Title: **High-risk HPV-positive and -negative high-grade cervical dysplasia: analysis of 5-year outcomes**

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Abstract

Objective: To evaluate the outcomes of high-risk (HR) HPV-positive and -negative women affected by high-grade cervical dysplasia.

Methods: This is a retrospective multi-institutional study. Medical records of consecutive patients with high-grade cervical dysplasia undergoing conization between 2010 and 2014 were retrieved. All patients included had at least 5 years of follow-up. A propensity-score matching was adopted in order to reduce the presence of confounding factors between groups. Kaplan-Meir and Cox hazard models were used to estimate 5-year outcomes.

Results: Overall, data of 2,966 women, affected by high-grade cervical dysplasia were reviewed. The study population included 1,478 (85%) and 260 (15%) women affected by HR-HPV-positive and HR-HPV-negative high-grade cervical dysplasia. The prevalence of CIN2 and CIN3 among the HR-HPV-positive and -negative cohort was similar (p=0.315). Patients with HR-HPV-positive high-grade cervical dysplasia were at higher risk of 5-year recurrence (after primary conization) that HR-HPV-negative patients (p<0.001, log-rank test). Via multivariate analysis, HR-HPV-negative women were at low risk of recurrence (HR: 1.69 (95%CI: 1.05, 4.80); p=0.018, Cox Hazard model). A propensity-score matched comparison was carried out in order to reduce biases that are related to the retrospective study design. In comparison to HR-HPV-negative patients, thosewith HR-HPV-positive CIN3 was associate with a 8-fold increase in the risk of recurrence (p<0.001, log-rank test).

Conclusions: HR-HPV-negative high-grade cervical dysplasia is not uncommon, accounting for 15% of our study population. Those patients experience more favorable outcomes than patients with documented HR-HPV infection(s). Further prospective studies are needed to corroborate our data.

Keywords: HPV; negative; positive; CIN; conization

Introduction

Human papillomavirus (HPV) represents one of the most common sexually transmitted infection in developed countries [1]. More than 40 different HPV types might infect the genital areas in either men or women [1]. Historically, HPV types are classified in “non-oncogenic” or “oncogenic” types, on the basis on whether they correlated with the risk of developing cancer [1].The International Agency for Research on Cancer (IARC) working group identified 13 types of HPV classified as“oncogenic” (high-risk (HR) HPV types) [3]. They included: the alpha-5 type 51; alpha-6 types 56 and 66; alpha-7 types 18, 39, 45 and 59; and alpha-9 types 16, 31, 33, 35, 52 and 58 [2]. Of note, the majority of HPV infections (including HR ones) regress spontaneously. However, robust evidence supported that persistent HR-HPV infection might cause precancerous and cancerous transformation in the uterine cervix [3-5] as in other organs (including the lower genital tract and the head &neck district) [4].

The role of HPV in determining precancerous/cancerous transformation of the uterine cervix is well established [1-7]. Hence, many developed countries incorporated in their guidelines the adoption HPV-based cervical screening programs [6]. In 2012, the US Preventive Services Task Force (USPSTF) recommended screening women aged 21 to 65 years for cervical cancer with cytology every 3 years, with an option for women >30 years for HR-HPV co-testing (cytology and cervical swab for HR-HPV) every 5 years [7]. Additionally, there is consistent evidence that primary HR-HPV testing (in the initial round of screening) led to a statistically significant increased detection of high-grade cervical dysplasia in comparison to cytology alone, thus supporting the adoption of HR-HPV testing [6]. However, several experiences suggested that 10-15% of women with high-grade cervical dysplasia are classified as HR-HPV-negative [8, 9]. The presence of false-negative results, low viral load or the presence of other HPV (not detected) types might explain this finding [8, 9], as well as the controversial topic of non HPV related CIN2-3 lesions [10, 11]. Although the prevalence of HR-HPV-negative lesions is not negligible, the clinical significance and behavior of HPV-negative lesions is still unknown. Hence, in the present paper we aimed to evaluate the long-term

impact of being diagnosed with HPV-positive and -negative lesions in a large group of women treated with conization for high-grade cervical dysplasia.

Methods:

This is a retrospective multi-institutional study conducted in Italy from 2010 and 2014. Institutional Review Board (IRB) approval was obtained (#5720). The list of participant centers is reported in supplemental material 1. For the purpose of the present study, we collected chart of consecutive patients with newly diagnosed high-grade cervical dysplasia treated in Italy from 01/01/2010 to 12/31/2014.

The inclusion criteria were: (i) newly histological diagnosed moderate /severe cervical dysplasia (HSIL/CIN2/CIN3); (ii) squamous cell lesions; (iii) cervical conization performed with laser or LEEP between 2010 and 2014; (iv) patients with available 5-year follow-up data (for non recurring patients; while, patients who recurred were included even if they did not complete the five-year follow-up period). For the study purpose only consecutive series of patients were accepted. Exclusion criteria were: (i) age <18 years; (ii) consent withdrawal; (iii) execution of ablative procedure; (iv) diagnosis of invasive cancer at the time of conization; (v) execution of cold knife conization; (vi) glandular lesions; (vii) ongoing pregnancy; and (viii) history of total hysterectomy. The main outcome measure of the study is to assess recurrence rate in HR-HPV-positive and –negative women undergoing conization due to high-grade cervical dysplasia. Secondary endpoint measure was to identify specific risk factors for recurrence in HPV-positive and -negative patients. Chart of patients undergoing conization during the years 2010 – 2014 were retrospectively reviewed. Demographic details, data about HPV type(s) detected, as well as data on treatment for the occurrence of cervical dysplasia were collected. HPV types were considered as high-risk according to the data of the International Agency for Research on Cancer (IARC) [12]. During the study period, different surgeons performed all the procedures across the participant centers.

157 However, no differences in the facilities and services related to patients' care were present. Details
158 of follow-up schedule and examination were reported elsewhere [13].
159 All patients included had conization due to high-grade cervical dysplasia (HSIL/CIN2/CIN3). All
160 conization were performed under direct colposcopic guidance. According to institutional protocols,
161 patients were evaluated colposcopically in outpatients' clinic at 3 (in case of positive margins) – 6
162 (in case of negative margins) months after conization. Briefly, patients had a follow-up scheduled
163 including Pap-smear, colposcopy and colposcopic-guided biopsy if clinically indicated, every 6
164 months for the first 2 years, and annually thereafter (until 5 years). Generally, in patients with
165 documented HPV infections, HPV testing was performed at the first examination after conization.
166 Various HPV testing methods were used in different departments (all of them were FDA approved).
167 The most used HPV testing methods included Hybrid Capture® 2 (hc2), Cobas®, and CLART®
168 [14]. Persistence of HPV infection was defined as the persistence of HPV detected at the first
169 clinical examination following conization (generally at 6 months). Persistence / recurrence after
170 conization was defined as the diagnosis of a new HSIL/CIN2/CIN3+ requiring secondary
171 conization. Persistence of cervical dysplasia was defined by the diagnosis of HSIL/CIN2/CIN3+ at
172 the first evaluation following conization; while, patients with recurrent cervical dysplasia had at
173 least one negative examination between conization and the diagnosis during follow-up of
174 HSIL/CIN2/CIN3+. Low grade cervical lesions (LSIL/CIN1) were not considered as recurrent
175 disease.

176 **Statistical methods**

177 Data are summarized using basic descriptive statistics. Since this is a retrospective comparison
178 between two groups, possible allocation biases might impair the quality of the results reporting.
179 Therefore, we performed a propensity score analysis. Propensity-score analysis aims to reduce
180 biases rising from different covariates. In order to perform this analysis, we developed a
181 multivariable logistic regression model. Age, type of lesion (CIN2 vs. CIN3), type of conization
182 (laser conization vs. LEEP), and vaccination status were included in the model. Detailed description

of propensity-score matching is described elsewhere [13]. HR-HPV negative patients were matched 1:1 to a group of HR-HPV positive patients. Propensity-score matching analysis attempts to minimize the inherent biases of a retrospective study design. We used a caliper width ≤0.1 standard deviations (SDs) of the logit odds of the estimated propensity score. Basic descriptive statistics were used to describe the two populations. Differences in categorical variables were analyzed using the Fisher exact test. Odds ratio (OR) and 95% confidence intervals (95%CI) were calculated for each comparison. T-test and Mann-Whitney test were used to compare continuous variables as appropriate. Recurrence-free survival was estimated using Kaplan-Meier model. The log-rank test was used to compare the risk of developing recurrence and the risk of death between the two groups over the time. P values <0.05 were considered statistically significant. Statistical analysis was performed with GraphPad Prism version 6.0 (GraphPad Software, San Diego CA) and IBM-Microsoft SPSS version 20.0 (SPSS Statistics. International Business Machines Corporation IBM 2013 Armonk, USA) for Mac.

Results

Overall, data of 2,966 women, affected by high-grade cervical dysplasia (HSIL/CIN2/CIN3) treated in Italy from 01/01/2010 to 12/31/2014, were evaluated. After the exclusion of 1,228 (41..4%) women who were not tested for HPV before conization, the remaining 1,738 (58.6%) women were included in the analysis. The study population included 1,478 (85%) and 260 (15%) women affected by HR-HPV-positive and HR-HPV-negative high-grade cervical dysplasia, respectively. Figure 1 shows the flow of patients into the study design.

The population of women with known HR-HPV status (n=1,738), included 440 (25.3%) and 1,298 (74.7%) patients with diagnosis of CIN2 and CIN3, respectively. The prevalence of CIN2 and CIN3 among the HR-HPV-positive and -negative cohort was similar (p=0.315). The latter group included 59 (22.7%) patients with CIN2 and 201 (77.3%) patients with CIN3. Baseline characteristics of patients with HPV-positive and HPV-negative high grade lesions are reported in Table 1.

Recurrence rate in HR-HPV positive and negative patients was 5.8% (n=86) and 0.8% (n=2), respectively (p<0.001). Figure 2 shows 5-year recurrence-free survival for HR-HPV-positive and -negative patients (p<0.001, log-rank test). According to margin status (Figure 3), we observed that in case of positive margins at conization, patients with HR-HPV negative lesions are less likely to develop recurrent disease in comparison to patients with HR-HPV positive lesions, but the difference is not statistically significant (p=0.212, log-rank test). While in case of negative margins at conization, patients with HR-HPV-negative lesions experienced significant better outcomes than the HR-HPV-positive counterpart (p=0.006, log-rank test).

Via multivariate analysis, HR-HPV-negative women were at low risk of recurrence (HR: 1.69 (95%CI: 1.05, 4.80); p=0.018,). Other factors influencing recurrence were: diagnosis of CIN3 instead of CIN2 (HR: 1.80 (95%CI: 1.09, 2.96); p<0.001, Cox Hazard model), and HPV persistence following conization (HR: 1.91 (95%CI: 1.21, 3.05); p<0.001,). In this analysis, co-infections (yes vs. no) and the presence of HPV16/18 in comparison to other HR HPV types were not associated with the risk of recurrence.

Propensity-matched comparison

A propensity-score matched comparison was carried out in order to reduce biases that are related to the retrospective study design. Table 2 reports baseline characteristics. As the results of the propensity-score matched comparison, baseline patients characteristics of the two groups of patients were balanced (p>0.60 *per* every variable). Looking at the crude number of events, we observed that the presence of a HR-HPV infection was associated with a statistically significant increase in the risk of high-grade cervical dysplasia recurrence after primary conization (2/260 (0.76%) vs. 18/260 (6.92%); p=0.0003). Five-year recurrence-free survival for HR-HPV-positive and -negative patients included in the propensity-score matched analysis is displayed in Figure 4A.

HR-HPV-positive and -negative CIN3

In order to avoid possible biases related to the inclusion of CIN2, we restricted our analysis to patients affected by CIN3 (201 HR-HPV-negative patients who were matched 1:1 to 201 HR-HPV-positive using a propensity-score matched algorithm). Looking at the crude number of events, we observed that presence of a HR-HPV infection was associated with a 8-fold increase in the risk of high-grade cervical dysplasia recurrence (2/201 (0.99%) vs. 16/201 (7.98%); p=0.0011,). Five-year recurrence-free survival for HR-HPV-positive and -negative patients included in the propensity-score matched analysis is displayed in Figure 4B.

Discussion

The present study investigates the clinical implication of being diagnosed with a HR-HPV -positive and -negative high-grade cervical dysplasia, thus reporting a number of noteworthy findings. First, HR-HPV-negative cervical dysplasia is not uncommon, accounting for about 15% of our study population. Second, the prevalence of HR-HPV-negative patients was similar between patients with CIN2 and CIN3. Third, HR-HPV-negative patients were more likely to experience better outcomes than the HR-HPV-positive counterpart.

Growing evidence suggests that the prevalence of HR-HPV-negative patients is not negligible, accounting for about 10-15% of women with high grade cervical dysplasia [8, 9]. Our study corroborated this data. However, only limited evidence exists on the outcomes of patients affected by high-grade cervical dysplasia according to HR-HPV status [8]. Hence, the clinical management of all these patients is still similar, regardless of their HR-HPV status. [9].

Ashman et al., evaluated a total of 943 Pap-smears with the diagnosis of HSIL [15]. HR-HPV was detected in 883 patients (93.6%) [15]. Overall, 24 out of 45 patients with HR-HPV-negative HSIL had histologically confirmed CIN2+ lesions [15]. Recently, Castle et al., reviewed current evidence on the clinical characteristics of HPV-positive and -negative precancerous lesions [8]. The review collected data from 3,089 and 307 HR-HPV-positive and -negative women from 19 studies [8]. Pooled data suggested that patients with HR-HPV-negative CIN2+ are less likely to have a high-

grade colposcopic impression, higher prevalence of low-risk HPV types, and a lower cancer risk than HR-HPV-positive CIN2+ [8]. Our study alone, reporting the Italian situation in the years from 2010 to 2014, provide a large amount of data (including 1,478 (85%) and 260 (15%) women affected by HR-HPV-positive and HR-HPV-negative high-grade cervical dysplasia) on this issue.

Although HPV could "virtually" be considered the primary cause of almost all cervical cancers, (HR) HPV is not detected in several women with cervical dysplasia. Several reasons might explain these findings: (i) false negative results; (ii) low viral load (especially in women with lesions but who are clearing infection); (iii) infection from low-risk HPV types; and (iv) the presence of other HPV types or subtypes not detectable by conventional tests [16, 17], (v) the questionable possibility of CIN2-3 arising from non-HR-HPV related pathways [10, 11]. These features and the inherent biases of the retrospective study design are the main limitations of the present investigation. Additionally, other limitations included: (i) the adoption of different tests, evaluated in different laboratories, even though all were FDA-approved; (ii) the lack of comparison between specificity, sensitivity and accuracy of various tests performed; (iii) the lack of data about the time interval between HPV testing and conization; (iv) the lack of data between last sexual intercourse and HPV testing. In fact, emerging data suggested that detection of HPV in genital samples might result from the deposition of HPV by infected sexual partners [18].

Other four points deserve to be addressed. First, the role of conization in women with CIN2 (especially, those who are HR-HPV-negative). In the recent years, we know about the non-uncommon spontaneous regression of CIN2 (especially, in p16 negative cases) [16]; wait-and-see approach could be a valuable option for young patients affected by CIN2 without colposcopic suspect for CIN3+ lesions [19, 20]. For this reason, we performed a secondary analysis on CIN3 only. Second, our study population included 11 out of 260 HR-HPV-negative patients who were detected with HR-HPV after conization. We can speculate that those patients were characterized by a low viral load or false negative results [8]. However, we have not excluded those patients from the analyses in order to a provide a more reliable portrait of a real life setting [19-21]. Third, it might be

possible that although in presence of documented histological lesions, the immune system of HPV-negative patients is actively clearing HPV. Then, we can speculate that those cases would regress spontaneously. Fourth, in our analysis, patients with HR-HPV-negative lesions undergoing conization with negative surgical margins are at low risk of recurrence. We can speculate that those patient are not requiring strict follow-up. However, owing to the retrospective nature of this study, further prospective evidence is needed to design appropriate follow-up schedules.

The main strength of the paper is the evaluation of a large real world data investigating the long-term outcomes of HR-HPV-positive and -negative women with high-grade cervical dysplasia. To date, no other studies evaluate the same issues, reporting long-term outcomes following primary conization. Additionally, the adoption of a propensity-score matching allowed us to evaluate a fair comparison between HR-HPV-positive and -negative patients.

In conclusion, the present paper investigated long-term outcomes of patients with HR-HPV-positive and -negative high-grade cervical dysplasia undergoing cervical conization. The results of this study highlighted that about 10-15% of patients with high-grade cervical dysplasia are HR-HPV-negative. The adoption of HPV testing alone would miss the diagnosis of 10-15% HPV-negative high-grade cervical dysplasia. HR-HPV-negative patients are at low risk of recurrence both for CIN2 and CIN3 lesions; while HR-HPV-positive patients experienced a 8-fold increase risk of recurrence than HR-HPV-negative counterpart. These data would be useful to tailor observation schedules after conization. Owing to the retrospective nature of the present investigation, our data should be evaluated with caution. Further prospective studies are needed to corroborate our research.

Author contribution:

Conceptualization: GB, VD, FS, AC, FR., Methodology: All authors.; Project administration: FR, FS; Supervision: FR.; writing – original draft: All authors; writing – review & editing: all authors.

Conflicts of interest:

The Authors declare no conflicts of interest.

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Legend to Figure:

Figure 1: Study design

Figure 2: Recurrence free survival in HR-HPV-positive and -negative women

Figure 3: Recurrence rate according to margin status: (A) positive; (B) negative

Figure 4: Propensity-score matched cohort of HR-HPV-positive and -negative women: patients affected by CIN2 and CIN3 (A); and in patients with CIN3 only (B)

References:

1- Satterwhite CL, Torrone E, Meites E, et al. Sexually transmitted infections among US women and men: Prevalence and incidence estimates, 2008. Sexually Transmitted Diseases 2013; 40:187-193.

2- Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, ElGhissassi F; WHO International Agency for Research on Cancer. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005;6:204

3- Kelly H, Weiss HA, Benavente Y, de Sanjose S, Mayaud P; ART and HPV Review Group. Association of antiretroviral therapy with high-risk human papillomavirus, cervical intraepithelial neoplasia, and invasive cervical cancer in women living with HIV: a systematic review and meta-analysis. Lancet HIV. 2018;5(1):e45-e58. doi:10.1016/S2352-3018(17)30149-2

4- Joura EA, Giuliano AR, Iversen OE, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med. 2015;372(8):711-723. doi:10.1056/NEJMoa1405044

5- Abbas KM, van Zandvoort K, Brisson M, Jit M. Effects of updated demography, disability weights, and cervical cancer burden on estimates of human papillomavirus vaccination impact at the

338 global, regional, and nationallevels: a PRIME modellingstudy. Lancet GlobHealth. 2020;8(4):e536-
339 e544. doi:10.1016/S2214-109X(20)30022-X
340 6- Melnikow J, Henderson JT, Burda BU, Senger CA, Durbin S, Weyrich MS. Screening for
341 Cervical Cancer With High-Risk Human Papillomavirus Testing: Updated Evidence Report and
342 Systematic Review for the US Preventive Services Task Force. JAMA. 2018;320(7):687–705.
343 doi:10.1001/jama.2018.10400ù
344 7- Moyer VA; U.S. Preventive Services Task Force. Screening for cervicalcancer: U.S. Preventive
345 Services Task Force recommendation statement [publishedcorrectionappears in AnnInternMed.
346 2013;158(11):852]. Ann Intern Med. 2012;156(12):880-891. doi:10.7326/0003-4819-156-12-
347 201206190-00424
348 8- Castle PE, Pierz AJ, Adcock R, et al. A Pooled Analysis to Compare the ClinicalCharacteristics
349 of Human Papillomavirus-positive and -Negative CervicalPrecancers [published online ahead of
350 print, 2020 Jul 12]. CancerPrev Res (Phila). 2020;10.1158/1940-6207.CAPR-20-0182.
351 doi:10.1158/1940-6207.CAPR-20-0182
352 9- Ca L, A C, E M, et al. High risk HPV DNA subtypes and E6/E7 mRNA expression in a cohort of
353 colposcopy patients from Northern Italy with high-grade histologically verified cervical lesions. Am
354 J Transl Res. 2012;4(4):452-457.
355 10- Petry KU, Cox JT, Johnson K, Quint W, Ridder R, Sideri M, et al. Evaluating HPV-negative
356 CIN2+ in the ATHENA trial. Int J Cancer. 2016 Jun 15;138(12):2932-9. doi: 10.1002/ijc.30032.
357 Epub 2016 Mar 2. PMID: 26851121; PMCID: PMC5069615.
358 11- Liu MZ, Hung YP, Huang EC, Howitt BE, Nucci MR, Crum CP. HPV 6-associated
359 HSIL/Squamous Carcinoma in the Anogenital Tract. Int J Gynecol Pathol. 2019 Sep;38(5):493-497.
360 doi: 10.1097/PGP.0000000000000556. PMID: 30312218.
361 12- IARC working group. Monographs. Human papillomaviruses. Available at:
362 https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100B-11.pdf Last accessed
363 07/07/2020

13- Bogani G, DI Donato V, Sopracordevole F, Ciavattini A, Ghelardi A, Lopez S, et al. Recurrence rate after loop electrosurgical excision procedure (LEEP) and laser Conization: A 5-year follow-up study. Gynecol Oncol. 2020 Sep 3:S0090-8258(20)33826-9. doi: 10.1016/j.ygyno.2020.08.025. Epub ahead of print. PMID: 32893030.

14- Bogani G, Raspagliesi F, Sopracordevole F, Ciavattini A, Ghelardi A, Simoncini T, et al. Assessing the Long-Term Role of Vaccination against HPV after Loop Electrosurgical Excision Procedure (LEEP): A Propensity-Score Matched Comparison. Vaccines (Basel). 2020 Dec 1;8(4):717. doi: 10.3390/vaccines8040717. PMID: 33271963; PMCID: PMC7711506

15- Ashman D, Zhang H, Li J, Austin M, Wang T, Pradhan D, Zhao C. HPV detection rates and histopathologic follow-up of patients with HSIL cytology in a large academic women's hospital laboratory. J Am Soc Cytopathol. 2020 May 6:S2213-2945(20)30074-0. doi: 10.1016/j.jasc.2020.04.010. Epub ahead of print. PMID: 32475726.

16- Reich O, Regauer S, Kashofer K. Possibly carcinogenic HPV subtypes are a cause of HSIL and negative clinical HPV tests - A European prospective single center study. Gynecol Oncol. 2020 Jul;158(1):112-116. doi: 10.1016/j.ygyno.2020.04.685. Epub 2020 Apr 27. PMID: 32354471.

17- de Thurah L, Bonde J, Lam JUH, Rebolj M. Concordant testing results between various human papilloma virus assays in primary cervical cancer screening: systematic review. Clin Microbiol Infect. 2018 Jan;24(1):29-36. doi: 10.1016/j.cmi.2017.05.020. Epub 2017 May 27. PMID: 28559000.

19-Malagón T, Burchell AN, El-Zein M, Guénoun J, Tellier PP, Coutlée F, Franco EL; HITCH Study Group. Estimating HPV DNA Deposition Between Sexual Partners Using HPV Concordance, Y Chromosome DNA Detection, and Self-reported Sexual Behaviors. J Infect Dis. 2017 Dec 5;216(10):1210-1218. doi: 10.1093/infdis/jix477. PMID: 28968731; PMCID: PMC5853413.

20- White CM, Bakhiet S, Bates M, Ruttle C, Pilkington LJ, Keegan H, et al. Exposure to tobacco smoke measured by urinary nicotine metabolites increases risk of p16/Ki-67 co-expression and

high-grade cervical neoplasia in HPV positive women: A two year prospective study. Cancer Epidemiol. 2020 Oct;68:101793. doi: 10.1016/j.canep.2020.101793. Epub 2020 Aug 22. PMID: 32841926.

21- Gustavsson I, Aarnio R, Berggrund M, Hedlund-Lindberg J, Sanner K, Wikström I, Enroth S, Olovsson M, Gyllensten U. Randomised study of HPV prevalence and detection of CIN2+ in vaginal self-sampling compared to cervical specimensc ollected by medical personnel. Int J Cancer. 2019 Jan 1;144(1):89-97. doi: 10.1002/ijc.31637. Epub 2018 Oct 29. PMID: 29943822.

Table 1: Baseline characteristics of the study population

	HR-HPV-positive (N=1,478)	HR-HPV-negative (N=260)
Age, years	42 (18, 70)	37 (23, 62)
BMI, Kg/mq	23 (14.4, 41.8)	20 (17, 40)
Last cytological assessment		
LSIL / ASCUS	23 (1.5%)	15 (5.7%)
HSIL / ASCH	1,073 (72.7%)	175 (67.4%)
Unknown	382 (25.8%)	70 (26.9%)
Type of cervical dysplasia		
CIN2	381 (25.8%)	59 (22.7%)
CIN3	1,097 (74.2%)	201 (77.3%)
Menopause		
No	1134 (76.7%)	232 (89.2%)
Yes	344 (23.3%)	28 (10.8%)
HPV status		
Negative	0	260 (100%)
Positive	1,478 (100%)	0
Margin status		
Negative	1260 (85.3%)	244 (93.8%)
Positive	218 (14.7%)	16 (6.2%)
Type of involved margin		
Endocervical positive	137 (9.2%)	13 (5%)
Ectocervcial positive	77 (5.2%)	5 (1.9%)
Surgical technique		
Laser conization	128 (8.7%)	56 (21.5%)
LEEP	1,350 (91.3%)	204 (78.5%)
Vaccination after conization		
No	1,401 (94.8%)	260 (100%)
Yes	77 (5.2%)	0
Detection of HR-HPV after conization		
No	752 (50.9%)	159 (61.2%)
Yes	376 (25.4%)	11 (4.2%)
Untested	350 (23.7%)	90 (34.6%)

Data are reported as median (range) or number (%). Abbreviation: BMI, body mass index; CIN, cervical intraepithelial neoplasia; HSIL; high-grade squamous intraepithelial lesion; LSIL, Low-grade squamous intraepithelial lesion; ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells - high-grade; ;HPV, human papillomavirus; LEEP, Loop Electrosurgical Excision Procedure.

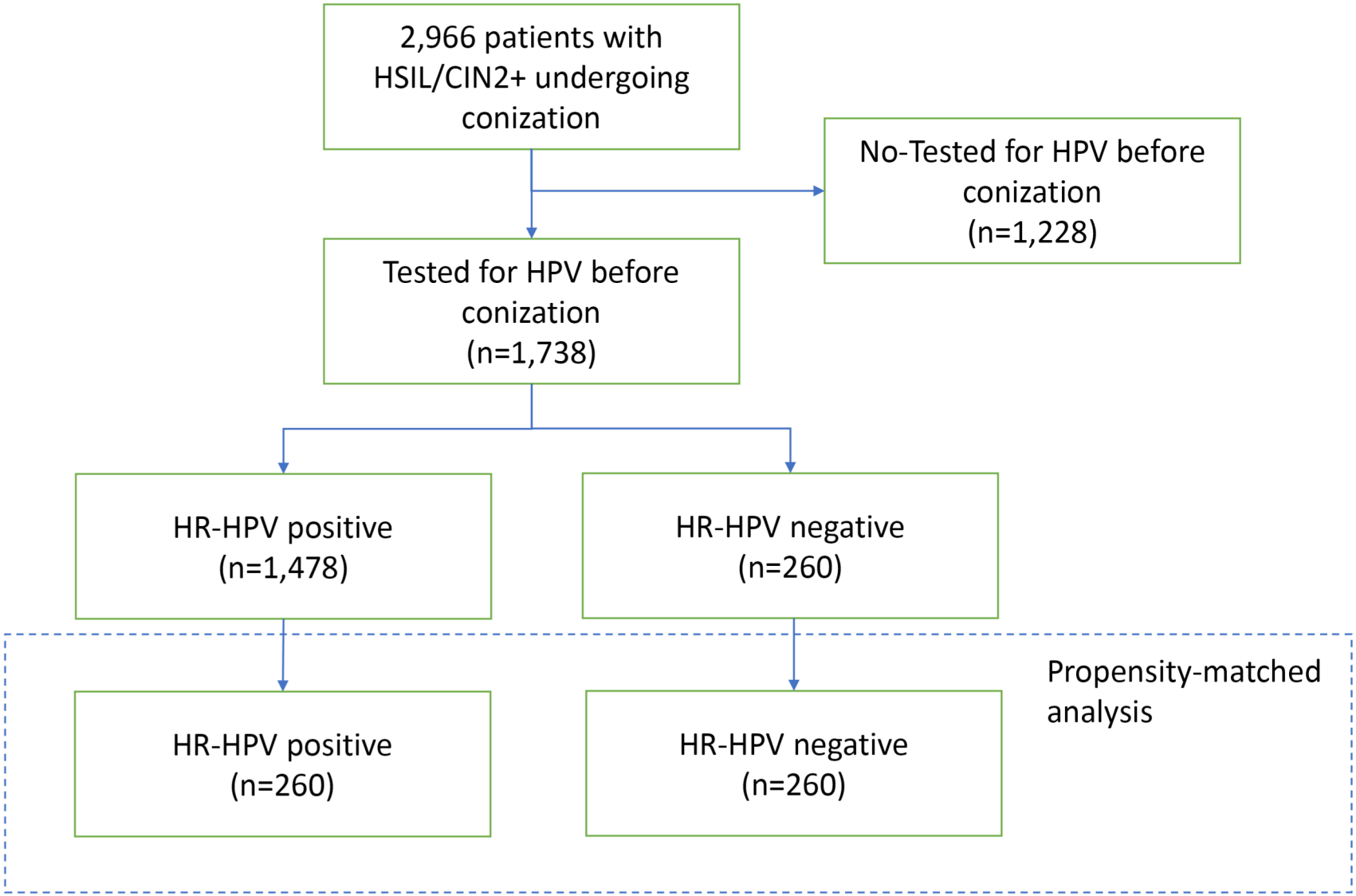
Table 2: Baseline characteristics of the propensity-score matched population

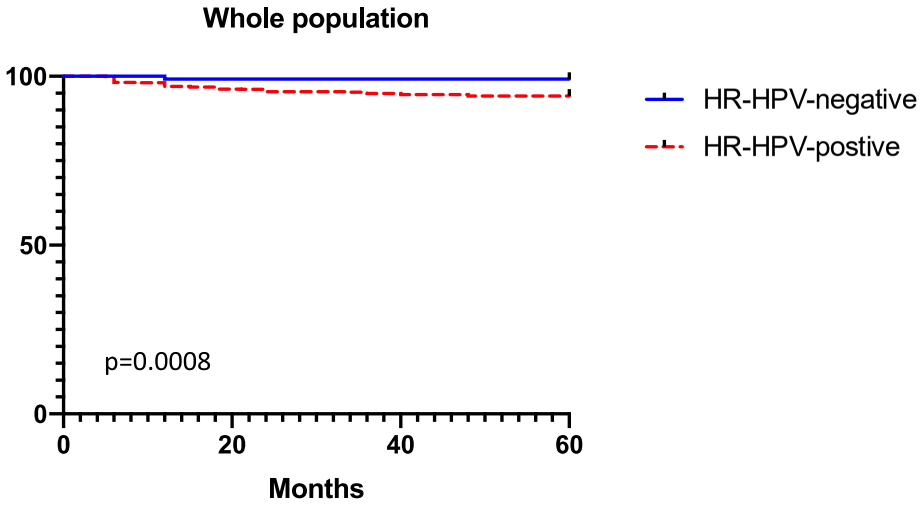
	HR-HPV-positive (N=260)	HR-HPV-negative (N=260)	p value
Age, years *	37 (22, 60)	37 (23, 62)	0.89
BMI, kg/mq *	20 (18, 40)	20 (17, 40)	0.87
Type of cervical dysplasia *			1.00
CIN2	59 (22.7%)	59 (22.7%)	
CIN3	201 (77.3%)	201 (77.3%)	
Menopause *			0.88
No	230 (88.5%)	232 (89.2%)	
Yes	30 (11.5%)	28 (10.8%)	
HPV status			<0.001
Negative	0	260 (100%)	
Positive	260 (100%)	0	
Margin status *			0.60
Negative	240 (92.3%)	244 (93.8%)	
Positive	20 (7.7%)	16 (6.2%)	
Type of involved margin			
Endocervical positive	15 (5.7%)	13 (5%)	0.69
Ectocervcial positive	8 (3%)	5 (1.9%)	0.39
Surgical technique *			0.82
Laser conization	53 (20.4%)	56 (21.5%)	
LEEP	207 (79.6%)	204 (78.5%)	
Vaccination after conization *			
No	260 (100%)	260 (100%)	1.00
Yes	0	0	
Detection of HR-HPV after conization			0.009
No	179 (68.8%)	159 (61.2%)	
Yes	20 (7.7%)	11 (4.2%)	
Untested	61 (23.5%)	90 (34.6%)	

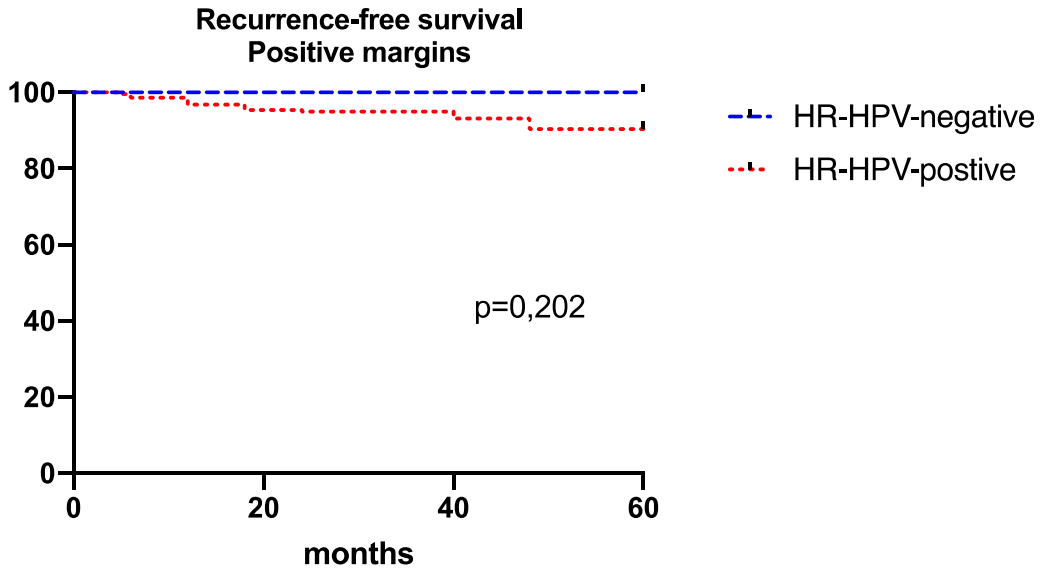
Data are reported as median (range) or number (%). Abbreviation: BMI, body mass index; CIN, cervical intraepithelial neoplasia; HSIL; high-grade squamous intraepithelial lesion; HPV, human papillomavirus; LEEP, Loop Electrosurgical Excision Procedure. *, variables included in the propensity-matched comparison.

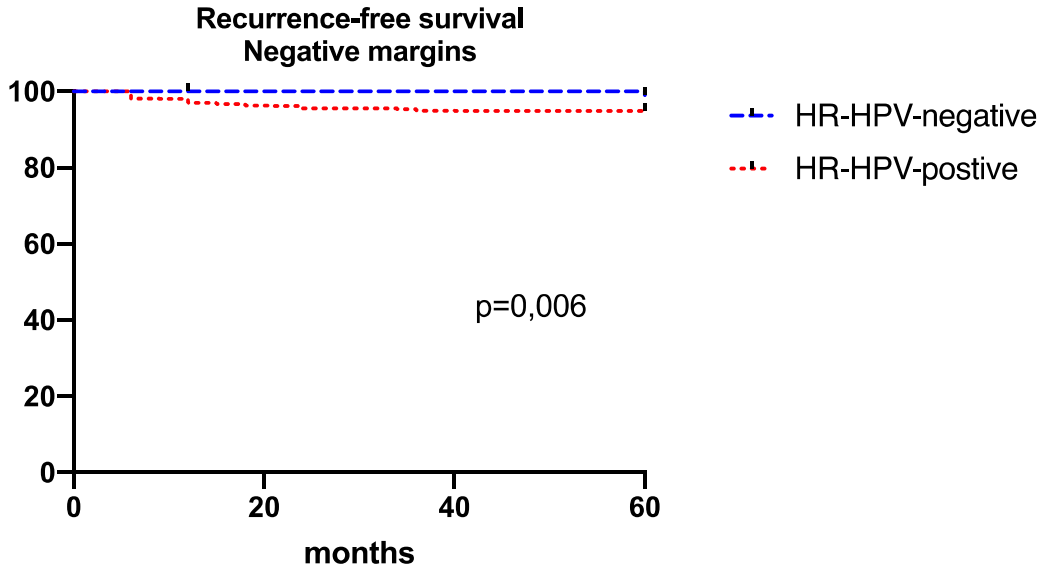
5. Figures

Click here to access/download;5. Figure;HRHPV curves.pptx

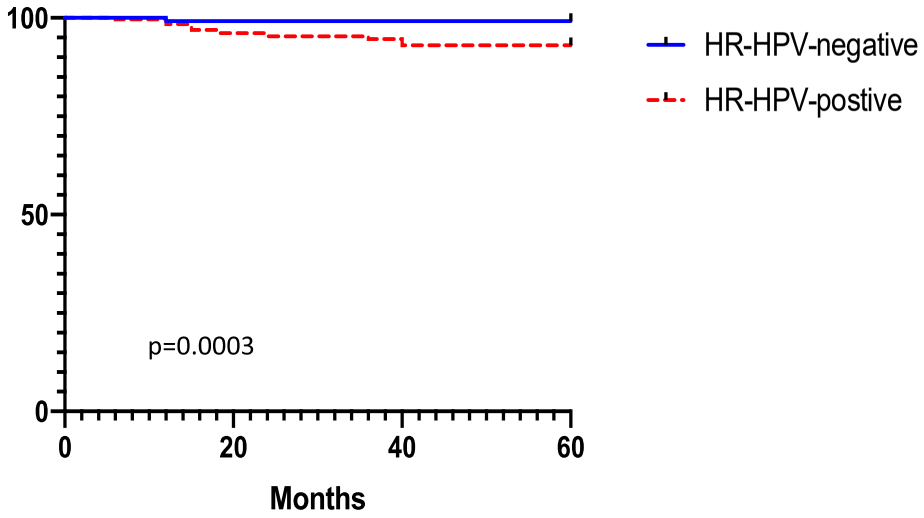



Whole population
100
50
0
p=0.0008
0
20
40
60
Months
HR-HPV-negative
HR-HPV-postive

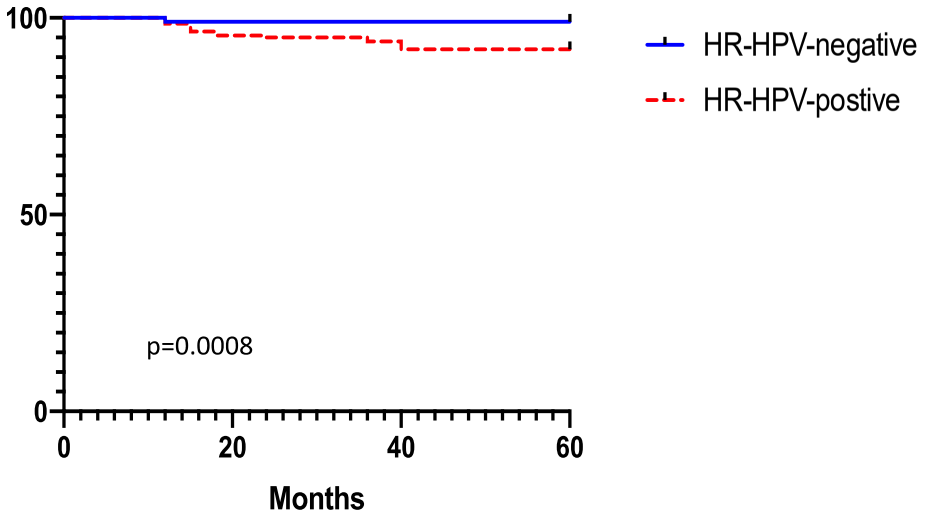

Recurrence-free survival
Positive margins
100
80
60
40
20
0
0
20
40
60
months
p=0,202
HR-HPV-negative
HR-HPV-postive


Recurrence-free survival
Negative margins
100
80
60
40
20
0
0
20
40
60
months
p=0,006
HR-HPV-negative
HR-HPV-postive

Propensity-matched analysis: CIN2+



Propensity-matched analysis: CIN3



Highlights

- Overall, 10-15% of patients with high-grade cervical dysplasia are HR-HPV negative
- Patients with HR-HPV-positive lesions experience a 8-fold increase in the risk of recurrence than HPV-negative patients
- In case of HR-HPV-negative patients, achieving complete excision of the lesion the risk of recurrence is negligible.