EJGO European Journal of Gynaecological Oncology

Prevalence and predictors of abnormal Papanicolaou smears in HIV-infected women

M. Vide Tavares, F. Coutinho Nunes, S. Saleiro, F. Mota, I. Torgal

Gynecology A Department, Coimbra Hospital and University Center, Coimbra (Portugal)

Summary

Purpose of investigation: To characterize the risk factors for abnormal cervical cytology among women with human immunodeficiency virus (HIV), and to determine the relationship between antiretroviral therapy (ART) and cytology results. *Materials and Methods:* Retrospective study of clinical data of 115 HIV-infected women between January 2008 and December 2011. Analysis of cervical smears history, as well as, epidemiologic, medical, and sexual factors, administration of ART, CD4 cells count, and HIV viral load were performed. *Results:* Mean age was 35.9 ± 6.5 years. Average time of HIV infection was 10.5 ± 4.5 years. HPV infection prevalence was 37.4%, the majority was high-risk. An abnormal Papanicolaou smear was found in 43.5%. Atypical squamous cell of undetermined significance (ASC-US) was reported in 7.8%, low-grade squamous intraepithelial lesions (LSIL) in 32.2%, and high-grade squamous intraepithelial lesions (HSIL) in 3.5%. HPV infection was the only statistical predictor of abnormal cytology ($p < 0.001$; OR = 0.042). ART, CD4 cells count, and HIV viral load did not correlate to regression of abnormal cytology. *Conclusion:* These women should be followed-up according to current cervical cancer screening guidelines, independently of the therapy, CD4 cells count, and HIV viral load.

Key words: HIV-infected women; Cytology; Human papillomavirus (HPV); Predictors.

Introduction

Invasive cervical cancer and its precursors are the most significant gynecologic manifestations of human immunodeficiency virus (HIV) infection. Innumerous reports [1-4] suggest that HIV-infected women have higher incidence of squamous intraepithelial lesions (SIL) than the general population, with an increased risk of developing invasive cervical neoplasia. The contribution of immunosuppression and other risk factors to the increased incidence of SIL in HIV-infected women remains controversial. Antiretroviral therapy (ART), especially highly active antiretroviral therapy (HAART), undoubtedly lowered the incidence of innumerous opportunistic diseases related to HIV infection, such as Kaposi's sarcoma and non-Hodgkin lymphoma. However, its impact on the incidence of SIL and cervical cancer has not been yet clarified [5-8].

The objectives of this study were to evaluate the prevalence of human papillomavirus (HPV) infection and characterize possible risk factors for abnormal cervical cytology among women with HIV infection, and to determine the potential relationship between antiretroviral therapy and cytology results.

Materials and Methods

The present study was approved by the Ethical Committee of the Coimbra Hospital and University Center, a tertiary care hospital with a Cervical-Vulvar Unit. The authors conducted a retrospective analysis of HIV-infected women in their Outpatient Clinic. These patients were commonly referred to the unit by the Infectious Disease Specialist who followed them. The authors analyzed the data of 131 patients (16 were excluded because of incomplete data), between January 2008 and December 2011, including cervical smear history as well as epidemiologic (age, years of HIV infection, race, smoking habits, drug abuse), medical (mode of transmission of HIV, other infections like hepatitis B or C), and sexual factors (age at first intercourse, number of sexual partners, history of prostitution, and HPV infection), administration of ART, CD4 cells count, and HIV viral load.

In the present clinical protocol, all HIV-infected women were advised to have a Papanicolaou smear taken every year. All smears were taken by gynecologists and ThinPrep technology was used systematically. They were processed in the pathology department and classified based on the Bethesda System guidelines. According to the present protocol, colposcopy was offered to all women with cervical cell abnormalities. If indicated, lesions were further evaluated by biopsy. Definitive treatment was provided as indicated.

In order to analyze progression or regression of the cytological abnormalities, the authors considered two consecutive Papanicolaou smears with a time interval not inferior to one year. For this study they analyzed 83 patients who met the criteria. Regression was considered when the second cytology (C2; follow-up) had lower-grade abnormalities than the first (C1; baseline); no change, when both had the same classification; progression, when the second cytology had higher-grade abnormalities than the first.

Women were asked if they were taking ART and also to specify the medication. CD4 cells count and HIV viral load were taken into consideration, if done two months earlier, or after the clinical examination and Papanicolaou smear.

Revised manuscript accepted for publication April 28, 2014

Table 1. — *Sociodemographic characteristics and HIV progression markers for abnormal cytological results.*

Variable	Frequency (%)	NILM	Abnormal	*p* value
Race				n.s.
White	95 (82.6)	55 (57.9)	40 (42.1)	
Black	18 (15.7)	8 (44.4)	10 (55.6)	
Other	2 (1.7)	2 (100)	0	
Age of first intercourse				n.s.
12-15 years	28 (24.3)	17 (60.7)	11 (39.3)	
16-18 years	56 (48.7)	29 (51.8)	27 (48.2)	
> 18 years	31 (27)	19 (61.3)	12 (38.7)	
Number of sexual partners				n.s.
1	27 (23.5)	18 (66.7)	9 (33.3)	
2-5	57 (49.6)	30 (52.6)	27 (47.4)	
>5	31 (27)	17 (54.8)	14 (45.2)	
Transmission				n.s.
Sexual	81 (70.4)	46 (56.8)	35 (43.2)	
Needle sharing	6 (5.2)	3 (50)	3 (50)	
Blood transfusion	5 (4.3)	4 (80)	1 (20)	
Unknown	23 (20)	12 (52.2)	11 (47.8)	
Other infections				0.08
None	99 (86.1)	56 (56.6)	43 (43.4)	
HCV	6 (5.2)	4 (66.7)	2 (33.3)	
HBV	3 (2.6)	2 (66.7)	1 (33.3)	
Other	7 (6.1)	3 (42.9)	4 (57.1)	
History of prostitution				n.s.
Yes	5 (4.3)	2 (40)	3 (60)	
No	110 (95.7)	63 (57.3)	47 (42.7)	
Drugs abuse				0.13
Yes	22 (19.1)	10 (45.5)	12 (54.5)	
No	93 (80.9)	55 (59.1)	9 (40.9)	
Smoking				n.s.
Yes	22 (19.1)	11 (50)	11 (50)	
No	93 (80.9)	54 (58.1)	39 (41.9)	
HPV infection				<0.001
Yes	43 (37.4)	8 (18.6)	35 (81.4)	
No	72 (62.6)	57 (79.2)	15 (20.8)	
Antiretroviral therapy				
Yes	77 (67)	41 (53.2)	36 (46.8)	n.s.
No	38 (33)	24 (63.2)	14 (36.8)	
CD4 cells count (cells/mm^3)				
<200	5 (4.3)	1 (20)	4 (80)	n.s.
200-350	31 (27)	18 (58.1)	13 (41.9)	
>350	79 (68.7)	46 (58.2)	33 (41.8)	

Legend:
HCV: hepatitis C; HBV: hepatitis B; HPV: human papillomavirus;
NILM: negative cytological results for intraepithelial lesions;

Table 2. — *Changes in cervical cytology status in consecutive Papanicolaou smears.*

Results	C1	C2	Comparison between C1 and C2		
			Regression n (%)	No change n (%)	Progression n (%)
NILM	46	55	-	34 (73.9)	12 (26.1)
ASCUS	8	2	7 (87.5)	0	1 (12.5)
LSIL	27	26	13 (48.1)	14 (51.9)	0
HSIL	2	0	2 (100)	0	-

Legend:
C1 and C2: first and second cytology;
time interval between C1 and C2 not inferior to one year;
regression: C2 < C1; no change: C1 = C2; progression: C2 > C1;
NILM: negative cytological results for intraepithelial lesions;
ASCUS: atypical squamous cell of undetermined significance;
LSIL: low-grade squamous intraepithelial lesions;
HSIL: high-grade squamous intraepithelial lesions.

Statistical analysis

Statistical analysis was performed using SPSS v.20.0. Univariate analysis was used to determine which variables were associated with abnormal cytology. Chi-square tests were used to test categorical variables. Variables at least marginally associated with abnormal cytology ($p < 0.15$) in univariate analyses were further investigated in multivariate logistic regression models. Analyzing the statistical significance of the predictors in the regression models, the reference value of $p < 0.05$ was adopted. With a sample of 115 patients and considering an α level of 0.05, the power of the study to detect a medium W effect size of 0.30 was 78% for contingency tables with three degrees of freedom, 83% for contingency tables with two degrees of freedom, and 90% for contingency tables with one degree of freedom.Odds ratio (OR) and 95% confidence intervals (CI) were also estimated.

Results

A total of 115 HIV-infected patients were analyzed. Table 1 shows epidemiologic data, risk factors, and correlates them to different cytological results. In this population, 43.5% had abnormal Papanicolaou smears. An atypical squamous cell of undetermined significance (ASC-US) was reported in 7.8%, low-grade squamous intraepithelial lesions (LSIL) in 32.2% and high-grade squamous intraepithelial lesions (HSIL) in 3.5%. Negative cytological results for intraepithelial lesions (NILM) were found in 56.5%. The prevalence of HPV infection was 37.4%.

Mean age of the patient population was 36.98 ± 6.52 [20-50] years. Mean time of infection by HIV was 10.48 ± 4.47 [1-24] years. There was no significant difference in race, age of first intercourse, number of sexual partners, mode of HIV transmission, history of prostitution, smoking habits, and the cytological results. Other infections and drug abuse were marginally associated with abnormal cytology ($p = 0.08$ and 0.13, respectively). Only HPV infection could be considered a statistical predictor of abnormal cytology ($p < 0.001$, OR = 0.042) and a risk factor. Women with a combination of three risk factors – other infections, HPV infection, and drug abuse – were 35% more likely to have abnormal cytology.

Table 2 shows the changes in cervical cytology status in consecutive Papanicolaou smears. Considering NILM, 26.1% had progression but the majority (73.9%) was stable. On the other hand, the majority of LSIL did not change and persisted. The only two cases of HSIL showed regression, because the second cytology was performed after treatment (the two biopsies revealed cervical intraepithelial neoplasia (CIN) 3 and a conization were carried out).

Table 3. — *Antiretroviral therapy and changes in cervical cytological status.*

Cytological result C1	Antiretroviral therapy C1	Antiretroviral therapy C2	Comparison between C1 and C2		
			Regression n (%)	No change n (%)	Progression n (%)
Abnormal	On ART	On ART	10 (38.5)	15 (57.7)	1 (3.8)
		Off ART	0	1 (100)	0
	Off ART	On ART	1 (20)	4 (80)	0
		Off ART	3 (60)	2 (40)	0
Normal	On ART	On ART	-	20 (80)	5 (20)
		Off ART	-	1 (100)	0
	Off ART	On ART	-	6 (75)	2 (25)
		Off ART	-	8 (72.7)	3 (27.3)

Legend:
C1 and C2: first and second cytology;
time interval between C1 and C2 not inferior to one year;
regression: C2 < C1; no change: C1 = C2; progression: C2 > C1.

Table 4. — *HIV viral load and changes in cervical cytological status.*

HIV viral load (number of copies/mL)		Comparison between C1 and C2		
		Regression n (%)	No change n (%)	Progression n (%)
C1	<50	14 (63.6)	26 (54.2)	5 (38.4)
	50-10^3	4 (18.2)	13 (27.1)	4 (30.8)
	10^3-10^5	4 (18.2)	9 (18.7)	4 (30.8)
C2	<50	17 (77.3)	34 (70.8)	9 (69.2)
	50-10^3	3 (13.6)	7 (14.6)	1 (7.7)
	10^3-10^5	2 (9.1)	7 (14.6)	3 (23.1)

Legend:
C1 and C2: first and second cytology;
time interval between C1 and C2 not inferior to one year;
regression: C2 < C1; no change: C1 = C2; progression: C2 > C1.

Table 5. — *HIV viral load and changes in cervical cytological status.*

CD4 cells count (cells/mm^3)		Comparison between C1 and C2		
		Regression n (%)	No change n (%)	Progression n (%)
C1	<200	1 (4.5)	3 (6.3)	0
	200-350	7 (31.8)	11 (22.9)	4 (30.8)
	>350	14 (63.6)	34 (70.8)	9 (69.2)
C2	<200	1 (4.5)	3 (6.3)	2 (15.4)
	200-350	3 (13.6)	5 (10.4)	1 (7.7)
	>350	18 (81.8)	40 (83.3)	10 (76.9)

Legend:
C1 and C2 = first and second cytology;
time interval between C1 and C2 not inferior to one year;
regression: C2 < C1; no change: C1 = C2; progression: C2 > C1

Table 6. — *Adjusted odds ratio and significance of antiretroviral therapy, CD4 cells count and HIV viral load in regression or progression of the cytological results.*

Predictors	*p* value	Odds ratio
Antiretroviral therapy	0.993	1.009
CD4 cells count	1.169	1.268
HIV viral load	0.464	0.025
Antiretroviral therapy * CD4 cells count; * HIV viral load	0.999	
	Cox & Snell R2= 0.09	Nagelkerke R2=0.14

Tables 3, 4, and 5 show the association between ART, CD4 cells count and HIV viral load, and changes in cervical cytological status, respectively. About 44.8% of women that were not on ART at baseline began treatment. The majority of women were under ART, had CD4 cells count superior to 200/mm^3 and almost negative viral load. The present authors found no differences in regression or progression of the cytological results when the three conditions were analyzed in isolation or combined (Table 6).

Discussion

The prevalence of abnormal Pap smears in HIV infected women was 43.5% in the present study, which is similar to many others described in the literature [2-4]. The prevalence of HPV infection in this study (37.4%) was not as high as others have described, mainly Heard *et al.* [9] who found a prevalence of HPV infection in European women with HIV infection near 49.5% (46.3-52.8%). However, the prevalence of HPV infection in the present study was clearly associated with abnormal cytological results and could be considered a statistical predictor of disease and a risk factor, which is in agreement with other reports in the literature [2, 3].

The present study showed, in agreement with others [2, 3], that in addition to HPV and HIV infection, no other characteristics usually associated with abnormal Papanicolaou smears abnormalities, such as early intercourse, smoking habits, number of sexual partners, and sexually transmitted infection, were significant.

The prevalence of HSIL was 3.5% which corresponds to only two cases. After the abnormal Pap smear and a colposcopy suggesting a high-grade lesion, a biopsy was performed and the histology revealed CIN 3. A conization was offered in both cases and the histology revealed a CIN 3 with free margins. Follow-up with Papanicolaou smear and colposcopic examination showed regression of the cytological abnormalities. No case of cervical cancer was found in the present study. The low incidence of high-grade cytological abnormalities and absence of cervical cancer could be explained by a highly cohort of women with great compliance to cervical cancer screening and intervention.

A synergistic action of HIV-induced immunosuppression and HPV infection may actually favor the onset of precancerous cervical lesions. HAART regimens have dramatically improved HIV prognosis. However, while systemic benefits are unquestionable, the beneficial actions that it may exhibit locally, on the cervix, are still unclear [6]. Re-

cent studies [7- 8, 10-11] have indicate that HAART may show a beneficial effect on cervical lesions but this evidence is still under debate. In the present study, the majority of women were under retroviral therapy but not necessarily HAART.

Although the most used therapy to treat HIV infection is HAART, a proportion of the present study population was receiving an older combination of antiretroviral drugs because of their long history of disease. Moreover, most of the present patient population had CD4 cells count superior to 200/mm^3 and negative viral load despite the antiretroviral therapy. Furthermore, the authors found no differences in regression or progression of the cytological results when analyzed for antiretroviral therapy, CD4 cells count, and HIV viral load. In agreement with Soncini *et al.* [12], the actions of HIV infection and antiretroviral therapy on the cervix are not clear. On the other hand, some publications clearly showed that antiretroviral therapy, such as HAART, induces high levels of CD4 cells count and negative HIV viral load influencing the regression of abnormal cytological results [13, 14], and even decreasing HPV prevalence [15].

The present study had several limitations. It was a retrospective analysis of clinical data of patients followed in the Outpatient Clinic with no control group. The present sample size (115 HIV positive patients) was too limited to draw meaningful conclusions.

Conclusion

It is clear that HIV-infected women have a higher risk of developing cervical neoplasia. After the advent of HAART, several studies were performed to increase our knowledge on the effect of this therapy on abnormal cytological results and cervical lesions. The present study and similar studies reinforce that HPV is a predictor and risk factor for cervical cellular abnormalities in HIV-infected women. Hence, HIV infected women under HAART should be followed-up according to current cervical cancer screening guidelines, independently of the therapy, CD4 cells count, and HIV viral load.

References

[1] Ellerbrock T.V., Chiasson M.A., Bush T.J., Sun X.W., Sawo D., Brudney K., et al. "Incidence of cervical squamous intraepithelial lesions in HIV-infected women". *JAMA,* 2010, *283*, 1031.

[2] Massad L.S., Riester K.A., Anastos K.M., Fruchter R.G., Palefsky J.M., Burk R.D., *et al.*: "Prevalence and predictors of squamous cell abnormalities in Papanicolaou smears form women infected with HIV-1". *J. AIDS,* 1999, *21*, 33.

[3] Maiman M., Fruchter R., Sedlis A., Feldman J., Chen P., Burk R.D., *et al.*: "Prevalence, risk factors and accuracy of cytology screening for cervical intraepithelial neoplasia in women with Human Immunodeficiency Virus". *Gynecol. Oncol.,* 1998, *68*, 233.

[4] Minkoff H.L., Eisenberger-Matityahu D., Feldman J., Burk R., Clarke L.: "Prevalence and incidence of gynecologic disorders among women infected with human immunodeficiency virus". *Am. J. Obstet. Gynecol.,* 1999, *180*, 824.

[5] Paramsothy P., Jamieson D.J., Heiling C.M., Schuman P.C., Klein R.S., Shah K.V., *et al.*: "The effect of highly active antiretroviral therapy on human Papillomavirus clearance and cervical cytology". *Obstet. Gynecol.,* 2009, *113*, 26.

[6] Del Mistro A., Bertorelle R., Franzetti M., Cattelan A., Torrisi A., Giordani M.T., *et al.*: "Antiretroviral therapy and the clinical evolution of human Papillomavirus-associated genital lesions in HIV-positive women". *Clin. Infect. Dis.,* 2004, *38*, 737.

[7] Heard I., Potard V., Costagliola D.: "Limited impact of immunosuppression and HAART on the incidence of cervical squamous intraepithelial lesions in HIV-positive women". *Antiviral Therapy,* 11, 109.

[8] Adler D.H., Kakinami L., Modisenyane T., Tshabangu N., Mohapi L., Bruyn G.D., *et al.*: "Increased regression and decreased incidence of human pappilomavirus-related cervical lesions among HIV-infected women on HAART". *AIDS,* 2012, *26*, 1645.

[9] Heard I., Cubie H.A., Mesher D., Sasieni P.: "MACH-1 Study Group. "Characteristics of HPV infection over time in European women who are HIV-1 positive". *BJOG,* 2013, *120*, 41.

[10] De Vuyst H., Mugo N.R., Chung M.H., McKenzie K.P., Nyongesa-Malava E., Tenet V., *et al.*: "Prevalence and determinates of human pappilomavirus infection and cervical lesions in HIV-positive women in Kenya". *Br. J. Cancer,* 2012, *107*, 1624.

[11] Zeier M.D., Botha M.H., Merwe F.H., Wilson I.E., Schalkwyk M., Grange M., *et al.*: "Progression and persistence of low-grade cervical squamous intraepithelial lesions in women living with Human Immunodeficiency Virus". *J. Low Genit. Tract Dis.,* 2012, *16*, 243.

[12] Soncini E., Zoncada A., Condemi V., Antoni A.D., Bocchialini E., Soregotti P.: "Reduction of the risk of cervical intraepithelial neoplasia in HIV-infected women treated with highly active antiretroviral therapy". *Acta Biomed.,* 2007, *78*, 36.

[13] Minkoff H.L., Ahdieh L., Massad L.S., Anastos K., Watts D.H., Melnick S., *et al.*: "The effect of highly antiretroviral therapy on cervical cytologic changes associated with oncogenic HPV among HIV-infected women". *AIDS,* 2001, *15*, 2157.

[14] Kreitchmann R., Bajotto H., Silva D.A., Fuchs S.: "Squamous intraepithelial lesions in HIV-infected women: prevalence, incidence, progression and regression". *Arch. Gynecol. Obstet.,* 2013, *5*, 1.

[15] Minkoff H.L., Zhong Y., Burk R., Palefsky J.M., Xue X., Watts D.H., *et al.*: "Influence of adherent and effective antiretroviral therapy use on Human Papillomavirus infection and squamous intraepithelial lesion in HIV-positive women". *J. Infect. Dis*., 2010, *201*, 681.

Address reprint requests to:
M. VIDE TAVARES
Coimbra Hospital and University Center
Gynecology Department A,
Praceta Mota Pinto, 3000 Coimbra (Portugal)
e-mail: m.vide@hotmail.com