Predictive factors for the detection of CIN II-III in the follow-up of women with CIN I

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Summary

Purpose of investigation: To determine which factors may increase the risk that women diagnosed with CIN I may later develop CIN II-III. *Methods:* A prospective study of 174 women with a grade 1 intraepithelial lesion (CIN I) confirmed by biopsy, with a follow-up time of at least one year. The following factors were studied: age, HPV infection, HPV infection by a high-risk genotype, the HPV genotypes involved, coinfection by several HPV genotypes and duration of follow-up. These factors were correlated with later detection of CIN II-III by biopsy during follow-up. Statistical analysis was performed using SPSS. *Results:* CIN II-III was detected at the follow-up in 24 of 174 women included in the study (13.7%), in four cases by colposcopically directed biopsy and in 20 by LLETZ. Correlation of the factors studied with the incidence of CIN II-III in this group showed that the only statistically significant factors were overall HPV infection and HPV infection by genotypes 31 and 70 (Chi-square and Fisher's test, $p < 0.05$, respectively), while the duration of follow-up came close to statistical significance (Student's test, $p = 0.052$). *Conclusion:* HPV infection and duration of follow-up are predictive factors for the detection of CIN II-III in follow-up care for women with CIN I.

Key words: Papillomavirus; CIN I; CIN II-III; Risk factors; Cervix.

Introduction

Follow-up and treatment of women with a low-grade squamous intraepithelial lesion (SIL) is often complex. On the one hand, these lesions have a low potential – approximately 1% [1] – for becoming malignant, and a risk of progression to CIN II-III of 5-11% [1, 2]. On the other hand, although low-grade SIL is initially detected in cytology and confirmed by colposcopy and directed biopsy, high-grade SIL is not infrequently found in subsequent follow-up testing [3]. According to one study [4], occult or de novo high-grade SIL was found in 23-55% of cases. For this reason, the management of low-grade SIL in young women tends to be very conservative, since it is believed that low-grade SIL is simply an indicator of HPV infection, and since this infection is often transitory [5, 6] the lesion will subside. After age 40, however, treatment tends to be more aggressive and the transformation zone is excised, since it is assumed that in such cases the infection is persistent and the lesion is less likely to resolve spontaneously [7]. In such cases, there is increased risk that it may develop into high-grade SIL, or that there may be occult high-grade SIL.

The objective of this study was to determine which factors may increase the risk that women with low-grade SIL will present later with high-grade SIL, in order to develop objective parameters that will facilitate identification of those patients who should be treated

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Material and Methods

This was a prospective study of a group of women who were referred by their primary care physicians for abnormal cytology results. All the patients underwent repeat cytology, human papillomavirus (HPV) testing, and colposcopy. In cases of atypical colposcopy results, they also underwent a biopsy. For the study group, 174 women were chosen who had a grade 1 intraepithelial lesions (CIN I) confirmed by colposcopically guided biopsy. All women had been followed for at least one year.

The following factors were studied in the study group: age, HPV infection, HPV infection by a high-risk genotype, the HPV genotypes involved, co-infection by several HPV genotypes and duration of follow-up. These factors were correlated with detection of CIN II-III in a colposcopically directed biopsy, limited excision of the transformation zone (LETZ), or cone biopsy performed during the follow-up period.

LETZ was indicated in cases of persistent CIN I for a period longer than one year.

Colposcopy: Colposcopy was performed following application of 2% acetic acid solution. The classification proposed by the International Federation of Cervical Pathology and Colposcopy (FCPC) in Rome in 1990 [8] was used.

Human papillomavirus detection and genotyping: Samples were obtained using a swab applied to the surface of the cervix. The sample was then dissolved in 0.5 ml saline solution (pH 7.2) and HPV genotyping was performed using a new microarray-based molecular technique (Genomica®) that permits detection of the 35 most prevalent genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, 89).

Statistical analysis

Statistical analysis was carried out using the SPSS program (version 12.0), Fisher's exact test, Chi-square and the Student's t-test. The level of statistical significance was established at $p < 0.05$.

Results

The patients included in the study were 174 women. Their average age was 33.89 years, with an age range between 16 and 62 years. In 134 cases, the reason for referral was low-grade SIL. Reasons for referral in the remaining cases are shown in Table 1. Mosaic pattern was the abnormal colposcopic image most frequently detected, accounting for 61 cases (35%), followed by leukoplakia in 32 cases (18.3%) and punctation in 17 cases (9.7%). Colposcopically guided biopsies were performed in all women of the group and diagnosis of CIN I was confirmed in the all the patients. HPV infection was detected in 119 (68.3%) women using a microarray-based molecular technique (Genomica®). High-risk or probable high-risk genotypes were present in 84% of cases. The most frequently isolated genotype was 16, followed by 53 and 51 (Table 2). Infection by more than one HPV genotype was found in 63 (36.2%) women.

During follow-up, 47 women with abnormal colposcopic images underwent a second colposcopically guided biopsy. In 37 cases the results of the second biopsy showed CIN I, in four cases CIN II-III, and in six cases the results were negative. In 101 cases, treatment was indicated. LETZ was performed in 87 cases and conization in eight, and six women underwent LETZ followed by cone biopsy, four of them because the surgical margin was involved.

CIN II-III was detected in 24 women (13.7%), in four cases by colposcopically directed biopsy and 20 by LETZ. Of the 24 women, 18 were infected by high-risk HPV genotypes, but genotype 16 was detected in only six women and genotype 18 in one case (Table 2).

The mean duration of follow-up was 712 (SD 392.25) days.

Statistical analysis was performed to correlate detection of CIN II-III with the variables studied (Table 3). Only HPV infection ($p = 0.03$, Chi-square test), infection by HPV genotype 31 ($p = 0.02$, Fisher's exact test) and infection by type 70 ($p = 0.035$, Fisher's exact test) were statistically significant. Association with HPV genotype 53 came close to statistical significance ($p = 0.065$; Fisher's exact test). Another parameter of near-statistical significance was the duration of follow-up. The longer the follow-up period, the greater the risk of developing CIN II-II ($p = 0.052$; Student's t-test).

The other parameters studied – age, the presence of high-risk HPV infection, and co-infection by various HPV genotypes – did not significantly increase the risk of detecting CIN II-III.

Table 1. — *Initial cytology results of the 174 women included in the study and percentage of HPV infections in each cytologic diagnosis*.

Cytoloty results	Number	Percentage	HPV infection
Low-grade SIL	149	85.6%	67.1%
AGUS	1	0.5%	0%
ASCUS	24	13.7%	66.6%

Table 2. — *Genotypes isolated in the 174 women with an initial diagnosis of CIN I and in the 24 women in whom CIN II-III was detected*.

	Total group		*24 women with CIN II-III*	
Genotype	Number	Percentage*	Number	Percentage*
16	32	18.3%	6	25%
53	27	15.5%	7	29.1%
51	26	14.9%	4	16.6%
66	20	11.5%	2	8.3%
31	13	7.4%	5	20.8%
33	10	5.7%	3	12.5%
56	10	5.7%		
58	9	5.1%		
52	8	4.6%		
61	8	4.6%	1	4.1%
84	8	4,6%	1	4.1%
18	7	4%	1	4.1%
6	6	3.4%	2	8.3%
70	6	3.4%	3	12.5%
81	6	3.4%		
11	4	2.2%		

*The percentages do not add up to 100 because 36.2% of the women were infected by more than 1 HPV genotype.

Table 3. — *Statistical analysis of the possible factors correlated with CIN II-III detection*.

Factors	CIN III positive[1]	CIN III negative[2]	Test	p value
HPV Infection	21/24	98/150	Chi-square	0.03
inf by a high-risk genotype*	18/21	84/98	Fisher's	0.3
Genotype 6 inf	2/24	4/150	Fisher's	0.19
Genotype 11 inf	0/24	4/150	Fisher's	1
Genotype 16 inf	6/24	26/150	Fisher's	0.39
Genotype 18 inf	1/24	6/150	Fisher's	1
Genotype 31 inf	5/24	8/150	Fisher's	0.02
Genotype 33 inf	2/24	8/150	Fisher's	0.63
Genotype 35 inf	0/24	2/150	Fisher's	1
Genotype 39 inf	0/24	1/150	Fisher's	1
Genotype 42 inf	0/24	5/150	Fisher's	1
Genotype 44 inf	1/24	3/150	Fisher's	0.45
Genotype 45 inf	0/24	1/150	Fisher's	1
Genotype 51 inf	4/24	22/150	Fisher's	0.76
Genotype 52 inf	0/24	8/150	Fisher's	0.6
Genotype 53 inf	7/24	20/150	Fisher's	0.065
Genotype 54 inf	1/24	3/150	Fisher's	0.45
Genotype 56 inf	0/24	10/150	Fisher's	0.36
Genotype 58 inf	0/24	9/150	Fisher's	0.61
Genotype 59 inf	0/24	1/150	Fisher's	1
Genotype 61 inf	1/24	7/150	Fisher's	1
Genotype 62 inf	0/24	1/150	Fisher's	1
Genotype 64 inf	0/24	1/150	Fisher's	1
Genotype 66 inf	2/24	18/150	Fisher's	1
Genotype 68 inf	0/24	1/150	Fisher's	1
Genotype 70 inf	3/24	3/150	Fisher's	0.035
Genotype 73 inf	0/24	2/150	Fisher's	1
Genotype 81 inf	0/24	6/150	Fisher's	1
Genotype 82 inf	1/24	1/150	Fisher's	0.25
Genotype 84 inf	1/24	7/150	Fisher's	1
Coinfección**	12/21	51/98	Chi-square	0.64
Mean age	32.25 years	34.15 years	Student's-t	0.39
Mean follow-up	861 days	690.5 days	Student's-t	0.052

inf = infection.
* only cases with high-risk genotype infection.
** only cases with coinfection.
[1] group with progression to CIN II-III (number of cases/total).
[2] group with no progression to CIN II-III (number of cases/total).

Discussion

According to the literature, the prevalence of HPV infection in women with cytology results showing low-grade SIL ranges between 52.5% and 76.1% [9, 10]. In our study, the prevalence of this infection was 67.1%. Another study reports that CIN I detected by biopsy is more frequently associated with HPV infection in as many as 93% of cases [3]; this percentage was considerably lower in our study, 68.3%.

It is noteworthy that 85.7% of the genotypes infecting women with CIN I were high-risk types, and by contrast with other studies of women with CIN II-III in which by far the dominant HPV genotype was 16 [9, 11], in our study there was no great difference in the frequency of genotypes 16, 51 and 53. Genotypes 6 and 11, which are more closely associated with transitory HPV infection, represented a very small percentage of all genotypes detected in our study.

The percentage of women treated for CIN I who were later diagnosed with CIN II-III was also similar to the tendency observed in the literature, between 23% and 55% [4]. In our study, this was the case for 24 of the 101 women (23.7%).

Age was not a statistically significant risk factor for CIN II-III in our cases. This places in question the recommendation that women over the age of 40 are at higher risk and should be treated for CIN I [7]. The average age of the women with CIN I and those with CIN II-III was quite similar, 34 and 32 years, respectively. Al-Nourhji *et al*. [12] also observed that women whose cytology results showed low-grade SIL and were later diagnosed with CIN II-III confirmed by biopsy were younger than those not diagnosed with CIN II-III (an average age of 25 as opposed to 30 years). Age, therefore, does not eliminate the risk of developing CIN II-III for women with a cytology diagnosis of low-grade SIL. For these reasons, we would recommend follow-up colposcopy, and in the event of an abnormal result, a biopsy to establish the diagnosis. In our study, during follow-up colposcopy and colposcopically directed biopsy detected four (16.6%) of the 24 cases of CIN II-III.

The only risk factors for detection of CIN II-III were overall HPV infection, and HPV infection by genotypes that are not, oddly, among the most prevalent: one that is probable high-risk, 70, and 31, a high-risk genotype. Infection of high-risk genotypes 16 and 18 were not correlated in the study with risk of detecting CIN II-III in women diagnosed with CIN I.

The duration of follow-up is also important. Though not statistically significant, it came close ($p = 0.052$). The longer the follow-up period, the greater the risk, probably because CIN I is persistent.

In conclusion, age is a relatively unimportant factor in deciding which patients with CIN I should be treated. HPV testing and genotyping may be useful in deciding whether or not to treat, not as an isolated factor but in conjunction with other clinical parameters (cytology, colposcopy and biopsy results) and the persistence of the lesion. Infection of high-risk genotypes other than 16 and 18 are correlated in the study with risk of detecting CIN II-III.

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References

[1] Ostor A.G.: "Natural history of cervical intraepithelial neoplasia. A critical view". *Int. J. Gynecol. Pathol*., 1993, *12,* 186.
[2] Syrjänen K.J.: "Spontaneous evolution of intraepithelial lesions according to the grade and type of the implicated HPV". *Eur. J. Obstet. Gynecol. Reprod. Biol*., 1996, *65*, 45.
[3] Gonzalez-Bosquet E., Esteva C., Muñoz-Almagro C., Lailla J.M.: "Comparison of conization and limited excision of the transformation zone (LLETZ) in the treatment of squamous intraepithelial lesion (SIL) of the uterine cervix". *Eur. J. Gynaecol. Oncol*., 2008, *29*, 123.
[4] Alonso I., Torné A., Puig-Tintoré L.M., Esteve R., Quinto L., Garcia S. *et al*.: "High-risk cervical epithelial neoplasia grade 1 treated by loop electrosurgical excision: follow-up and value of HPV testing". *Am. J. Obstet. Gynecol*., 2007, *197,* 359.
[5] Denis F., Hanz S., Alain S.: "Clearance, persistence and recurrence of HPV infection". *Gynecol. Obstet. Fertil*., 2008, *36,* 430.
[6] Rodríguez A.C., Schiffman M., Herrero R., Wacholder S., Hildesheim A., Castle P.E. *et al*.: "Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections". *J. Natl. Cancer Inst*., 2008, *100,* 513.
[7] Spitzer M., Apgar B., Brotzman G.: "Management of histologic abnormalities of the cervix". *Am. Fam. Physician*, 2006, *73*, 105.
[8] Dexeus S., Cararach M., Dexeus D.: "The role of colposcopy in modern gynecology". *Eur. J. Gynaecol. Oncol*., 2002, *23,* 269.
[9] Gonzalez-Bosquet E., Almagro M.M., Mora I., Suñol M., Callejo J., Lailla J.M.: "Prevalence of human papilloma virus infection of the uterine cervix in women with abnormal cervical cytology". *Eur. J. Gynaecol. Oncol*., 2006, *27*, 135.
[10] Levert M., Clavel C., Graesslin O., Masure M., Birembaut P., Quereuy C., Gabriel R. *et al*.: "Human papillomavirus typing in routine cervical smears. Result from a series of 3,778 patients". *Gynecol. Obstet. Fértil*., 2000, *28,* 722.
[11] Gonzalez-Bosquet E., Esteva C., Muñoz-Almagro C., Ferrer P., Pérez M., Lailla J.M.: "Identification of vaccine human papillomavirus genotypes in squamous intraepithelial lesions (CIN2-3)". *Gynecol. Oncol*., 2008, *111,* 9.
[12] Al-Nourhji O., Beckmann M.J., Markwell S.J., Massad L.S.: "Pathology correlates of a Papanicolaou diagnosis of low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion". *Cancer,* 2008, *114,* 469.

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