients. In the HIV- control group, among 72 specimens (15 cervical punch biopsies, 54 cervical cones, and 3 hysterectomy specimens) from 72 different HIV- patients analysed, 24 corresponded to LSIL, 29 to HSIL, and 19 to SCC. In the study group, HIV was detected in blood of 23 of the 40 patients, specifically in 6/13 HIV+ patients with 21 LSIL samples, 7/11 patients with 23 HSIL samples, and 10/16 SCC patients with 16 SCC samples.

Compared with LSIL, SCC patients had a significantly higher prevalence of detectable HIV (p=0.0409).

Regarding blood $CD4^+$ T cell count and $CD4^+/CD8^+$ ratio, HSIL patients displayed significantly lower $CD4^+$ absolute counts (p=0.0434) and $CD4^+/CD8^+$ ratio (p=0.0378) compared with LSIL patients. No further differences in these parameters were observed regarding SCC, but for several of these patients, data was not available in clinical records, potentially influencing results obtained in the comparisons (**Table 1** and **Figure 1**).

Characterization of HPV types in histological specimens

Assessment of high-risk HPV types in cytological specimens was simultaneously performed at the time of histological sampling. In the HIV+ population, high-risk HPV was identified in 80% of patients with LSIL, 95% of patients with HSIL, and 89% of patients with SCC, compared to 80%, 100%, and 86% of patients in the HIV- population, respectively (**Table 3** and **Figure 2**). Differences observed in the distribution of HPV types between HIV+ and HIV- populations were statistically significant (p=0.006), with infection with high-risk

types other than 16, 18, and 45 being more prevalent in HIV+ patients and infection with HPV16 type more prevalent in HIV- patients (**Table 3**).

In subgroup analyses, high-risk HPV types other than 16, 18, and 45 were present in 9/21 (43%) patients with LSIL, 10/20 (50%) patients with HSIL, and 1/6 (17%) of patients with SCC in the HIV+ population, compared with 3/20 (15%), 4/16 (25%), and 0/6 (0%) patients in the HIV- population, respectively (**Table 3**).

Characterization of $CD4^+$ and $CD8^+$ T cell populations in histological specimens

$CD4^+$- and $CD8^+$-positive T cells were found in histological specimens both from the epithelium and stroma in all LSIL, HSIL, and SCC samples (**Table 4**, **Figures 1a, b, c, d**).

In HIV+ patients, $CD4^+$ T cells were found in the epithelial compartment of all histological lesions. They all had a similar score, mostly 1 (**Figures 1a**, **b**). Regarding the stromal compartment, SCC presented higher $CD4^+$ T cell scores than LSIL and HSIL, and HSIL were more frequently scored higher than LSIL. All LSIL were scored 1 or 2, SCC lesions were scored 2 or 3, and HSIL were more frequently scored 2. In the epithelial compartment, no significant differences were found between LSIL, HSIL, and SCC patients regarding the three $CD4^+$ T cell distribution scores, while in the stromal compartment SCC patients presented higher $CD4^+$ T cell scores compared with LSIL and HSIL patients (LSIL vs SCC, p=0.0002; HSIL vs SCC, p=0.0215).

Regarding $CD8^+$ T cells, significant differences were found in the epithelial compartment between LSIL and HSIL (p=0.0043). LSIL were scored 1 and 2, while both HSIL and SCC were mostly scored 2, with a few cases scored 1 or 3. In the stroma, lower $CD8^+$ T cell scores were found in LSIL, which was significantly different from HSIL (p=0.0218) and SCC (p=0.0295).

In HIV- patients, most cases of $CD4^+$ T cells in the epithelial compartment were scored 1. In stroma, $CD4^+$ T cell scores gradually increased from LSIL to SCC lesions. LSIL patients were mostly scored 1 and occasionally 2, while all HSIL and most SCC patients were scored 3. In these patients, the distribution of cervical $CD4^+$ T cells according to histological subgroups was similar to the previously described in HIV+ patients, with no significant differences in $CD4^+$ scores between histological subgroups in the epithelial compartment. In contrast, stroma scores showed a gradual increase from LSIL to SCC, with significant score differences found in almost all comparisons (p<0.0001 for LSIL vs HSIL and LSIL vs SCC; p=0.0560 for HSIL vs SCC).

Regarding $CD8^+$ T cell distribution, differences were found between HIV- and HIV+ patients. In epithelial compartment, $CD8^+$ T cells showed no significant score differences between LSIL, HSIL, and SCC. All subgroups predominantly displayed scores of 1, despite a slightly higher prevalence of $CD8^+$ T cells in LSIL. In stromal compartment, $CD8^+$ T cell distribution in HIV- patients followed a similar pattern of $CD4^+$ T cells in the same compartment, with a gradual score increase from LSIL to SCC and statistically significant differences between lesions (p<0.0001 for LSIL vs HSIL and LSIL vs SCC). No significant differences were found between HSIL and SCC (p=0.2429).

Discussion

In this study, the local immune microenvironment of intra-epithelial lesions and cervical carcinomas from HIV-infected women was characterized, using HIV-uninfected women as control group.

In accordance with previous reports,[24,25] this study shows that HIV- women with SCC are older than their HIV+ counterparts. It should be acknowledged that, in this patient series, most HIV+ women with SCC (10/16) were newcomers to our health care service, mainly originating from other countries, and not previously exposed to ART or monitored for blood $CD4^+$ T cell count or HIV viral load. Probably for the same reason, SCC patients had a significantly higher prevalence of detectable HIV load compared with LSIL patients.

The CD4+ immune cell depletion observed in the blood of HIV+ individuals has been associated with neoplasm development, namely cervical intraepithelial neoplasia in HIV+ women with $CD4^+$ counts <200 cells/ul.[24] This was confirmed in this study, in which lower $CD4^+$ T cell counts and $CD4^+/CD8^+$ ratios in blood were associated with HSIL in the cervix.

There has been a growing awareness on the role of the immune system in recognizing and destroying neoplastic cells and this way preventing the growth of several neoplasms.[26,27] Nonetheless, recent studies also show that the immune system may simultaneously contribute to tumor development by selecting resistant tumor clones, promoting immunosuppression and cell proliferation, and increasing metastatic potential.[28,29] This is considered one of the hallmarks of cancer, addressed in numerous publications addressing.[30,31]

$CD8^+$ T cells play a major role in anti-tumor immunity, recognizing tumor-associated antigens and eliminating tumor cells. It has been shown that a T cell increase in tumor microenvironment is correlated with better prognosis in several cancers, such as breast, colorectal, lung, and melanoma.[32,33] However, tumors may develop mechanisms to evade immune surveillance.[28,29] $CD4^+$ T cells are equally important, as they are necessary to promote the development of $CD8^+$ cytotoxic T and memory T cells, helping T cells to destroy tumors.[26,27]

The normal $CD4^+$ and $CD8^+$ T cell profile is characterized by a higher density of cells per mm^2 in the stromal compared to the epithelial compartment in normal uterine cervix, but the proportion of these cell subsets is similar in both compartments.[34] This profile is modulated by female hormonal status.[35]

Cervical $CD4^+$ and $CD8^+$ T cell infiltrates suffer alterations along infectious and neoplastic processes. In cervix, the main trigger is HPV infection. The immunophenotype, density, and distribution of lymphoid infiltrates depend on the malignant potential of high-risk HPV-associated lesions, and lymphoid subset characteristics correlate with the natural history of these lesions.[34,36–38]

Compared to normal cervical tissue, $CD4^+$ T cells predominate in regressing LSIL, both within the stroma and epithelium, with the highest $CD4^+/CD8^+$ ratio observed in persistent LSIL compared with HSIL and invasive carcinoma, with $CD8^+$ largely exceeding $CD4^+$ cells in invasive cancers.[34,36]

In invasive cervical carcinoma, tissue lymphocytes are mainly of $CD8^+$ phenotype, with reversed $CD4^+/CD8^+$ ratios in cervical lymphoid infiltrates. This correlates with human cervical carcinoma progression. $CD4^+$ T cells and $CD4^+/CD8^+$ ratio in tumors are significantly lower in patients with lymph node metastases compared to patients without lymph node metastases.[39] Shah et al. also reported better prognosis for cervical cancer patients with higher $CD4^+/CD8^+$ ratios, with a 5-year survival rate significantly higher in patients with high $CD4^+/CD8^+$ ratios compared with patients with low $CD4^+/CD8^+$ ratios.[16,38] In the present study, this was also observed in the control group of HIV- patients, confirming an increased number of stromal $CD8^+$ T cells in HSIL and SCC compared with LSIL.

Study data shows that $CD4^+$ and $CD8^+$ T cell counts in the local cervical microenvironment differ according to histological lesion type, with more severe lesions showing an increase in $CD8^+$ T cells in both HIV+ and HIV- patients.

It is established that HIV-infected women have a median three-fold higher incidence of cervical lesions and more commonly progress to cervical invasive neoplasia compared to HIV- women.[40] This has been associated with progressive destruction of $CD4^+$ T cells and loss of cell-mediated immunity in the HIV+ population, compromising the response to HPV infection and potentially accelerating the neoplastic process.[41]

In line with our results, a previous study supports the idea that an imbalance between $CD4^+$ and $CD8^+$ T lymphocytes in the cervix of HIV+ patients with SIL may contribute to increased progression to cancer.[21]

In this research, significant differences in CD4+ and CD8+ T cell populations were found both in specimens from HIV+ and HIV- groups. In HIV+ patients, LSIL had lower $CD8^+$ T cell scores both in the epithelium and stroma compared with HSIL and SCC. In HIV- patients, a gradual increase was observed in stromal $CD8^+$ cell scores from LSIL to SCC. These findings support the notion that $CD8^+$ T cell recruitment is important in anti-tumor immune response and increased lesion severity.[42–44] The higher density of $CD8^+$ T cells in the epithelium and stroma of HSIL may be directly associated with an increased recruitment of cytotoxic T cells and the more aggressive nature of these lesions. However, presence of an enriched cytotoxic compartment is not associated with lesion regression and non-progression to invasive carcinoma.

$CD8^+$T cell scores were significantly higher in HIV+ women with HSIL (epithelial and stromal CD8+) compared with HIV- women with HSIL, and the same was true for SCC, with significant differences also found between the epithelial and stromal compartments in HIV+ and HIV- patients. Moreover, $CD4^+$ T cell scores were significantly lower in the epithelium and stroma of HIV+ patients with HSIL compared with HIV- patients. Regarding SCC, similar $CD4^+$ T cell scores were found in the epithelial compartment, but HIV+ patients presented lower $CD4^+$ T cell scores in stroma.

Our results confirm an increased recruitment of immune CD4+ and CD8+ T cells in lesions of HIV+ compared with HIV- patients and suggest a more aggressive nature of lesions, reduced HPV infection clearance, and diminished lesion regression in HIV+ patients.

Our study highlights the relevance of HPV types and T lymphocytes in the HIV+ patient population. The importance of HPV infection in carcinogenesis has been attributed to E5, E6, and E7 HPV oncoproteins and how they regulate the immune response in infected patients.[45] In this study, HIV+ patients had different HPV types associated with intra-epithelial lesions and cancer progression, confirming that HIV+ populations have specificities and should be followed and treated with specific protocols.

An interesting finding from this study is that $CD4^+$ T cell depletion in the cervix mucosa seems to mirror circulating $CD4^+$ T cell depletion, with lower blood $CD4^+$ lymphocyte counts and $CD4^+/CD8^+$ ratio particularly evident in HSIL.

This research also showed that, in patients under cART, evidence of a reconstituted circulating $CD4^+$ compartment (of at least 200 $CD4^+$ T cells/uL) was not enough to prevent development of pre-invasive and invasive squamous lesions of the cervix. These results agree with those by Das et al. reporting that, even in individuals under ART, progression to cancer cannot always be prevented and the ratio of $CD4^+/CD8^+$ T lymphocyte infiltration in cervical cancer is significantly lower than in peripheral blood, especially in advanced stages of disease.[46] This is probably due to persistent alterations in the lymphocytic population of the local microenvironment. Curiously, in 2017 McBride et al. suggested that the $CD4^+/CD8^+$ ratio may be a better marker of disease progression, morbidity, and mortality risk in HIV patients.[47]

Interestingly, Perdomo-Celis and colleagues reported that $CD8^+$ T cells from patients on cART exhibit decreased degranulation capacity.[48] This defect was mainly seen in effector memory cells with high CD57 expression. Thus, although cART can achieve HIV viral suppression, $CD8^+$ T cells from HIV+ patients under cART may have an alteration in their cytotoxic program commonly associated with alterations in cellular activation, differentiation, exhaustion, and inflammation. This may explain why cART is not completely effective in the prevention of cervical HSIL and cancer development, as observed in this study.

Further studies on the function of different lymphocytes present in the cervix may better clarify this hypothesis and enable the development of new therapies, eventually linked to the impaired function of local lymphocytes, including regulatory immune cells.

The present scenario may change, as vaccines are being developed to improve the immune response to HPV-induced lesions and cancer. Welters et al. reported that the HPV16 E6 and E7 synthetic long peptide vaccine can vigorously enhance the number and activity of HPV16-specific $CD4^+$ and $CD8^+$ T cells.[49] The expansion of both types of HPV16 (tumor)-specific T cells indicates the potential of this type of vaccine as immunotherapy for HPV16-induced progressive infections, lesions, and malignancies.

Several issues still need to be addressed, as vaccination may not induce development of HPV-specific T cells capable of attacking the tumor. Indeed, as shown in this study, vaccine spectrum should consider other high-risk HPV types distinct from 16 and 18, which are more prevalent in HIV+ patients with SIL than in HIV- patients.

The fact that, in HIV+ patients, SILs already show prominent alterations of density and CD4+/CD8+ T cell ratio, even in patients with cART-reconstituted immune system, may explain the sustained aggressive behavior of these lesions, and bring into consideration the use of therapeutic vaccines at this stage.

Although this study has not addressed the full immune complexity of HIV+ women's cervix, retrieved data suggests the occurrence of alterations in the immune cell infiltrate of cervix microenvironment consisting in significantly increased $CD8^+$ T cell populations and higher prevalence of different high-risk HPV types other than 16 and 18. This suggests that specific clinical follow-up protocols should be developed taking into account different types of high-prevalent HPV infection. Additionally, therapeutic strategies seeking to mirror the natural immune response and the promising diagnostic use of digital pathology software to prospectively explore cervical $CD4^+$ and $CD8^+$ T cells populations in HIV+ patients may be relevant for cancer prevention in these patients.

Conflict of interest

The authors have no competing interests to declare.

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Ethical approval

Written informed consent was retrieved from all patients and the study was approved by the ethics committee of Hospital Garcia de Orta (HGO-CGO 23/2014), Institute of Oncology Francisco Gentil de Lisboa (IPO-UIC/996), and Nova Medical School (NMS-03/2015/CEFCM).

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Table 1. Clinicopathological characteristics of HIV+ and HIV- patient populations according to lesion type.

Population	Lesion type	Age (years)	Known Infection duration (years)	Histological specimen type	HIV viral load*	$CD4^+$ T cell count* (cells/µL)	$CD4^+/CD8^+$ ratio*
HIV+	LSIL n=21	38.0 [35.0; 46.0]	7.0 [3.0; 11.0]	B = 19 (90.5%) C = 2 (9.5%) H = 0 (0.0%)	Detectable: 6 (28.6%) Non-detectable: 15 (71.4%)	469.0 [269.5; 619.0]	53.0 [42.0; 91.0]
	HSIL n=23	36.0 [29.0; 42.0]	6.5 [2.0; 12.3]	B = 9 (39.1%) C = 13 (56.5%) H = 1 (4.4%)	Detectable: 7 (38.9%) Non-detectable: 11 (61.1%) Not available: 5	220.0 [87.5; 546.5]	36.0 [20.5; 63.5]
	SCC n=16	44.5 [43.0; 55.3]	7.5 [4.5; 13.0]	B = 10 (62.5%) C = 6 (37.5%) H = 0 (0.0%)	Detectable: 10 (66.7%) Non-detectable: 5 (33.3%) Not available: 1	219.0 [109.5; 594.0]	40.0 [18.0; 61.0]
HIV-	LSIL n=24	37.5 [32.3; 45.8]	n/a	B = 2 (8.3%) C = 22 (91.7%) H = 0 (0.0%)	n/a	n/a	n/a
	HSIL n=29	38.0 [32.5; 47.5]	n/a	B = 3 (10.3%) C = 25 (86.2%) H = 1 (35%)	n/a	n/a	n/a
	SCC n=19	65.0 [36.0; 71.0]	n/a	B = 7 (36.8%) C = 9 (47.4%) H = 3 (15.8%)	n/a	n/a	n/a

B, biopsy; C, cone; H, hysterectomy; HIV, human immunodeficiency virus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma. Continuous Variables presented as Median [IQR]. *Same year of collection

Table 2. Type of specimen and cervical lesion diagnosis in the HIV+ study population. Patients providing more than one sample are highlighted in grey.

HIV + Patients	Specimens			Diagnosis			
	Biopsy	Cone	Hysterectomy	LSIL	HSIL	SCC	Total
1	3			3			3
2	1			1			1
3	1			1			1
4	1			1			1
5	1			1			1
6	1			1			1
7	2			1	1		2
8	1			1			1
9	2				2		2
10	2			1	1		2
11	3	1		1	3		4
12	3			2	1		3
13	1			1			1
14	1				1		1
15	3			3			3

16	1			1			1
17	1			1			1
18	3	2		1	3		4
19		1			1		1
20		1			1		1
21		1			1		1
22	1					1	1
23	1					1	1
24	1					1	1
25	1					1	1
26		1				1	1
27		1				1	1
28		1				1	1
29		1				1	1
30	1					1	1
31	2	1			3		3
32	1				1		1
33	1				1		1
34	1				1	1	1

35	2	1			2	1	3
36		1				1	1
37	1					1	1
38	1					1	1
39	1					1	1
40			1			1	1
Total	**46**	**13**	**1**	**21**	**23**	**16**	**60**

Table 3. High-risk HPV types according to lesion subgroups.

	LSIL		**HSIL**		**SCC**	
HPV type	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-
16	2	11	5	12	3	4
18/45	0	0	0	0	0	1
16+18/45	0	1	0	0	1	0
16,18+45+other high-risk types	5	1	4	0	1	1
Other high-risk, non 16,18+45 types	9	3	10	4	1	0
High-risk negative	5	4	1	0	0	0
Number of tested specimens	21	20	20	16	6	6

HIV, human immunodeficiency virus; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma

Table 4. $CD4^+$ and $CD8^+$ T cell populations identified in histological specimens and respective score.

Histological diagnosis	Score	**$CD4^+$**				**$CD8^+$**			
		HIV+ epithelium	HIV- epithelium	HIV+ stroma	HIV- stroma	HIV+ epithelium	HIV- epithelium	HIV+ stroma	HIV- stroma
LSIL	1	20/21	23/24	10/21	16/24	9/21	21/24	1/21	24/24
	2	1/21	1/24	11/21	8/24	12/21	3/24	12/21	0/24
	3	0/21	0/24	0/21	0/24	0/21	0/24	8/21	0/24
HSIL	1	23/23	23/29	6/23	0/29	1/23	18/29	0/23	1/29
	2	0/23	6/29	14/23	0/29	19/23	11/29	5/23	23/29
	3	0/23	0/29	3/23	29/29	3/23	0/29	18/23	5/29
SCC	1	14/16	16/19	0/16	0/19	3/16	14/19	0/16	0/19
	2	2/16	3/19	9/16	3/19	11/16	5/19	3/16	12/19
	3	0/16	0/19	7/16	16/19	2/16	0/19	13/16	7/19

HIV, human immunodeficiency virus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma

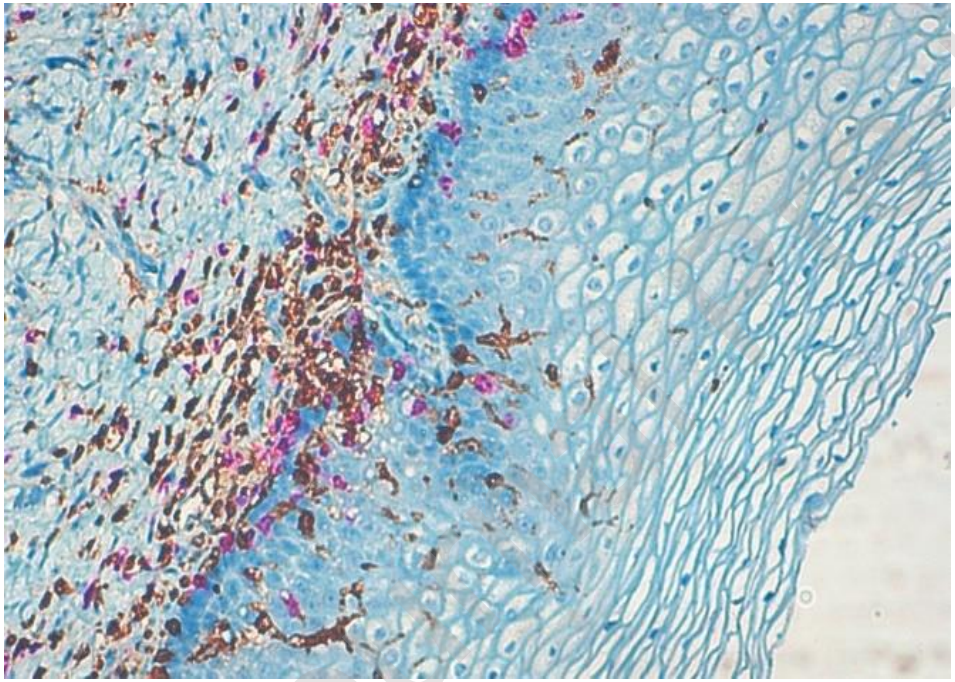

Fig. 1a

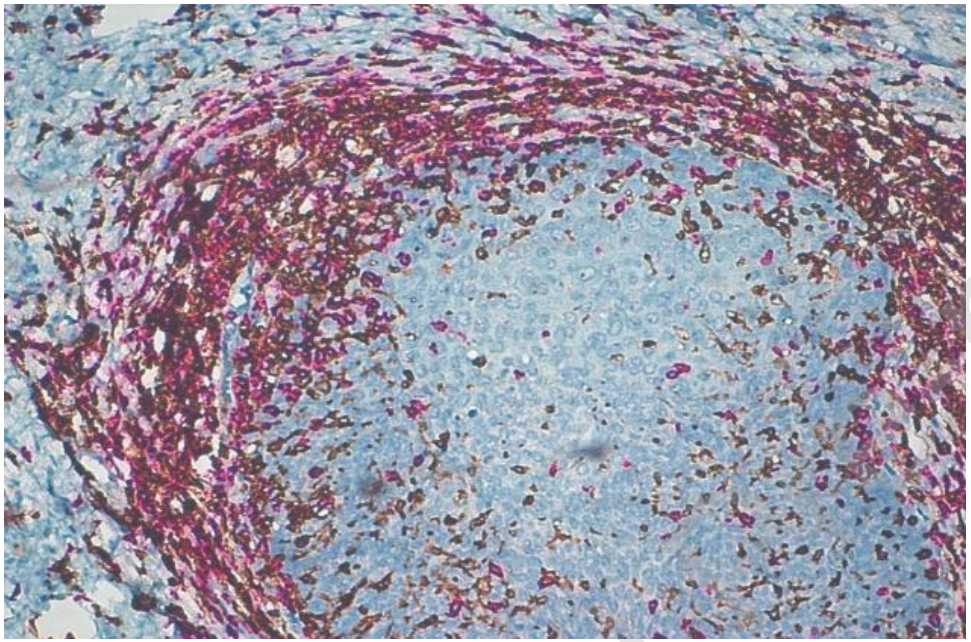

Fig. 1b

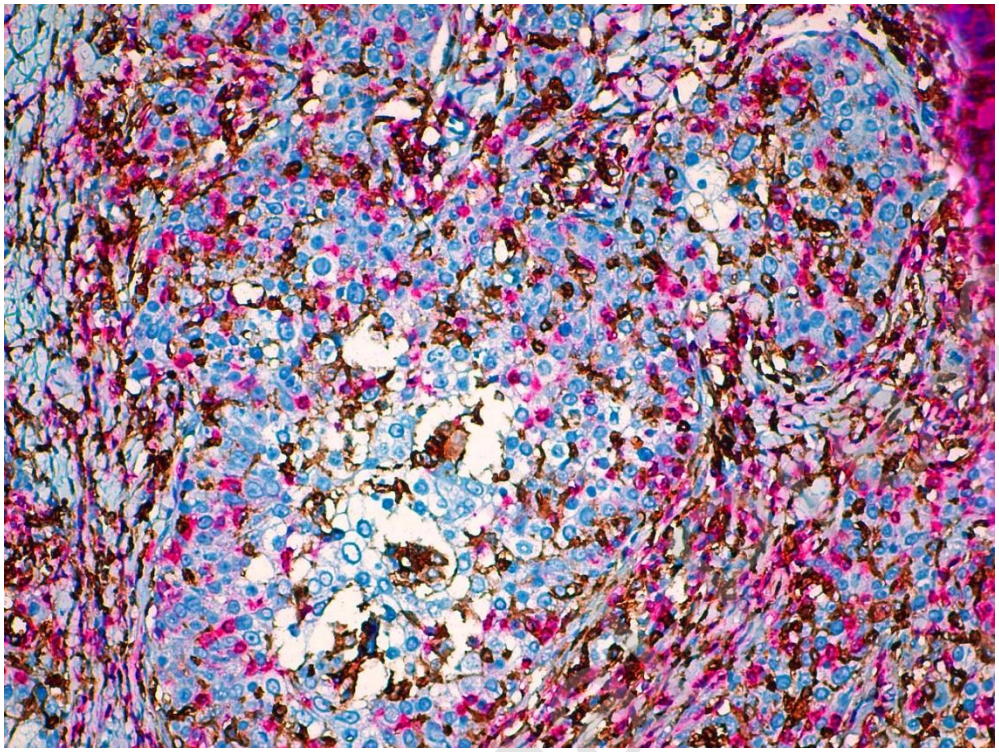

Fig. 1c

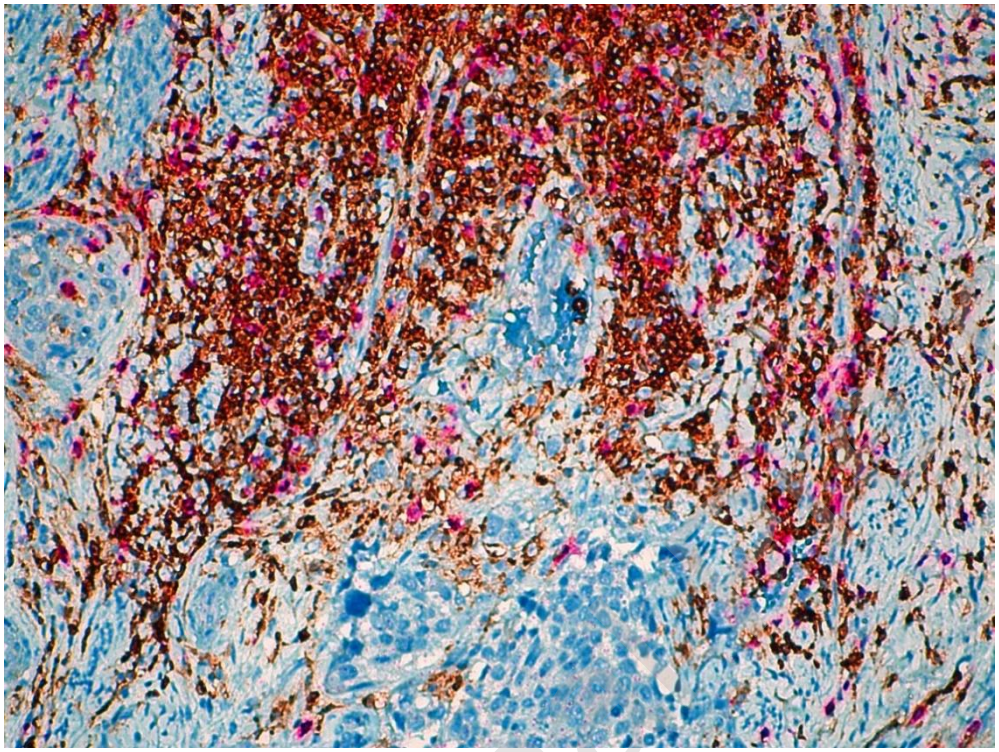

Fig. 1d

Figure 1. CD4+ (brown) and CD8+ (red) dual immunohistochemical staining in cervical lesions.

a) Low-grade squamous cell lesion in an HIV+ patient showing few intraepithelial and stromal CD4+ cells and CD8+ cell predominance in the stroma and basal layer; **b)** HIV+ patient with glandular colonization by high-grade squamous intraepithelial lesion, with heavy intraepithelial (basal layer) and stromal CD4+ and CD8+ cell infiltrate with CD8+ cell predominance; **c)** HIV+ squamous cell carcinoma showing CD8+ cell predominance, both in the

epithelium and stroma; **d)** HIV- patient with squamous cell carcinoma with low CD8+ cell density both in the epithelium and stroma compared with the HIV+ patient depicted in c).