Artículos originales

Determinants of LSIL Regression in Women from a Colombian Cohort

Determinantes de la regresión de lesiones cervicales de bajo grado en una cohorte de mujeres colombianas

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Abstract

Objective: To analyze the role of Human Papillomavirus (HPV) and other risk factors in the regression of cervical lesions in women from the Bogotá Cohort. ***Methods:*** 200 HPV positive women with abnormal cytology were included for regression analysis. The time of lesion regression was modeled using methods for interval censored survival time data. Median duration of total follow-up was 9 years. ***Results:*** 80 (40%) women were diagnosed with Atypical Squamous Cells of Undetermined Significance (ASCUS) or Atypical Glandular Cells of Undetermined Significance (AGUS) while 120 (60%) were diagnosed with Low Grade Squamous Intra-epithelial Lesions (LSIL). Globally, 40% of the lesions were still present at first year of follow up, while 1.5% was still present at 5 year check-up. The multivariate model showed similar regression rates for lesions in women with ASCUS/AGUS and women with LSIL (HR= 0.82, 95% CI 0.59-1.12). Women infected with HR HPV types and those with mixed infections had lower regression rates for lesions than did women infected with LR types (HR=0.526, 95% CI 0.33-0.84, for HR types and HR=0.378, 95% CI 0.20-0.69, for mixed infections). Furthermore, women over 30 years had a higher lesion regression rate than did women under 30 years (HR= 1.53, 95% CI 1.03-2.27). The study showed that the median time for lesion regression was 9 months while the median time for HPV clearance was 12 months. ***Conclusions:*** In the studied population, the type of infection and the age of the women are critical factors for the regression of cervical lesions.

Key words: Human papillomavirus, cervical intraepithelial neoplasm, risk factors, follow-up studies

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Resumen

Objetivo: Analizar el papel del virus del papiloma humano (VPH) y otros factores en la regresión de lesiones del cuello del útero en mujeres de la cohorte de Bogotá, Colombia. ***Métodos:*** El tiempo medio de seguimiento fue nueve años. Se incluyeron 200 mujeres VPH positivas con citología anormal. El tiempo de regresión de lesión fue modelado mediante análisis de supervivencia censurando por intervalos. ***Resultados:*** 80 mujeres (40%) tuvieron células escamosas atípicas de significado indeterminado (ASCUS) o células glandulares atípicas de significado indeterminado (AGUS) y 120 (60%) tuvieron lesiones escamosas intraepiteliales de bajo grado (LEI-BG). El 40% de las lesiones estaban presentes en el primer año de seguimiento, mientras que el 1,5% aún estaba a los cinco años. Se observaron tasas similares de regresión para ASCUS/AGUS y LEI-BG (HR=0,82, IC 95% 0,59-1,12). Mujeres infectadas con VPH de alto riesgo y aquéllas con infecciones mixtas tuvieron tasas inferiores de regresión de las lesiones que las mujeres con VPH de bajo riesgo (HR=0,526, IC 95% 0,33-0,84, para los VPH de alto riesgo, y HR=0,378, IC 95% 0,20-0,69, para las infecciones mixtas). Las mujeres mayores de 30 años tuvieron una mayor tasa de regresión de lesiones que las menores de 30 (HR= 1,53, IC 95% 1,03-2,27). El tiempo medio de regresión de las lesiones fue 9 meses, y el tiempo medio para la eliminación del VPH fue 12 meses. ***Conclusiones:*** En la población estudiada, el tipo de infección y la edad de las mujeres son factores críticos para la regresión de lesiones cervicales.

Palabras Clave: virus del papiloma humano, regresión de lesiones cervicales, factores de riesgo, estudios de seguimiento

Introduction

Although cervical cancer is considered a preventable disease, there are some major problems related to its development through the premalignant stages. One is the difficulty of predicting which lesion will progress and which will regress. HPV is the principal risk factor associated with the development of cervical cancer and its precursor lesions. However, HPV presence alone is insufficient cause for the disease. Other factors related to the virus and to the host are likely to be involved in the carcinogenic process. Viral factors may include type, viral load, viral integration status and presence of variants. Likely host factors include age, immunological stage, number of sexual partners, parity and use of oral contraceptives. The majority of these approaches have been obtained from cross-sectional studies.

Low grade Squamous Intra-Epithelial Lesions (LSILs) may either progress to High grade Squamous Intra-Epithelial Lesions (HSILs) and invasive cervical cancer or regress to a normal state (1-5). Few studies have evaluated persistence or regression of low grade cervical lesions over time. Moreover, few follow-up studies have evaluated the behavior of these lesions in relation to HPV presence and other viral and host risk factors, even though these factors are very important for clinical management (6-8). Consequently, there is a need to study the roles played by HPV and other risk factors in the development, persistence or regression of these lesions over time. Furthermore, discrepancies exist among studies to date regarding whether or not risk factors for HPV infection are the same as or different from those of LSILs. Using biomarkers and risk factors to aid in the prediction of persistence rates, regression rates and duration of pre-invasive stages of cervical cancer could be important for the development of more efficient prevention programs. This in turn could help to decrease the number of cases that progress to high grade lesions, decrease the time and number of follow ups, reduce treatment costs and lower anxiety among affected women.

In 1993, the National Cancer Institute of Colombia, in collaboration with the International Agency for Research on Cancer (IARC), started a population-based cohort study on the natural history of HPV infections and cervical neoplasia in a group of low-income women from Bogotá, Colombia. This country has one of the highest cervical cancer rates in the world and a high prevalence of HPV infections (9).

Herein, we present results on women with low grade cervical lesions and HPV infections whose median duration of total follow-up was nine years.

Analysis of factors affecting the rate of regression of low grade cervical lesions is focused on the possible roles of HPV types, viral loads and various female characteristics.

Methods

From November 1993 to November 1995 the Colombian National Cervical Cancer Institute conducted a population census in four Bogotá health districts. Two thousand women aged 18-85 were randomly identified and invited to participate in the cohort study. In order to increase information on sexually active adolescents, 200 sexually-active women aged 13-17, all of whom had visited an adolescent clinic for contraceptive counseling with no medical referral, were also invited to participate. Recruitment and data collection methods have been fully described elsewhere (9,10). Briefly, at recruitment, participants answered a structured questionnaire on socio-demographic characteristics, sexual behavior, reproductive history, smoking, and dietary habits. After interview, all women were asked to undergo gynecological examination, provide a cervical scrape (for cytological evaluation and HPV DNA test) and give a 10 ml blood sample. Of 2200 women who were invited to join the study, 53 refused to participate, 8 were ineligible due to mental illness, hysterectomy, or history of cervical cancer; ultimately, 2139 women joined the study. Informed consent was obtained from all study participants. The ethical committees of the Colombian National Cancer Institute and the IARC approved the study protocol.

Follow up consisted of a visit every 6-9 months until March, 2004. At each visit, a short follow up questionnaire and a cervical scrape for cytological evaluation and HPV testing were obtained. Follow up ended in March, 2004, or upon diagnosis of cervical intra-epithelial neoplasia (CIN) III, whichever occurred first. Cervical colposcopic was performed in all women who had repeated cytological diagnoses of low grade SIL or cytological evidence of HSIL. Colposcopically guided cervical biopsies were performed for women with cytological or colposcopic evidence of HSIL. Women with confirmed CIN III diagnosis underwent adequate treatment. HPV status was not known during the follow up and did not influence clinical management.

The analysis described here was carried out on a subset of the study cohort. We selected 200 women with low grade cervical abnormalities (low grade squamous intraepithelial cervical lesions or ASCUS/AGUS) and were HPV positive with their respective follow-up. We excluded women who had not had at least one follow up visit. Regression of a lesion was defined as a change from an abnormal diagnosis to a normal cytology diagnosis between two consecutive visits.

Testing for HPV was done using a standard GP5+/GP6+ PCR-EIA based assay (11). Briefly, HPV positive samples were subjected to EIA-HPV group-specific analysis using cocktail probes for high-risk (HR) and low-risk (LR) HPVs (12). The HR HPV cocktail probe consisted of oligoprobes for HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The LR HPV probe consisted of oligoprobes for HPV 6, 11, 40, 42, 43, 44, 81, 82 (MM4 and Iso 39 subtype), 83, 84, 71, CP6108, 26, 34, 53, 54, 55, 57,61, 70, 72, 73. HPV types with unknown oncogenic potential --- types 26, 53, 73, 34 and Iso 39 --- were classified in the results as HR types. During follow-up a new GP5+/6 +PCR–RLB analysis was developed and used to type the same 37 different HPV types detected by EIA. In this validation process, some samples from our cohort were tested using both techniques. Ninety six percent agreement was observed between the PCR-RLB analysis and the PCR-EIA assay. Specimens from the first 4 visits were typed with PCR-EIA and those from visits 5 and 6 were typed with PCR-RLB (13).

The PCR-EIA was also used semi-quantitatively to assess the relative amount of HPV DNA in cervical scrapes. This was possible due to the linear relationship found between amounts of DNA and optical densities (OD) in the range of 10 to 10^6 genome equivalents. Viral loads of the samples were categorized as low (OD < 0.5), medium (0.6 < OD < 1.5) or high (OD > 1.5). They were also analyzed by tertiles.

Statistical methods

The time of lesion regression was modeled using methods for interval censored survival time data. The survival function, which describes the

probability that a cervical lesion has cleared as a function of time, was estimated using the Kaplan-Meier estimator. Different risk factors potentially associated with persistence and/or regression of cervical lesions were considered. They included kinds of lesions, HPV infection (high risk, low risk and mixed infections), viral load, age, number of regular sexual partners during follow-up, oral contraceptive use, intrauterine device use, parity and smoking. We carried out Cox regression analysis to estimate the age-adjusted Hazard Ratios (HRs) and 95% confidence intervals (CIs) upon clearance of cervical lesions for all potential risk factors. Finally, only variables significantly related to the rate of regression were included in a multivariate Cox model.

Results

Two hundred HPV positive women with abnormal cytology with one or more follow-up visits were included in the analysis. In total, we tested 2563 cervical specimens from enrolment and follow-up visits. Median duration of total follow-up was 9 years and median duration of follow-up for regresión analysis was 6.5 years. Median interval between visits was 8.4 months, and median number of visits was thirteen.

Study population characteristics are presented in Table 1. Sixty percent of women's cytology showed LGSIL; whereas 40% were diagnosed with ASCUS/AGUS. Distributions for women according

Figure 1. Low grade cervical lesion regression in women from the Bogotá Cohort.

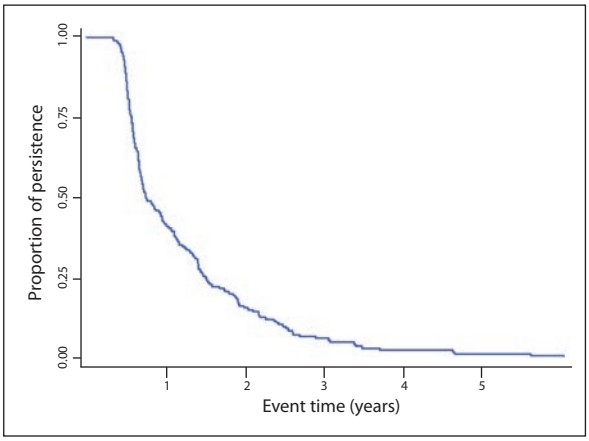


Table 1. Study population characteristics

Characteristics	No.	% Total
Total	200	100
Cytological Diagnosis		
ASCUS/AGUS	80	40
LSIL	120	60
Age (years) +		
< 25	41	20.5
25-29	36	18
30-34	45	22.5
35-39	35	17.5
40 or more	43	21.5
Type of Infection		
High Risk (HR)	151	75.5
Low Risk (LR)	27	13.5
Mixed (HR/LR)	22	11
Number of sexual partners during follow-up		
None	167	83.5
1	19	9.5
+1	14	7
Parity		
None	13	6.5
1 - 2	111	55.5
3 or more	75	37.5
Missing values	1	0.5
Oral Contraceptives		
No	174	87
Yes	17	8.5
Missing values	9	4.5
IUD (Intrauterine device)		
No	142	71
Yes	49	24.5
Missing values	9	4.5
Viral load		
Low	71	35.5
Median	64	32
High	65	32.5
Smoking Habit		
Never	135	67.5
At some time	56	28
- Former smoker	26	
- Current smoker	30	
Missing values	9	4.5

(HR) types: 16, 18, 26, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 ISO39

(LR) types: 6, 11, 40, 42, 43, 44,82(MM4), 83(MM7), 84(MM8), 71(CP8061), CP6108, 81(CP8304), 54, 55, 57, 61, 70, 72, 73

to age groups were similar to overall distribution. Most women were infected with high risk types (75.5%), reported no new sexual partners during follow-up (83.5%) and had 1 or 2 full term pregnancies (55.5%); oral contraceptives were used by 8.5%, and 24.5% used IUD. Only 28% reported having smoked

The probability of regression of low grade cervical lesions as a function of time is presented in Figure 1. Globally, 40% of lesions were still present at 1 year follow-up, 15% at 2 year follow-up and 1.5% at 5 year follow-up.

Percentages of low grade cervical lesions which regressed as a function of time in accordance with different risk factors are presented in Figures 2 to 5. Regression of lesion was more likely in women with low risk infections than in women with high risk and mixed HPV infections. Lesion regression was also more likely in women over 30 and among smokers women than in younger women (≤ 30) and among smokers. Other factors studied —number of sexual partners during follow-up, parity, IUD use and viral loads— were not associated with probabilities of lesion regression.

Crude ratios and multivariate analysis on regression of low grade cervical lesions with several characteristics are presented in Table 2. The multivariate model showed similar lesion regression rates in women with ASCUS/AGUS compared to those with LSIL (HR= 0.82, 95% CI 0.59-1.12). Women with high risk types or mixed infections had lower lesion regression rates than women infected with LR types (HR=0.526, 95% CI 0.33-0.84, for high risk types and HR=0.378, 95% CI 0.20-0.69, for mixed infections). Similarly, women over 30 had higher lesion regression rates than women younger than 30 (HR= 1.53, 95% CI 1.03-2.27).

An age stratified analysis appears in Table 3. Women over 30 with 3 or more parities had a lower lesion regression rate than nulliparous women (HR= 0.23, 95% CI 0.07-0.72). There was a tendency for smokers under age 30 to have a higher lesion regression rate than for non smokers in the same age group (HR= 1.77, 95% CI 0.98-3.19).

Figure 2. Low grade cervical lesion regression according to cytological diagnosis in women from the Bogotá Cohort

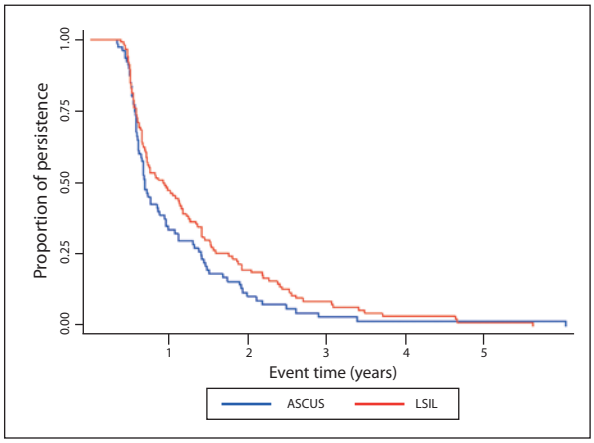
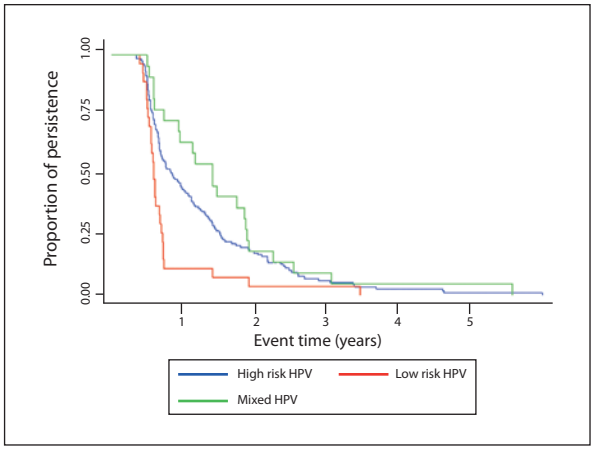


Figure 3. Low grade cervical lesion regression according to HPV infections in women from the Bogotá Cohort

A. High risk, low risk, mixed infections



B. Single High risk, Single Low risk, Multiple High risk, Multiple Low risk, Mixed (HR and LR)

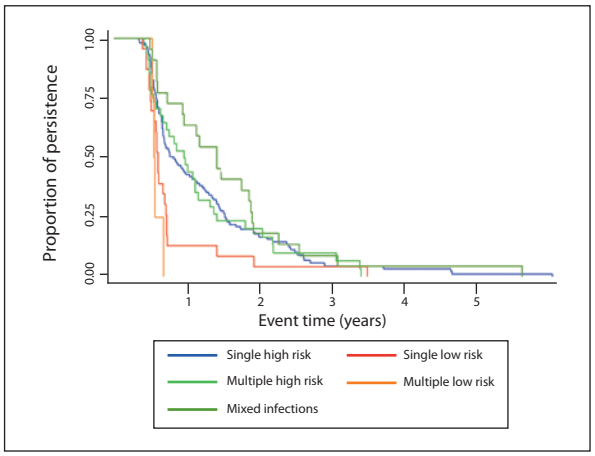


Figure 4. Low grade cervical lesion regression according to age in women from the Bogotá Cohort.

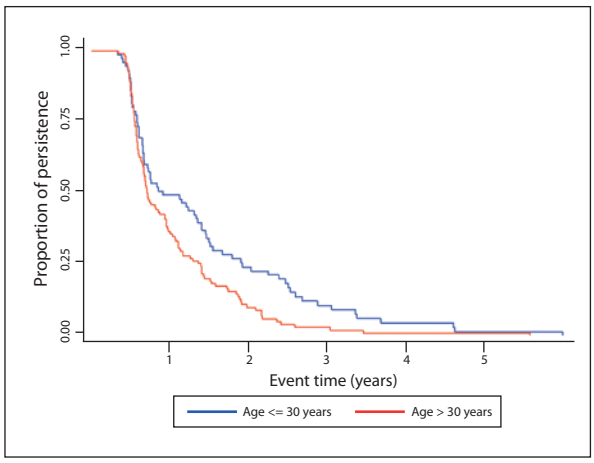
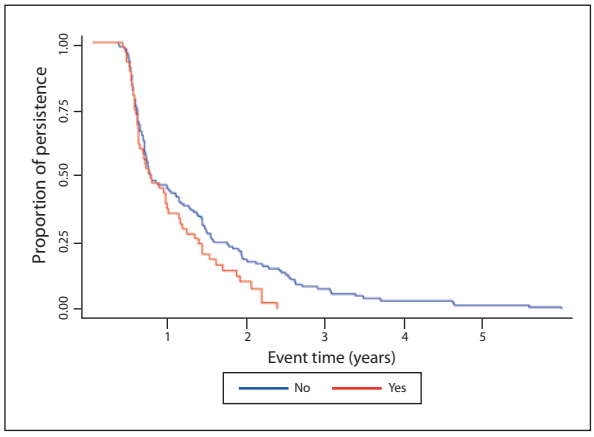
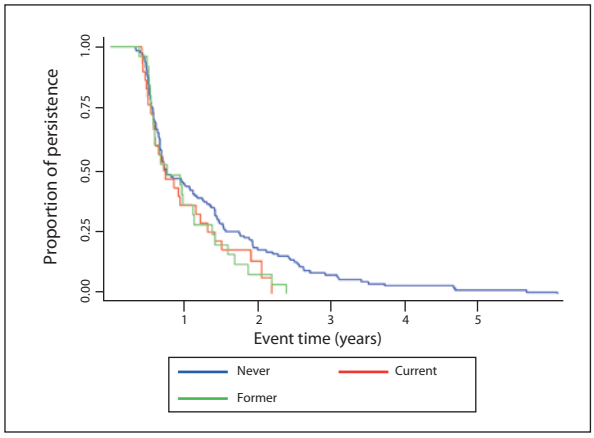


Figure 5. Low grade cervical lesion regression according to smoking habits in women from the Bogotá Cohort

A. Smoking Habit YES or NO



B. Smoking Habit (Never, Current, Former)



We also compared HPV clearance time with cytological regression time. In 96 women (48%), cytological regression and HPV clearance occurred at the same time. In 61 women (31.5%), cytological regression occurred before HPV clearance. In 43 women (21.5%) HPV clearance occurred before cytological regression. Median lesion regression time was 9 months while median HPV clearance time was 12 months.

Discussion

Long term follow-up studies are the best way to study the natural history of HPV infection and the development of cervical lesions because they allow researchers to analyze different risk factors related to the virus, host and environment over time thus resulting in better information about where, when, and how these factors are involved in the process.

In this report we analyzed the roles played by a number of factors in the regression of low grade cervical lesions. Factors included HPV types, viral load and various characteristics of women in a cohort of HPV positive women with abnormal cytology in Bogotá, Colombia. To date, this has been one of the longest running follow-up studies in a population at high risk for cervical cancer.

Our results indicate that women infected with HR HPV types and those with mixed infections (HR/LR) had lower lesion regression rates than women infected with LR types. Women with multiple LR infections had lesion regression rates similar to those women with a single LR infection, while women with multiple HR infections had lesion regression rates similar to those women with a single HR infection. Two follow-up studies, conducted by Nobbenhuis et. al. in 2001 and by Schlecht N et. al. in 2003, estimated rates of progression and regression of different cervical lesions according to HPV status. They showed lower regression rates for LGSIL and ASCUS lesions in women infected with HR types than in women infected with LR types or HPV negative (7,8). Although these results are similar to our results, neither of these studies described the role of mixed infections (HR/LR). Our study showed even lower regression rates for these cases than for women with HR infections.

The biological role that these infections play in the regression or progression of low grade cervical lesions remains unknown. A cohort study done in Brasil observed a higher risk of lesion progression in women infected by multiple HPV viral types than in women infected by a single HPV type. They

Table 2. Risk factors for regression of low grade cervical lesions in women from the Bogotá cohort

Characteristics	No	Crude Model	Multivariate Model
Total	200	HR (95% CI)	HR (95% CI)
Cytological Diagnosis			
ASCUS/AGUS	80	1	1
LSIL	120	**0.777 (0.58-1.04)**	0.820 (0.59-1.12)
Age (years) +			
<=30	77	1	1
>30	123	**1.493 (1.10-.02)**	**1.53 (1.03-2.27)**
Type of Infection			
Low risk (LR)	27	1	1
High risk (HR)	151	**0.501 (0.32-.73)**	**0.526 (0.33-0.84)**
Mixed (HR/LR)	22	**0.375 (0.21-.65)**	**0.378 (0.20-0.69)**
Number of sexual partners during follow-up			
None	167	1	1
1	19	0.760 (0.50-1.18)	0.91 (0.54-1.52)
+1	14	0.657 (0.39-1.16)	0.659 (0.38-1.11)
Parity			
None	13	1	1
1 - 2	111	0.816 (0.45-1.22)	0.827 (0.43-1.56)
3 or more	75	0.812 (0.44-1.21)	0.577 (0.28-1.16)
Missing values	1		
Oral contraceptives			
No	174	1	1
Yes	17	0.699 (0.40-.20)	0.772 (0.43-1.37)
Missing values	9		
IUD			
No	142	1	1
Yes	49	1.094 (0.77-1.53)	1.243 (0.82-1.87)
Missing values	9		
Viral load			
Low	71	1	1
Median	64	0.722 (0.43-1.20)	0.864 (0.50-1.49)
High	65	0.745 (0.49-1.12)	0.849 (0.55-1.30)
Smoking Habit			
Never	135	1	1
At some time	56	**1.37 (0.98-1.91)**	1.251 (0.88-1.77)
- Former smoker	26		
- Current smoker	30		
Missing values	9		

HR = Hazard ratio adjusted for age, cytological ,diagnosis, type of infection, number of sexual partners, parity, oral contraceptives , viral load and smoking habit

Table 3. Risk factors for regression of low grade cervical lesions in women from the Bogotá cohort by age groups

Characteristics	> 30 years Multivariate Model	<= 30 years Multivariate Model
Total	HR (95% CI)	HR (95% CI)
Cytological Diagnosis		
ASCUS/AGUS	1	1
LSIL	0.74 (0.48-1.14)	1.040 (0.61-1.77)
Type of Infection		
Low risk (LR)	1	1
High risk (HR)	0.635 (0.36-1.10)	0.287 (0.09-0.83)
Mixed (HR/LR)	0.432 (0.20-0.91)	0.227 (0.06-0.78)
Number of sexual partners during follow-up		
None	1	1
1	1.191 (0.53-2.67)	0.938 (0.47-1.84)
+1	0.666 (0.32-1.37)	0.864 (0.33-2.23)
Parity		
None	1	1
1 - 2	0.380 (0.12-1.17)	1.276 (0.53-3.03)
3 or more	0.236 (0.07-0.72)	1.344 (0.43-4.13)
Oral contraceptives		
No	1	1
Yes	0.741 (0.22-2.44)	0.818 (0.41-1.62)
IUD		
No	1	
Yes	1.572 (0.89-2.77)	11.01 (0.55-1.87)
Viral load		
Low	1	1
Median	0.86 (0.43-1.73)	0.775 (0.29-2.01)
High	0.81 (0.47-1.39)	0.852 (0.36-1.96)
Smoking habit		
Never	1	1
At some time	1.015 (0.64-1.59)	1.77 (0.98-3.19)

HR = Hazard ratio adjusted for age, cytological ,diagnosis, type of infection, number of sexual partners, parity, oral contraceptives , viral load and smoking habit

also observed that co-infections with HR/LR types incrementally influenced the risk of LSILS (14).

Our present findings indicate that cytological regression was influenced by age: women over 30 had higher lesion regression rates than women younger than 30 years (HR= 1.53, 95% CI 1.03-2.27). Although some reports have shown that increasing age is associated with higher risk of both HPV persistence and development of HGSIL (15-17), previous results from our cohort showed no association (18). Follow-up studies of mild and moderate dysplasias have also shown increased regression risk for low grade cervical lesions in women of older ages compared to regression risks for younger women (19-22). The observations in those studies are in agreement with our observations.

Our age stratified analysis suggests age-dependent association between low grade cervical lesion

regression and certain risk factors. Women aged 30 years or older with 3 or more parities had lower lesion regression rates than nulliparous women. Parity is a risk factor that some studies have shown to be associated with a higher risk of viral persistence and/or HGSIL (23,24). In an initial analysis of our cohort, which was not stratified by age, parity was also associated with lower HPV infection clearance rates compared with nulliparous women (25). Smoking has been moderately associated as a risk factor for high-grade cervical lesions and cervical cancer. However, reports on the role it plays in viral persistence and low-grade cervical lesions are contradictory (16, 26-29), so this question remains unclear. Some researchers consider the risk factors for HPV infection to be the same as those for low grade lesions, but other authors have proposed that these factors are different (26). Our results show that some risk factors associated with low grade lesion regression are different from the risk factors associated with clearance of HPV infections. They are also different from the risk factors associated with high grade cervical lesion progression and cancer. The only associated risk factor which appears throughout the entire carcinogenic process is the presence of HR infections. The majority of studies have shown HR infections to be associated with lower clearance and regression rates and also with higher risk of progression than low risk HPV infections. Discussion is needed regarding interactions between factor characteristics and host features which are important to the behavior and final effects produced by the factor during the carcinogenesis process. Tobacco use is clearly associated with high-grade cervical lesions and cancer, although there is no clear its association with HPV persistence or with the development of low grade lesions. The association between smoking and cervical cancer appears to increase in relation to certain characteristics including dose, duration of habit, intensity of aspiration, number of cigarettes smoked daily, host immune status, hormonal changes and genetic damage caused by carcinogens related to tobacco.

Although cytological regression and HPV clearance occurred at the same time in almost 50% of the women, the median lesion regression was 9 months whereas median time to HPV clearance was 12 months. These results are different from those reported by Nobbenhuis et al., 2001. They reported that HR HPV clearance preceded cytological regression by a mean time of 3 months. These differences are probably due to arbitrary definitions of HPV clearance and cytological regression. In the Nobbenhuis study regression of abnormal cytology was defined as a return to normal cytology (at least two consecutive cervical smears read as normal) and HR HPV clearance was defined when none of the HR HPV types from the previous visit were detected at the next visit. In our study regression of abnormal cytology was defined differently. We defined it as a change from an abnormal diagnosis to normal cytological diagnosis between two consecutive visits. This difference in definitions of cytological regression definitely alters the interpretation of results.

Discrepancies in cytological diagnosis are a principal problem in the analysis of low-grade cervical lesions. In our study expert cytologists read the slides. However, during the period in which we conducted our study, the Bethesda classification was implemented, which could have initially led to some inaccuracies. Our study underlines the importance of biological marker analysis which can provide additional information on what occurs in the cellular environment and indicate(s) which cervical lesions are most likely to progress to HGSIL and cervical cancer.

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