Correlations between Babes-Papanicolaou's findings and immunosuprression in Human Immunodeficiency Virus infected women

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Abstract

Objective(s). *To assess the frequency and correlations between immunosupression and cytological findings of Babes-Papanicolaou test in HIV infected women.* ***Methods.*** *Prospective study from January, the 1st 2011 to April, the 30th 2013, in the Clinical Infectious Hospital, Constanta, Romania, included 98 HIV infected women, 92.9% highly active antiretroviral therapy (HAART) recipients, who inform consented to Babes-Papanicolaou technique. Immunologic data were correlated with cervical cytological smears results, classified by 2001Bethesda System. Descriptive and bivariate statistics were performed using SPSS version 17.* ***Results.*** *41.83% of HIV infected females (n=41/98), mean aged 27.65 +/- 7.690 y.o. (limits: 15-60 years), 73.58% HAART recipients (n=39/53), presented abnormal results of cervical cytology. The risk of squamous cell lesions (SILs) is increased when CD4 cell counts are decreased <500 cells/µl (p< 0.02).* ***Conclusion(s).*** *The prevalence of SILs is higher among HIV infected women HAART recipients and strongly associated with lower CD4 cell counts.*

Keywords: *HIV, squamous intraepithelial lesions, Babes-Papanicolaou cervico-vaginal smear, HPV, HAART*

Introduction

The Joint United Nations Programme on HIV and AIDS (UNAIDS) Report on the Global AIDS Epidemic estimated, in 2012, that more than half of the 34 million persons infected with the HIV virus worldwide are women[(1)]. Among women aged 15-49 years, HIV/AIDS is the leading cause of death and cervical cancer is frequently reported as a leading causes of death in women from middle and low-income countries[(2)]. Comparing with HIV uninfected, infected women are five times more likely to have cervical squamous intraepithelial lesions (SILs), the precursors of the invasive cervical cancer[(3,4)]. The risk factors of cervical cancer are various and related to individual behavior (sexual habits, use of oral contraceptives, multiparity, tobacco smoking) and the Human Papillomavirus (HPV) infection. HPV is a sexually transmitted virus with more than 100 strains and 40% of them may cause cervical cancer, mainly types 16 and 18 considered to be responsible for 70% of cervical cancer globally[(2)]. Not all the HPV infected women develop cervical cancer because most HPV infections regress spontaneously. The cofactors which may increase the HPV oncogenity are HIV infection, immunodeficiency states, *herpes simplex* virus infection, tobacco smoking, early age at beginning sexual life, women with multiple sexual partners, chronic lower genital tract infection.

The immune status of HIV infected individuals is evaluated by CD4 T cell counts. Moderate immunosupresion was defined as CD4+ cell count between 200 and 500 cells/mm^3 and severe immunosupresion as CD4+ cell count <200 cells/mm^3. The use of highly active antiretroviral therapy (HAART) in HIV infected patients influences the immune status, reduces the risk of acquired immunodeficiency syndrome (AIDS) and death[(5)].

Methods

The present paper work is based on cytological and serological evaluation of a group of 98 HIV infected women from Clinical Infectious Hospital, Constanta, Romania, who inform consented to routine gynecological examinations and Babes-Papanicolaou screening of cervical lesions. The setting for a prospective study during January, the 1st 2011 to April, the 30th 2013 was Clinical Infectious Hospital, Constanta, Romania.

The authors proposed to assess the correlations between HIV viral loads, CD4 T cell counts, which expressed the immunosupression level, the use of highly active antiretroviral therapy (HAART) and cytological findings of Babes-Papanicolaou cervico-vaginal smears, and to determine the prevalence of test's abnormalities. Conventional samples for cytological examination were performed by Babes-Papanicolaou method of staining and analyzed in optical microscopy to detect pre-malignant and malignant cervical lesions.

Results of cervical cytology were classified, using the 2001 Bethesda Classification System[(6)], and reported as no abnormality detected (N=normal), reactive modifications (RM), squamous cell abnormalities [atypical

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squamous of undetermined significance (ASC-US), atypical squamous cells - cannot exclude HSIL (ASC-H), low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma], glandular cell abnormalities [Atypical Glandular Cells not otherwise specified (AGC or AGC-NOS)].

A part of women with abnormal cytology were referred for HPV DNA testing or diathermy loop electroresection with consecutive histopathological exams of cervical tissue samples.

To assess the degree of HIV immunosuppression, laboratory measurements of CD4 T cells and plasma HIV viral loads were considered 4 CD4 levels: <200, 201–499, 500-999 and >1,000 cells/µl and 6 HIV viral load levels: undetectable viral load (quantified as zero); <500; 500- 9,999; 10000-99,999; ≥100,000 copies/ml, ≥1 milion. Inclusion criteria consist in availability of at least 1 quantitative viral load test along with CD4 count after the HAART initiating or changing and timely related with Babes-Papanicolaou screening test. We categorized the cytological results of each woman by CD4 levels and HIV viral load.

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software (version 17.0: SPSS; Chicago, IL, USA). Descriptive statistics expressed values of outcomes as mean + standard deviation. Bivariate analysis was performed using the Pearson χ^2 test and Fischer Exact test for categorical variables. A p value <0.05 was considered statistically significant.

Results

The mean age of the 98 participants was 27.65 ± 7.690 y.o. (limits: 15-60 years) with a median of 24 years.

A half of women (n=53; 54%) were identified with abnormal cytology: 18 (18.36%) ASCUS, 6 (6.12%) AGCUS, 1 (1.02%) ASC-H, 14 (14.28%) LSIL, 16 (16.32%) HSIL.

There was no significant correlations between HIV infected women's age and CD4 cell count, HIV viral load or cervical cell results classified by Bethesda system (Figure 1).

The mean CD4 cell count was 572 ± 369.318 cells/µl (Table 1), 330 ± 181.423 cells/µl in AGC (limits: 125-468 cells/µl) and 484.70 ± 314.605 cells/µl (limits: 158-1049) in HSIL, with no significant correlation (Table 2; Figure 1).

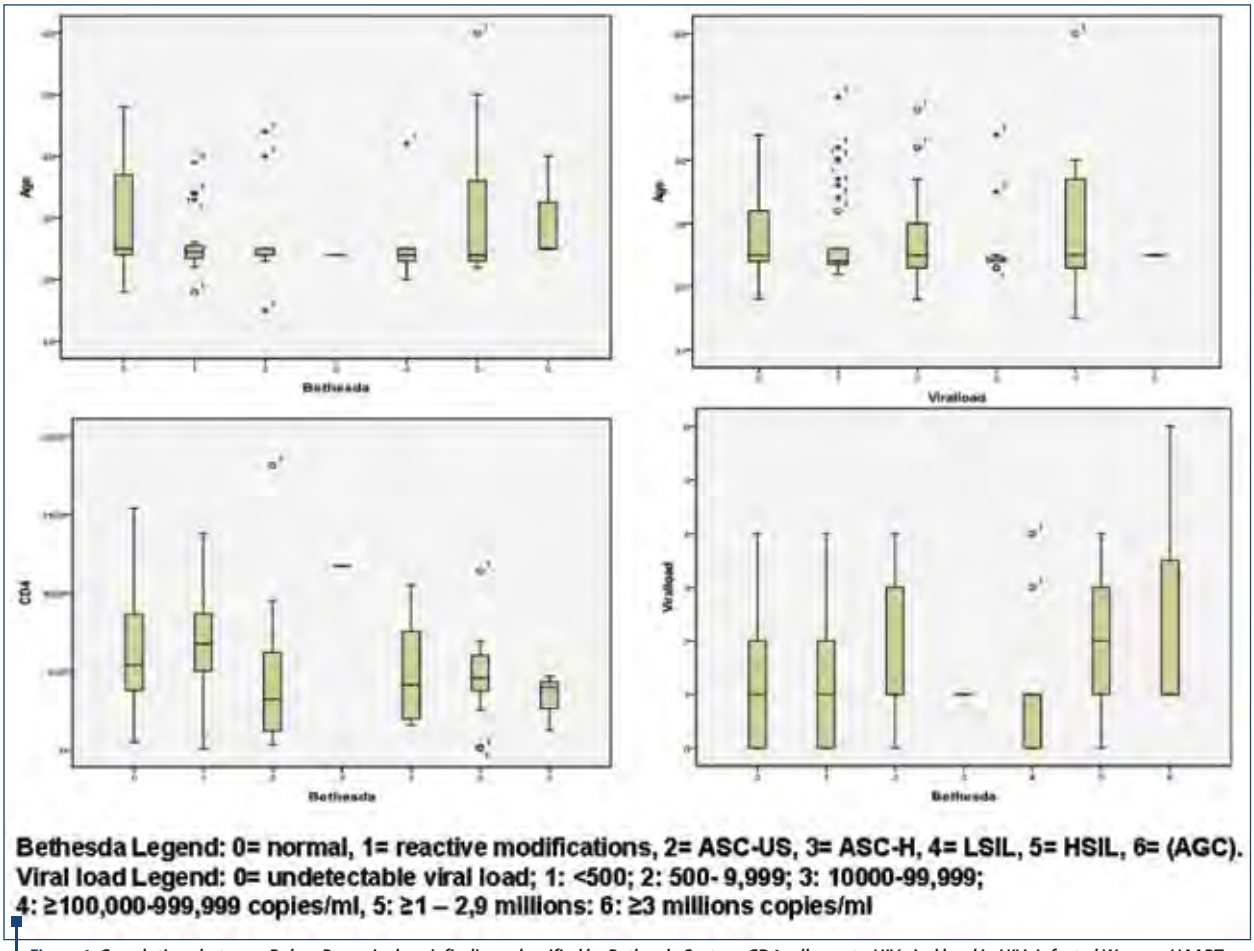

Figure 1. Correlations between Babes-Papanicolaou's findings classified by Bethesda System, CD4 cell counts, HIV viral load in HIV- Infected Women, HAART recipients (n=91)

Table 1 Descriptive statistics of CD4, viral load and age of HIV infected women along Bethesda classification system of Babes-Papanicolaou test

Bethesda Classification		CD4	HIVload	Age
Normal	Mean	616.66	31601.34	29.10
	N	29	29	29
	Std. Deviation	368.430	100608.224	7.816
	Median	540.00	50.00	25.00
	Minimum	47	0	18
	Maximum	1540	458822	48
Reactive changes	Mean	667.82	27653.75	25.86
	N	28	28	28
	Std. Deviation	344.664	101312.057	4.592
	Median	675.00	47.00	24.50
	Minimum	4	0	18
	Maximum	1380	518218	39
ASC-US	Mean	451.29	253809.50	25.93
	N	14	14	14
	Std. Deviation	477.027	911999.950	7.280
	Median	321.00	50.00	24.00
	Minimum	29	0	15
	Maximum	1814	3421551	44
ASC-H	Mean	1173.00	445.00	24.00
	N	1	1	1
	Std. Deviation	.	.	.
	Median	1173.00	445.00	24.00
	Minimum	1173	445	24
	Maximum	1173	445	24
LSIL	Mean	484.70	29592.90	25.40
	N	10	10	10
	Std. Deviation	314.605	88145.405	6.004
	Median	413.00	143,50	24.00
	Minimum	158	0	20
	Maximum	1049	280106	42
HSIL	Mean	474.92	84386.08	31.62
	N	13	13	13
	Std. Deviation	296.347	219908.024	12.299
	Median	456.00	1106.00	24.00
	Minimum	9	0	22
	Maximum	1141	797721	60
AGC	Mean	330.67	9058.33	30.00
	N	3	3	3
	Std. Deviation	181.423	15616.749	8.660
	Median	399.00	50.00	25.00
	Minimum	125	34	25
	Maximum	468	27091	40
Total	Mean	572.31	68006.58	27.65
	N	98	98	98
	Std. Deviation	369.318	360762.759	7.690
	Median	542.00	50.00	24.00
	Minimum	4	0	15
	Maximum	1814	3421551	60

Table 2 ANOVA Table of CD4, viral load and age means by Bethesda classification of cervical exam's findings

			Sum of Squares	df	Mean Square	F	P
CD4 * Bethesda	Between Groups	(Combined)	1253565.611	6	208927.60	1.587	0.160
		Linearity	492809.577	1	492809.57	3.744	0.056
		Deviation from Linearity	760756.034	5	152151.20	1.156	0.337
	Within Groups		11976843.206	91	131613.66		
	Total		13230408.816	97			
HIV load * Bethesda	Between Groups	(Combined)	6.006E11	6	1,001E11	0.758	0.605
		Linearity	1.569E10	1	1.569E10	0.119	0.731
		Deviation from Linearity	5.849E11	5	1.170E11	0.885	0.494
	Within Groups		1.202E13	91	1.321E11		
	Total		1.262E13	97			
Age * Bethesda	Between Groups	(Combined)	477.680	6	79.613	1.378	0.232
		Linearity	28.818	1	28.818	0.499	0.482
		Deviation from Linearity	448.863	5	89.773	1.554	0.181
	Within Groups		5258.524	91	57.786		
	Total		5736.204	97			

Table 3 Crosstab of Babes-Papanicolaou test's cytological findings Bethesda classified by HAART

		HAART		Total
		1=yes	2=no	
Bethesda system*	AGC	4	0	4
	ASC-H	1	0	1
	ASC-US	13	0	13
	HSIL	11	2	13
	LSIL	10	0	10
	N	26	3	29
	RM	26	2	28
Total		91	7	98

**no abnormality detected (N=normal), reactive modifications (RM), squamous cell abnormalities [atypical squamous of undetermined significance (ASC-US), atypical squamous cells – cannot exclude HSIL (ASC-H), low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma], glandular cell abnormalities [atypical Glandular Cells not otherwise specified (AGC)]*

The mean HIV RNA load was 68,006.58 ± 36,076.27 copies/ml (limits: 0- 34,215.51), median 50 copies/ml (Table 1). The highest mean value of viremia (25.380.95 copies/ml) was observed in ASC-US cases (n=14) (Table 2; Figure 1). HIV RNA was undetectable in 18 cases (18.36%).

Table 4 Crosstab of Babes-Papanicolaou test's cytological findings Bethesda classified by CD4 cell count groups

Number of cases		CD4 groups					Total
		0-49	50-199	200-499	500-999	>1000	
Bethesda System*	AGC	0	1	3	0	0	4
	ASC-H	0	0	0	0	1	1
	ASC-US	0	6	3	3	1	13
	HSIL	0	2	3	7	1	13
	LSIL	0	4	2	3	1	10
	N	0	4	8	13	4	29
	RM	1	2	4	16	5	28
Total		1	19	23	42	13	98

** no abnormality detected (N=normal), reactive modifications (RM), squamous cell abnormalities [atypical squamous of undetermined significance (ASC-US), atypical squamous cells – cannot exclude HSIL (ASC-H), low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma], glandular cell abnormalities [atypical Glandular Cells not otherwise specified (AGC)].*

Table 5 Crosstab of Babes-Papanicolaou test's cytological findings Bethesda classified by the viral load count

		Viral load (copies/mL)						Total
		0	<500	500-9,999	10,000- 99,999	100,000- 999,999	>1 milion	
Bethesda System*	AGC	0	3	0	0	0	1	4
	ASC-H	0	1	0	0	0	0	1
	ASC-US	3	4	2	3	1	0	13
	HSIL	3	3	3	2	2	0	13
	LSIL	3	5	0	1	1	0	10
	N	11	9	5	3	1	0	29
	RM	9	11	4	2	2	0	28
Total		29	36	14	11	7	1	98

**no abnormality detected (N=normal), reactive modifications (RM), squamous cell abnormalities [atypical squamous of undetermined significance (ASC-US), atypical squamous cells – cannot exclude HSIL (ASC-H), low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma], glandular cell abnormalities [atypical Glandular Cells not otherwise specified (AGC)].*

HAART was started in 91 HIV infected women and Babes Papanicolaous test revealed squamous cell abnormalities (SIL) in 34 of them (Table 3).

The cervical cell abnormalities consisted in: atypical squamous cells (ASC) of undetermined significance (ASC-US) (n=13/91; 14.28%); ASC-cannot exclude high grade squamous intraepithelial lesions [HSIL (ASC-H)] (n=1/91; 1.09%); nuclear atypia of squamous intraepithelial lesions (SIL) as low-grade (LSIL) (n=10/91; 10.98%) and high-grade (HSIL) (n=11/91; 12%); rare atypical glandular cells of undetermined significance (AGC-US) (n=4/98; 4%), and no invasive carcinoma was detected.

HAART was indicated in 95 cases, was voluntary stopped by 4 women and 91 continued the therapy. Among 3 naïve cases (28.57%), 2 presented HSIL. The risk of SIL occurrence among HIV infected women is related only with the decreased count of CD4 T cell <500 µl (n= 24/43; 55.81%

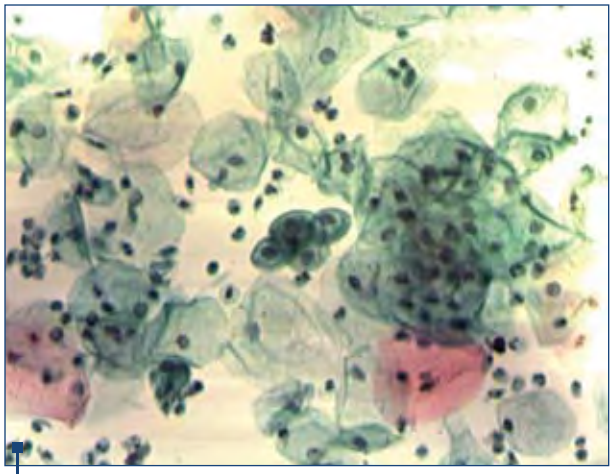

Figure 2. LSIL. Four koilocytes in the centre of the field, surrounded by normal superficial and intermediate squamous cells and some polimorphonucleare leucocytes (x200)

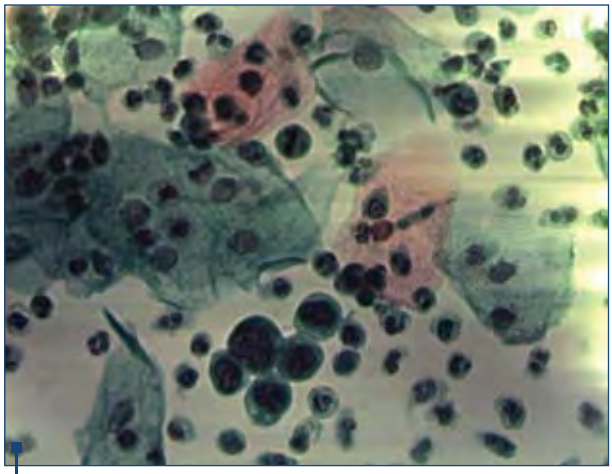

Figure 3. HSIL. Basal cells with enlarged, hyperchromatic and irregularly outlined nuclei in an inflammatory background (x400)

vs 17/55; 30.9%; OR=2.82; 1.14<OR<7.08; RR=1.81; 1.12<RR<2.91; 95%CI; χ^2=6.09; p< 0.02) (Table 4).

There was no correlation with the increased HIV viral load >10,000 copies/mL (n=11/19; 57.89% vs 30/79; 37.97%; OR=2.25; 0.73<OR<7.01; RR=1.52; 0.95<RR<2.45; 95%CI; χ^2=2.47; p< 0.12) (Table 5, Figure 1). Detection of HPV DNA performed by personal patients fee (n=3) allowed one positive detection of low risk HPV DNA genotype 42, 66 and one negative.

The cervical biopsies obtained after diathermy loop electroresection (ERAD) were performed in 8 (8.16%) cases but no cervical cancer was diagnosed.

Furthermore, in Figures 2 and 3, we can note the cytopathic effect of HPV: perinuclear empty cavity surrounded by cytoplasmic thickening associated with moderate nuclear enlargement.

Discussion

Schrager et al.[7] in 1989 and Vermund et al.[8] in 1991, reported that HPV infection and squamous intraepithelial lesions have greater rates in HIV seropositive women than in general population.

Many studies revealed an association between HIV infection and the increased risk for detection of low-grade and high-grade squamous intraepithelial lesions, HPV and cervical cancer[9,10].

HIV seems to be a risk co-factor of HPV infection and cervical cancer. Its oncogenity is influenced by the level of immune system.

It is considered that HIV-positive women with CD4+ cell counts < 500 cells/µl have a greater risk of HSIL than HIV-positive women with CD4 counts >500 cells/µl. We identified more cases of HSIL among higher 500 CD4 cells count recipients (8 versus 5) but without statistical significance.

High plasma HIV RNA levels and the absence of antiretroviral therapy are associated with an increased HSIL risk. Our study did not reveale a significant correlation between viral load count higher 10,000 copies/ml and any SIL risk.

Clinical implications consist in developing a better and rigorous follow-ups, and management for HIV positive women with abnormal Babes-Papanicolaou smears in order to improve the preventing and screening methodologies in HPV HIV co-infected women, with HPV genotyping. It is also recommended to integrate all these strategies in the National Program of Cervical Carcinoma Screening.

Conclusions

The prevalence of abnormal Babes-Papanicolaou conventional cervico-vaginal cytology was higher among HIV infected women HAART recipients from Constanta, Romania (37.36%). Lower CD4 cell counts were strongly associated with a high SILs risk of occurrence. ■

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