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Predicting Factors for High-Grade Cervical Dysplasia in Women With Low-Grade Cervical Cytology and Nonvisible Squamocolumnar Junction

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Abstract

Objective: To assess the risk of developing high-grade cervical dysplasia among women with low-grade cervical cytology and nonvisible squamocolumnar junction (SCJ) at colposcopic examination. **Methods:** Data of consecutive women with low-grade intraepithelial lesion(≤LSIL) undergoing colposcopic examination, which was unsatisfactory (due to the lack of the visualization of the entire SCJ), were retrospectively reviewed. The risk of developing high-grade cervical intraepithelial neoplasia (CIN2+) was assessed using Kaplan-Meier and Cox models. **Results:** Data of 86 women were retrieved. Mean (standard deviation [SD]) age was 36.3 (13.4) years. A total of 71 (82.5%) patients had high-risk human papillomavirus (HR-HPV) at the time of diagnosis. Among the 63 patients undergoing repetition of HPV testing, 15 (24%) and 48 (76%) women had positive and negative tests for HR-HPV at 12 months, respectively. We observed that 5 (33%) of 15 patients with HPV persistence developed CIN2+, while only 1 (2%) patient of 48 patients without HPV persistence developed CIN2+ (odds ratio [OR]: 23.5; 95% confidence interval [CI]: 2.46-223.7; $P < .001$). The length of HR-HPV persistence correlated with an increased risk of developing CIN2+ ($P < .001$; P for trend). High-risk HPV persistence is the only factor predicting for CIN2+ (hazard ratio: 3.19; 95% CI: 1.55-6.57; $P = .002$). **Conclusions:** High-risk HPV persistence predicts the risk of developing CIN2+ in patients with unsatisfactory colposcopic examination. Further studies are warranted in order to implement the use of HPV testing in patients with unsatisfactory colposcopy.

Keywords

HPV, high-grade dysplasia, unsatisfactory colposcopy, CIN

Introduction

Colposcopy allows identification of abnormal areas, including low- and high-grade cervical intraepithelial neoplasia (CIN1 and CIN2+, respectively).[1-3] Lesions are generally located in the transformational zone (TZ) close to the squamocolumnar junction (SCJ).[4] The evaluation of TZ and SCJ is paramount to assess the status of the uterine cervix, in case of human papillomavirus (HPV) infection. Therefore, the visualization of the SCJ is essential to assess the quality of colposcopic examination.

According to the old nomenclature, the lack of the visualization of the entire SCJ defined a colposcopy as "unsatisfactory." Although the new nomenclature refused the term "unsatisfactory colposcopy," the not complete visualization of the SCJ is still a concern.

It is estimated that the entire SCJ is not visualized in about 10% to 20% of colposcopic examinations, thus making these evaluation less precise than in case in which the SCJ is fully visualized. Several investigators recommended cone biopsy in those cases.[5,6]

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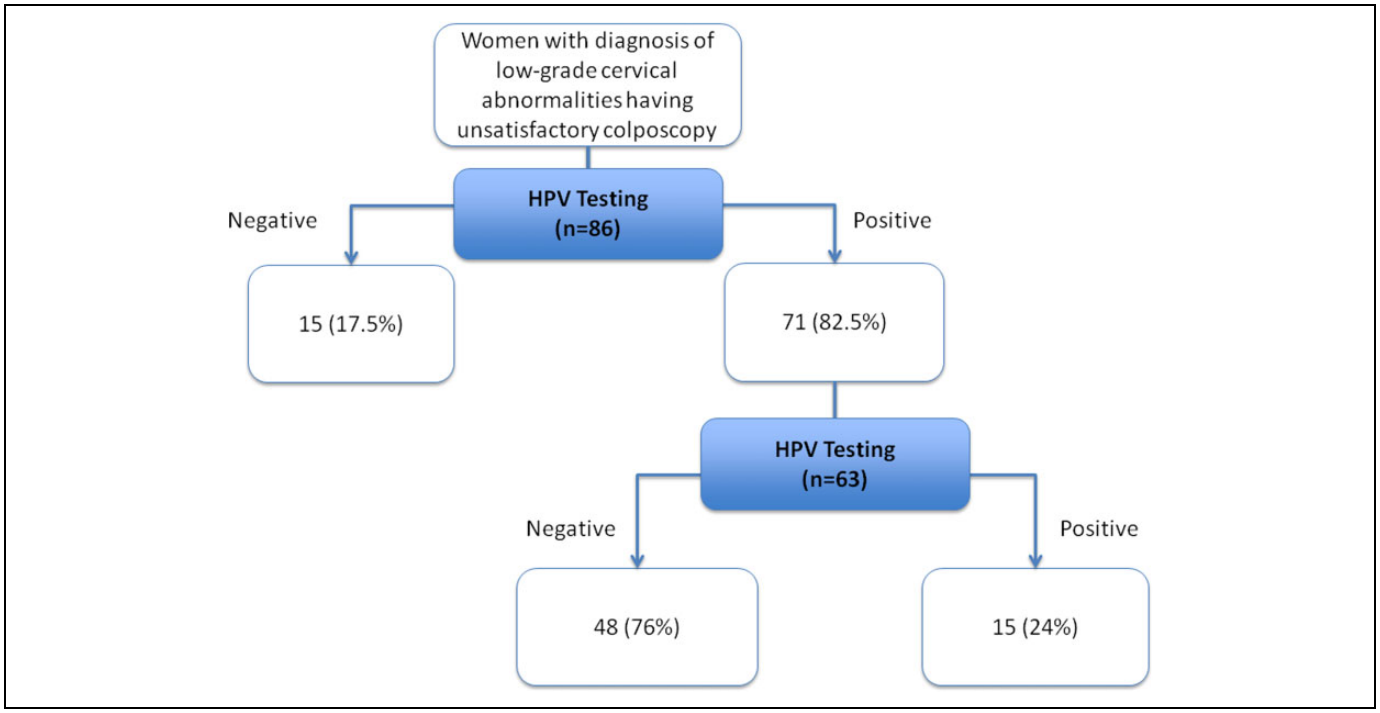

Figure 1. Study design.

In the present study, we sought to assess the risk of developing high-grade lesions (CIN2+) among women with low-grade cervical cytology and unsatisfactory colposcopic examination. Additionally, we aimed to identify factors predicting CIN2+ among this cluster of women.

Methods

We retrospectively reviewed records of all consecutive women undergoing HPV DNA testing between 2005 and 2015 at Gynecologic Oncology Unit of National Cancer Institute (Milan, Italy). The institutional review board approved this study.

Data of all patients included were abstracted from a dedicated database including all women undergoing HPV testing. Data were included prospectively into the database. All patients included gave written informed consent for the use of personal information for health research. Demographic details, data about HPV type(s) detected, and data on treatment for the occurrence of genital precancerous and cancerous condition were retrospectively reviewed. Low-grade cervical cytology included low-grade intraepithelial lesion (LSIL) and atypical squamous cells of undetermined significance. Unsatisfactory colposcopy was defined according to the old nomenclature realized by the International Federation for Cervical Pathology and Colposcopy (IFCPC).[4] Human papillomavirus types were considered as high risk (HR) according to the data of the International Agency for Research on Cancer.[7] High-risk HPV types classified as type 1 carcinogens included HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66. Figure 1 shows the flow of patients into the study design.

Age was categorized as the follows: age less than 30 years, between 30 and 50 years, and more than 50 years. On the basis of body mass index (BMI), women were categorized into normal weight (BMI less than 25 kg/m^2), overweight (BMI more than 25 less than 30 kg/m^2), and obese (BMI more than 30 kg/m^2). Tobacco use was defined as a dichotomous variable (yes vs no). Exclusion criteria were as follows: (1) age younger than 18 years, (2) withdrawal of consent, (3) history of HPV-related lesions (including cervical, vaginal, and vulvar dysplasia related to HPV infections) within the past 5 years, (4) pregnancy, and (5) previous hysterectomy. According to our institutional protocol, patients were evaluated colposcopically in outpatient clinic at 6 months after primary colposcopic examination. A dedicated team of gynecologic oncologists performed all gynecological and colposcopic examinations. Details regarding colposcopic examination, HPV testing, and HPV analysis are reported elsewhere.[8] The genotyping results were ranked hierarchically according to cancer risk: HPV 16 > HPV 18 > other carcinogenic HPV. Generally, HPV testing was performed at the time of first colposcopic examination. In our department, no specific guidelines suggested the repetition of HPV testing. However, the majority of women with the diagnosis of HR-HPV had another HPV testing at 6 or 12 months after primary examination.[8] Human papillomavirus infection persistence was defined as the persistence of an HPV type, detected at 2 consecutive time points, in accordance with the definition included in the meta-analysis of Koshiol et al.[9]

Data are summarized using basic descriptive statistics. The risk of developing CIN2+ was evaluated using Kaplan-Meir and Cox models. Hazard ratio and 95% confidence intervals (CIs) were

Table 1. Baseline Patient Characteristics.

Characteristics	N = 86
Age, median (IQR), years	32.5 (26, 45.2)
BMI, median (IQR), kg/m^2	22 (21, 24.2)
Tobacco use, n (%)	
No	68 (79%)
Yes	18 (21%)
Positivity for HPV at diagnosis, n (%)	71 (82.5%)
Positivity for HPV 16/18 at diagnosis, n (%)	37 (43%)
Positivity for HPV other than 16/18 at diagnosis, n (%)	34 (39.5%)
Patients undergoing another HPV testing after primary examination	63 (73.2%)
Persistence of HPV, n (%)	15 (17.4%)
Time of HPV persistence, median (IQR), months	6 (6-18)
Follow-up, median (IQR), months	29 (23-60)

Abbreviations: BMI, body mass index; HPV, human papillomavirus; IQR, interquartile range; SD, standard deviation.

calculated for each comparison. Univariate and multivariate analyses were performed when appropriate. All covariates with a P value less than .10, based on univariate analysis, were included in the multivariate model. Duration of follow-up was counted from date of LSIL detection and of CIN2+ diagnosis or date of last follow-up. Statistical analyses were performed using GraphPad Prism version 6.0 for Mac (GraphPad Software, San Diego, California) and IBM-Microsoft SPSS (SPSS Statistics. International Business Machines Corporation IBM. 2013. Armonk) version 20.0 for Mac.

Results

Data of 86 women diagnosed with low-grade cervical cytology were retrieved. Table 1 reports baseline characteristics of the study population. Mean (standard deviation [SD]) age was 36.5 (12.1) years. Seventy-one (82.5%) patients had HR-HPV at the time of diagnosis. Among the 63 patients undergoing repetition of HPV testing, 15 (24%) and 48 (76%) women had positive and negative tests for HR-HPV at 12 months, respectively. After a median follow-up of 29 (75% interquartile range: 23-60) months, 8 (9.3%) patients had conization due to CIN2+ (data not shown). No invasive cancer was diagnosed.

Table 2 reports factors predicting the risk of developing CIN2+. Considering factors predicting the risk of developing CIN2+ over time (median follow-up: 29 months), HR-HPV persistence correlated with the risk of developing CIN2+ (hazard ratio: 3.19; 95% CI: 1.55-6.57; $P = .002$). Similarly, length of HR-HPV persistence correlated with an increased risk of developing CIN2+ ($P < .001$; P for trend—data not shown). No other factors correlated with the risk of developing CIN2+. However, although it did not reach statistical significance, we observed that women with overweight (BMI between 25 and 30 kg/m^2) experienced a higher risk of developing CIN2+ (hazard ratio: 2.72; 95% CI: 0.54-13.6; $P = .22$).

Looking at the crude number of CIN2+ diagnosed, we observed that 5 (33%) of 15 patients with HPV persistence at 12 months developed CIN2+, while only 1 (2%) of 48 patients without HPV persistence developed CIN2+ (odds ratio: 23.5; 95% CI: 2.46-223.7; $P < .001$; Table 2). Type-specific HR-HPV infection did not influence the risk of developing CIN2+. In fact, comparing women having an infection from HPV 16, HPV 18, and other HR-HPV types, we observed a similar risk at 10-year follow-up ($P = .89$, log-rank test). Similarly, no differences were observed comparing HPV 16 and HPV 18 ($P = .61$, log-rank test).

Table 2. Factors Influencing the Risk of Developing CIN2+.[a]

Characteristics	Number of Patients	Number of CIN2+	Univariate Analysis, HR (95% CI)	P Value
Age, years				
Less than 30 years	33	4 (12.1%)	1.54 (0.38-6.19)	.53
Between 30 and 50 years	39	2 (5.1%)	0.42 (0.08-2.09)	.29
More than 50 years	14	2 (14.3%)	1.66 (0.33-8.23)	.53
BMI, kg/m^2				
Normal weight	54	4 (7.4%)	0.78 (0.24-5.59)	.12
Overweight	20	3 (15%)	2.72 (0.54-13.6)	.22
Obese	13	1 (7.7%)	0.04 (0.00-488.3)	.50
Tobacco use				.52
No	68	7 (10.3%)	Reference	
Yes	18	1 (5.5%)	0.50 (0.06-4.08)	
Positivity for HR-HPV at diagnosis				.79
No	15	1 (6.7%)	Reference	
Yes	71	7 (9.8%)	1.32 (0.16-10.8)	
Positivity for HPV 16/18 at diagnosis				.76
No	49	5 (10.2%)	Reference	
Yes	37	3 (8.1%)	0.80 (0.19-3.37)	
Positivity for HR-HPV other than 16/18 at diagnosis				.73
No	52	4 (7.7%)	Reference	
Yes	34	4 (11.7%)	0.73 (0.31-5.11)	
HR-HPV persistence				**.002**
No	48	1 (2%)	Reference	
Yes	15	5 (33%)	**3.19 (1.55-6.57)**	

Abbreviations: BMI, body mass index; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HR, hazard ratio; HR-HPV, high-risk human papillomavirus.
[a]Variables characterized by P value < .10 are presented in bold characters.

Discussion

The present study evaluated outcomes of women diagnosed with low-grade cervical cytology and characterized by an unsatisfactory colposcopic examination, thus reporting a number of noteworthy findings. First, we observed that women diagnosed with low-grade cervical cytology have a risk of about 30% of developing CIN2+ in case of positivity for HR-HPV, while in the absence of HR-HPV infection, this risk is negligible. Second, persistence of HR-HPV is the main factor predicting this risk. Third, type-specific HPV infection does not influence

patient outcomes. Additionally, in our series, other baseline factors (such as age or BMI) do not impact on the risk of developing CIN2+.

In the present study, we analyzed a particular subset of patients for whom colposcopic examination was not able to define the entire SCJ. According to the nomenclature introduced in 2002, these colposcopic examinations are defined as "unsatisfactory." On the other hand, colposcopy was defined "satisfactory" in case of complete visualization of the SCJ. In 2011, the new nomenclature realized by the IFCPC replaced the terms "satisfactory colposcopy" and "unsatisfactory colposcopy" with different degree of SCJ visibility. The term "unsatisfactory colposcopy" was abandoned because it has the connotation of an inadequate examination that needs to be repeated.[4]

Recently, an investigation published by our study group evaluated the risk of developing CIN2+ in consecutive 212 women diagnosed with high-risk HPV types with negative cytology results. We observed that HPV persistence correlated with both LSIL/CIN1+ ($P < .001$) and CIN2+ ($P < .001$) in univariate analyses. Moreover, a 6-month increase in HPV persistence was associated with increased risk of developing LSIL/CIN1+ ($P = .010$) and CIN2+ ($P = .012$) in multivariate analyses.[10] The data reported in this previous investigation are in agreement with those reported in the present study.

Although several studies investigated the rate of occult CIN2+ diagnosed in women with high-risk cytology and unsatisfactory colposcopy,[11-14] the present study is the first investigating the risk of developing CIN2+ over time in women for whom the trigger pap smear indicated the presence of low-grade abnormalities. Our data will be useful during patients' counseling. Obviously, our findings have to be confirmed by larger prospective investigations.

Interestingly, our study suggested that there was no difference between HPV 16 to HPV 18 versus other HR-HPV types in predicting the risk of developing CIN2+. We observed that in a 10-year follow-up, there was no difference between different HPV types involved. This finding is in contrast with other results published in the literature.[15] In fact, accumulating evidence suggested that HPV 16 and HPV 18 correlate with a greater risk of developing cervical dysplasia in comparison with other HPV types.[15] However, the relative small sample size of the 2 subgroups of patients (HPV 16-HPV 18 vs other HR-HPV types) might influence this finding.

The inherent biases of the single-center retrospective study design are the main weaknesses of the present study. The main strength of the present investigation is represented by the potential clinical implication of our results. In fact, our data might be useful for patients' counseling and scheduling their follow-up. Four points of the present investigation deserve to be addressed: (1) we observed the patients diagnosed with a low-risk cervical cytology experienced a relative high risk of developing CIN2+, in case of HR-HPV persistence. However, owing to the small sample size and the relative low power of the study, these data have to be confirmed in other large investigations. (2) Our experience might not reflect the common practice since our study population is a selected group of patients referred into a National Cancer Center, instead of territorial centers. (3) In our analysis, type-specific HPV types are not associated with the risk of developing CIN2+. Other experiences suggested that type-specific HPV type(s) correlates with patient outcomes.[8,13,14] (4) Smoking history might impact on patient outcomes. In the present study, tobacco use was defined as a dichotomous variable (yes vs no). We might speculate that magnitude of tobacco use as well as length of smoking might influence how this variable impact on our results.

In conclusion, the present study reported outcomes of women who had unsatisfactory colposcopy after the diagnosis of low-grade cervical cytological abnormalities. Our analysis suggested that HR-HPV persistence is the main factor predicting for the occurrence of CIN2+. In case of HR-HPV persistence, the risk of developing CIN2+ is not negligible. Further prospective studies are warranted in order to improve treatments of women with unsatisfactory colposcopy.

Authors' Note

Giorgio Bogani and Claudia Lombardo contributed to study design, data analysis, and manuscript writing. Francesca Taverna, Domenica Lorusso, and Francesco Raspagliesi contributed to study design, study planning, and manuscript writing. Antonino Ditto, Fabio Martinelli, and Mauro Signorelli contributed to study design, study conduct, and manuscript writing. Valentina Chiappa and Umberto Leone Roberti Maggiore contributed to data analysis and manuscript writing. Lavinia Mosca and Ilaria Sabatucci contributed to data collection and manuscript writing. Cono Scaffa contributed to data collection, study conduct, and manuscript writing.

Declaration of Conflicting Interests

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