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Original Article

Age-specific predictors of cervical dysplasia recurrence after primary conization: analysis of 3,212 women

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ABSTRACT

Objective: This study aimed to identify predictors of recurrence/persistence of cervical intraepithelial neoplasia grade 2+ (CIN2+) lesion (r-CIN2+) after primary conization.
Methods: Retrospective analysis involving all consecutive women having conization for CIN2+ between 1998 and 2018. The risk of r-CIN2+ was assessed using Kaplan-Meier and Cox models.
Results: Data of 3,212 women were retrospectively identified. After a mean follow-up of 47 (±22.2) months, 112 (3.5%) patients developed r-CIN2+. Mean time interval between prior conization and diagnosis of r-CIN2+ was 26.2 (±13.2) months. Via multivariate analysis, presence of high-risk human papillomavirus (HPV) types at the time of CIN2+ diagnosis, hazard ratio (HR)=3.40 (95% confidence interval [CI]=1.66–6.95) for HPV16/18 and HR=2.59 (95% CI=1.21–5.55) for HPV types other than 16/18, positive margins at primary conization, HR=4.11 (95% CI=2.04–8.26) and HPV persistence after conization, HR=16.69 (95% CI=8.20–33.9), correlated with r-CIN2+, independently. Considering age-specific HPV types distribution, we observed that HPV16/18 infection correlated to an increased risk of r-CIN2+ only in young women (aged ≤25 years; p=0.031, log-rank test); while in the older population (>25 years) HPV type(s) involved had not impact on r-CIN2+ risk (p>0.200, log-rank test).
Conclusion: HPV persistence is the main factor predicting r-CIN2+. Infection from HPV16/18 has a detrimental effect in young women, thus highlighting the need of implementing vaccination against HPV in this population. Further prospective studies are warranted for tailoring clinical decision-making for post-conization follow-up on the basis of risk factors.

Keywords: Human Papillomavirus 16; Conization; Papillomavirus Infections; Squamous Intraepithelial Lesions of the Cervix

INTRODUCTION

Cervical cancer represents one of the most important issues worldwide. The estimated incidence in Europe is 10.6 per 100,000 [1]. Cervical cancer represents a source of ongoing concern for the health care since it correlates with a high mortality rate. And it is one of the most preventable type of cancer because it develops over a long time [1].

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Author Contributions
Conceptualization: B.G., R.F.; Data curation: P.C., C.V., L.S.; Formal analysis: P.C.; Investigation: P.C., M.F.; Methodology: C.V.; Project administration: B.G.; Supervision: R.F.; Validation: D.A.; Writing - original draft: B.G.; Writing - review & editing: P.C., C.V., M.F., L.S., D.A., R.F.

Human papillomavirus (HPV) might cause cervical abnormalities and cancer [2]. Generally, persistent HPV infection might cause cervical dysplasia (also known as cervical intraepithelial neoplasia [CIN]) that might potentially evolve in cancer. Although the majority of women having HPV infection never develop CIN or cancer, a relatively large number of women is at risk of developing CIN. Women with CIN who had appropriate follow-up and treatments are at low-risk of developing cervical cancer [3]. However, recurrent CIN is a risk factor for developing cervical cancer. Additionally, re-treatment for recurrent CIN is associated with fertility and obstetrical issues in women who wish to preserve their childbearing potential.

Owing to the importance of identifying factor predicting recurrence, several studies aimed to assess potential predictors of CIN recurrence [4-8]. Positive cervical margins, down regulation of the immune system, smoking and HPV persistence after treatment are strongly associated with the risk of CIN recurrence [4-8]. In particular, HPV persistence is still considered the strongest factor associated with CIN recurrence [5-8]. Interestingly, although several investigations highlighted that various HPV types have different impact on the risk of developing CIN, no studies evaluate the role of type-specific HPV infection in predicting CIN recurrence. Here, we sought to investigate whether type-specific HPV infection(s) might play a role in the risk of developing recurrent CIN. Additionally, we aimed to identify risk factors of recurrent CIN along a large group of patients having conization for CIN.

MATERIALS AND METHODS

This is a retrospective study evaluating the risk of cervical dysplasia recurrence among women having conization. The Institutional Review Board of National Cancer Institute—Milan approved this study (IRB 68/12). We retrospectively reviewed records of all consecutive women undergoing conization between 1998 and 2018 at Gynecologic Oncology Unit of National Cancer Institute—Milan, Italy. Data of women undergoing conization for cervical intraepithelial neoplasia 2+ (CIN2+) were included in a dedicated database. All patients included gave written informed consent for the use of personal information for health research. Part of these data were included in our previous publications of our study group for different analysis [6,8].

Primary endpoint measure was to identify predictors for cervical dysplasia recurrence. Demographic details, data about HPV type(s) detected as well as data on treatment for the occurrence of cervical dysplasia were retrospectively reviewed. HPV types were considered as high-risk according to the data of the International Agency for Research on Cancer (IARC) [9]. Inclusion criteria were: 1) conization due to CIN2+ and 2) age ≥18 years. Exclusion criteria were: 1) withdrawal of consent; 2) presence of invasive genital cancer at diagnosis; and 3) ongoing pregnancy.

Data of patients having second conization following a prior conization executed in another center were not included in the present analysis. During the study period different expert surgeons performed all the procedures. No differences were present among the facilities available for patients' care and the referral pattern. Same surgical technique, which was laser conization, was used in all cases. Details of surgical treatment are reported elsewhere [6,8].

According to our institutional protocol, patients were evaluated colposcopically in outpatient clinic at 3 (in case of positive margins)–6 (in case of negative margins) months after primary conization. A dedicated team of gynecologic oncologists performed all gynecological and

colposcopic examinations following conization. Details of follow-up schedule and examination were reported elsewhere [6,8]. Briefly, patients had a follow-up scheduled every 6 months including Pap-smear, colposcopy and colposcopic-guided biopsy if clinically indicated, for the first 2 years, and annually thereafter. Generally, HPV testing was performed at the first examination after conization in patients with documented HPV infections. Persistence of HPV infection is defined as the persistence of HPV detected at the first clinical examination following conization (generally at 6 months). Details regarding HPV testing are reported elsewhere [6,8]. For the purpose of this study we investigated the presence of HPV (yes vs. no) and type of HPV involved (HPV16/18, other high-risk HPV types and other no high-risk HPV types) at the time of diagnosis of CIN2 + (i.e., before conization). HPV types were classified as high-risk according to the IARC. Coinfections referred to the presence of multiple HPV types in a patient. Persistence/recurrence (r-CIN2+) was defined as the emergence of CIN2+ lesion after the initial diagnosis of CIN2+. Persistence of cervical dysplasia was defined by the diagnosis of CIN2+ at the first evaluation following conization; while, recurrence had at least one negative examination between conization and the diagnosis of r-CIN2+.

Data are summarized using basic descriptive statistics. The risk of developing r-CIN2+ was evaluated using Kaplan-Meir and Cox hazard models. Hazard ratio (HR) and 95% confidence interval (CI) were calculated for each comparison. Univariate and multivariate analysis were performed when appropriate. All covariates with a p-value less than 0.10, based on univariate analysis were included in the multivariate model. Duration of follow-up was counted from date of first conization and date of last follow-up or secondary conization. Statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA), IBM-Microsoft SPSS (SPSS Statistics; IBM Corp., Armonk, NY, USA) version 20.0.

RESULTS

Data of 3,212 consecutive patients undergoing conization were retrospectively identified. Mean population age was 37.2 (±10.4) years. All patients had conization due to diagnosis of r-CIN2+. After a mean follow-up of 47 (±22.2) months, 112 (3.5%) patients developed r-CIN2+. Ten (8.9%) and 102 (91.1%) cases were classified as persistent and recurrent disease, respectively. Mean time interval between prior conization and diagnosis of r-CIN2+ was 26.2 (±13.2) months. **Table 1** reports baseline characteristics of the study population. Increased age correlated with a risk reduction in term of developing r-CIN2+ (p=0.019, log-rank test). Additionally, diagnosis of high-risk HPV types (including HPV16/18 and other high-risk types) at the time of CIN2+ diagnosis, positive margins and HPV persistence were associated with r-CIN2+ (**Fig. 1**). Via univariate analysis, age older than 25 years (HR=1.37; 95% CI=0.95–1.99; p=0.091), presence of both HPV16/18 (HR=2.50; 95% CI=1.66–3.78; p<0.001) or other high-risk HPV types (HR=1.65; 95% CI=0.97–2.80; p=0.061), coinfections (HR=5.85; 95% CI=3.87–8.85; p<0.001), positive margins at primary conization (HR=3.87; 95% CI=2.01–7.44; p<0.001) and high-risk HPV persistence after conization (HR=17.0; 95% CI=11.09–26.18; p<0.001) were associated with an increased risk of developing r-CIN2+ over the time. Via multivariate analysis, presence of both HPV16/18 (HR=3.40; 95% CI=1.66–6.95; p=0.001) or other high-risk HPV types (HR=2.59; 95% CI=1.21–5.55; p=0.014), positive margins at primary conization (HR=4.11; 95% CI=2.04–8.26; p<0.001) and high-risk HPV persistence after conization (HR=16.69; 95% CI=8.20–33.9; p<0.001) correlated with r-CIN2+, independently (**Table 2**). HPV persistence correlated with a high-risk of r-CIN2+. In patients with negative margins and without HPV persistence, the risk of developing r-CIN2+ was 2%, and this risk increased to 30% in case of

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Table 1. Baseline characteristics

Characteristics	Study population (n=3,212)
Age groups (yr)	
≤25	511 (15.9)
26–45	1,419 (44.2)
>45	1,282 (39.9)
HPV types (at diagnosis of CIN2+)	
16/18	747 (32.2)*
Other high-risk types	1,697 (73.2)*
16/18 and other high-risk types	365 (15.7)*
Negative test	241 (10.4)*
Not tested	892 (27.8)
Coinfections	430 (18.5)*
HPV persistence	98/2,079 HPV+ (4.7)
Re-conization	112 (3.5)
Follow-up (mo)	60 (6–120)

Values are presented as median (interquartile range) or number (%).
CIN2+, cervical intraepithelial neoplasia grade 2+; HPV, human papillomavirus.
*Based on 2,320 tested for HPV.

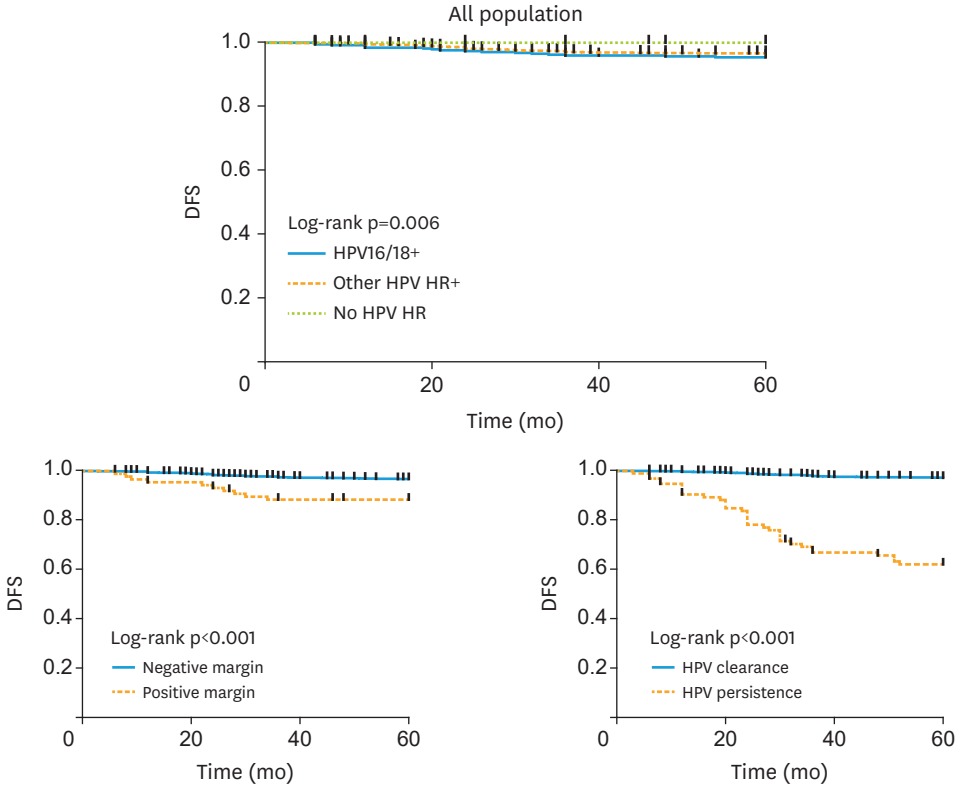

Fig. 1. Factors predicting the risk of cervical dysplasia persistence/recurrence. "HPV16/18+", "Other HPV HR+", and "No HPV HR" were evaluated at the time of CIN2+ diagnosis before conization.
CIN2+, cervical intraepithelial neoplasia grade 2+; DFS, disease free survival; HPV, human papillomavirus; HR, high-risk.

HPV persistence. In patients with positive margins and without HPV persistence, the risk of developing r-CIN2+ was 9%, and this risk increased to 80% in case of HPV persistence.

Considering the whole population, type of infection at the time of CIN2+ diagnosis before conization (HPV16/18 vs. other high-risk HPV types) did not impact the risk of developing r-CIN2+ (p=0.249, log-rank test). Considering age-specific HPV type distribution in women with

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Table 2. Age-specific HPV types distribution at the time of diagnosis of CIN2+ in all population and women with cervical dysplasia recurrence

Class of age	≤25 yr (n=314)		26–45 yr (n=1,054)		>45 yr (n=952)	
	REC	Tot	REC	Tot	REC	Tot
HPV16/18 (n=747)	14 (13)	108	29 (6.5)	443	6 (3.1)	195
Other high-risk HPV (n=1,697)	14 (6.1)	229	40 (5.8)	688	20 (2.6)	781
HPV coinfection (n=430)	13 (3)	64	24 (10.3)	233	13 (9.8)	133

Values are presented as number (%).
CIN2+, cervical intraepithelial neoplasia grade 2+; HPV, human papillomavirus; REC, recurrent cervical dysplasia.

Table 3. Age-specific predictors of cervical dysplasia recurrence

Class of age	≤25 yr	REC	p-value	>25, ≤45 yr	REC	p-value	>45 yr	REC	p-value
HPV status	n=277*		<0.001	n=934*		<0.001	n=868*		<0.001
Persistence	10	10 (100.0)		61	13 (21.3)		27	11 (40.7)	
Clearance	267	9 (3.4)		873	36 (4.1)		841	12 (1.4)	
Margin	n=496†		0.201	n=1,380†		0.003	n=1,227†		0.136
Positive	19	2 (10.5)		40	6 (15.0)		29	2 (6.9)	
Negative	477	20 (4.2)		1,340	47 (3.5)		1,198	25 (2.1)	

Values are presented as number (%).
HPV, human papillomavirus; REC, recurrent cervical dysplasia.
*Considering patients with available data on HPV status; †Considering patients with available data on margin status.

CIN2+, we observed that infection from HPV16/18 correlated with an increased risk of r-CIN2+ only in young women (aged ≤25 years; p=0.031, log-rank test); while in the older population (>25 years) various HPV types had not impact on r-CIN2+ risk (p>0.200, log-rank test). **Table 3** shows how risk factors (i.e., HPV and margin status) are influenced by age. Similarly, **Fig. 2** shows the association of HPV types and the risk of developing r-CIN2+, according to age.

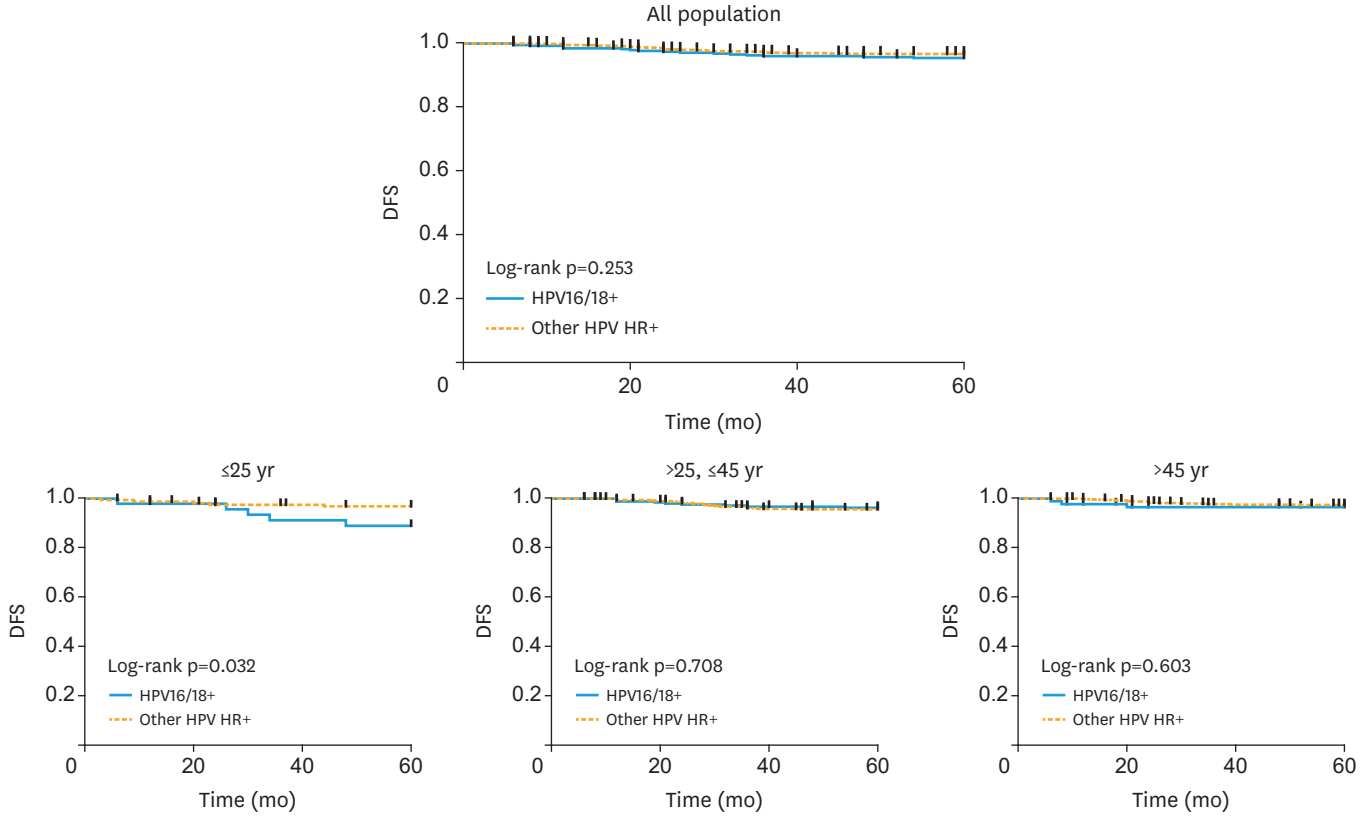

Fig. 2. Age-specific HPV types distribution and effects on cervical dysplasia recurrence risk. "HPV16/18+", "Other HPV HR+", and "No HPV HR" were evaluated at the time of CIN2+ diagnosis before conization.
CIN2+, cervical intraepithelial neoplasia grade 2+; DFS, disease free survival; HPV, human papillomavirus; HR, high-risk.

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DISCUSSION

The present study investigated factors predicting the risk of r-CIN2+ in a large cohort of unselected women undergoing cervical conization. The present study, we reviewed chart of more than 3,200 women having conization, thus observing a number of noteworthy findings. First, presence of high-risk HPV (both HPV16/18 or other HPV types) correlated with an increased risk of r-CIN2+. Second, we corroborated recent evidence highlighting the importance of HPV persistence in predicting the risk of recurrence. Our study demonstrates that HPV persistence is associated with r-CIN2+, much more than margin status. In case of negative margins, HPV persistence was associated with an approximately 15-fold increase in risk of developing r-CIN2+. In case of positive margins, HPV persistence was associated with an approximately 8-fold increase in risk of developing r-CIN2+.Third, we observed an age-specific impact of high-risk HPV types. In our series, infection from HPV16/18 correlated to an increased risk of r-CIN2+ only in young women (aged ≤25 years); while in the older population various HPV types had not impact in influencing r-CIN2+ risk.

Historically, margins status was the only factor considered to be predictive for CIN recurrence [4]. Accumulating evidence underline that HPV persistence represents the main variable predicting the risk of r-CIN2+ [5,7,10]. The Fondazione IRCCS Istituto dei Tumori—HPV study group and other investigators suggested the importance of HPV persistence in patients treated for HPV-related lesions [5-8,10]. In our previous investigation, adopting 2 different artificial intelligence in a series of 1,273 patients having conization, we observed that HPV persistence is one of the main factors predicting for r-CIN2+ [6]. Other investigators corroborated these results [5,10]. Zhang et al., [7] reviewing data of more than 500 patients having conization observed that positive margins and persistence of HPV after treatment determinate a 6- and 20-fold increase in the risk of recurrent disease, respectively. Recently, a review and meta-analysis analyzed data of 44,446 women collected from 96 studies in order to assess the risk of therapeutic failure associated with the histological status of the margins of the tissue excised to treat cervical dysplasia [10]. The authors observed that the risk of r-CIN2+ was 6.6% (95% CI=4.9–8.4) and was increased with positive compared with negative resection margins (relative risk=4.8; 95% CI=3.2–7.2). Post conization HPV clearance was associated with a risk of r-CIN2+ of 0.8%, whereas this risk was 3.7% in patients with negative margins [10].

Another point deserving attention is age-specific HPV types distribution and their impact on risk of r-CIN2+. In our study, we observed that type of high-risk HPV do not impact on the risk of r-CIN2+. But focusing on young women (aged less than 25 years) presence of HPV16/18 instead other HPV types correlates with an increased risk of recurrence. Growing evidence suggest how various HPV types might have a different impact on the basis of age [11-13]. Aro et al., [14] evaluated HPV types distribution among a cohort of 1,279 women with cervical dysplasia [11]. The authors observed that HPV type distribution was distinctly polarized by age with HPV16/18 being markedly HPV16/18-related lesions more prevalent young women. In this study HPV16/18-related cervical dysplasia were 64%, 58%, and 35% in women aged <30, 30–44, and ≥45 years of age, respectively. This would be a very interesting findings especially in the light of the growing adoption of primary prevention in young women. That might potentially reduce the prevalence of HPV16/18 (and also other high-risk types) in this cluster of women [13,14]. Vaccination against HPV might play an important role even after conization in reducing the occurrence of new r-CIN2+. Interestingly, a recently published Italian experience (SPERimentazione ANti HPV Zona Apuana project) suggested that HPV

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vaccination, performed after conization, reduces r-CIN2+ rate (1.2% vs. 6.4% in vaccinated vs. non-vaccinated women) [15]. Although vaccination would be useful in reducing the risk of r-CIN2+, in our series (over the study period), no patients had vaccination against HPV.

The large study population is the main strength of the present paper. While, the inherent biases of the single centre, retrospective study design represent the main weaknesses of the present investigation. Another important limitations were: 1) the inclusion of a large proportion of patients (28%) who were not tested for HPV. Although this point represents a limitation this reflect the current practice in a "real life" setting. 2) we have to highlight that some patients might had second conization at outside centers, the recurrence rates represent better estimates.

In conclusion the present paper investigated risk factors for r-CIN2+, thus observing that HPV persistence is the main factor predicting r-CIN2+. Additionally, positive margins and high-risk HPV infection have a role in increasing r-CIN2+ risk. Patients with positive margins and with HPV persistence are at high-risk of recurrence. Infection from HPV16/18 have a detrimental effect in women, thus highlighting the need of implementing vaccination against HPV in young women. Further prospective studies are warranted in order to identify patients at low- and high-risk of recurrence, thus tailoring clinical decision-making and triaging strategies for post-conization follow-up.

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