Performance of Different Follow-Up Strategies and Genotype-Based Recurrence Risk After Treatment of Cervical High-Grade Squamous Intraepithelial Lesion

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Objective: Our aim was to evaluate the performance of different follow-up strategies after treatment for cervical intraepithelial neoplasia (CIN) 2 or 3, including human papillomavirus (HPV) detection, cytology, or colposcopy, as well as their combinations. Additionally, we compared the influence of the persistence of HPV 16/18 versus that of other high-risk HPV genotypes (HR-HPV) in the recurrence risk.

Methods: Retrospective register-based study, including women who had an excision of the transformation zone for CIN2 or CIN3 at our institution, between January 2011 and December 2022. The outcome assessed was histopathological recurrence/persistence of CIN2 or worse.

Results: Of the 721 women included, 6.8% (49/721) had recurrence/persistence. The sensitivity, specificity, and positive and negative predictive values of the HPV test were 97.4%, 80%, 22.3%, and 99.8%, respectively, whereas for cotesting (HR-HPV and cytology), 86.8%, 90.1%, 34.4%, and 99.1%, respectively. The referral rates for colposcopy were 24.3% and 14.2%, respectively. The sensitivity of colposcopy was low (40.0%).

Women who were initially positive for non-16/18 genotypes at baseline who became HPV16/18 positive during follow-up, had a statistically significant increased risk of CIN2 or worse, compared with those who tested positive only for other HR-HPV genotypes during both stages (hazard ratio = 4.98; 95% CI = 1.66–14.91).

Conclusions: Human papillomavirus testing is the best strategy for follow-up after treatment of cervical HSIL. The addition of cytology triage decreases by more than 40% the referrals for colposcopy, without significantly missing cases of recurrence/persistence. Human papillomavirus 16/18 in the follow-up, regardless of being previously positive, is associated with higher risk of recurrence/persistence of HSIL.

Key Words: high-grade squamous intraepithelial lesions, cervical intraepithelial neoplasia, human papillomavirus, HPV genotypes, excision of the transformation zone, HPV test, cytology, colposcopy

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Women diagnosed with cervical high-grade squamous intraepithelial lesions (HSIL), encompassing cervical intraepithelial neoplasia (CIN) 2 or 3, are usually treated with an excisional procedure (excision of the transformation zone [ETZ]) to prevent progression to invasive disease.[1,2]

While the rate of success of ETZ is high, it is consensual that a "test of cure" is needed before the woman can be returned to "routine" screening. The most recommended strategies for immediate follow-up, currently, are based in the detection of high-risk (HR) human papillomavirus (HPV)—either isolated or associated with cytology (cotesting). The American Society for Colposcopy and Cervical Pathology 2019 guidelines recommend HPV testing at 6 months after treatment, and then annually, until obtaining 3 consecutive negative tests.[2] In other countries, the follow-up protocols are less demanding, allowing discharge after 1 negative HPV test.[3]

It is acknowledged that, even after successful treatment, the risk of CIN2 or worse (CIN2+) will remain higher than in the general population for at least 25 years, and this is the rationale behind the recommendation of continuing surveillance for at least this period (even if it exceeds the recommended age to stop screening), testing every 3 years.[2] Previous studies revealed a posttreatment risk of CIN2+ ranging from 4.8% to 15%.[4–8] The persistence of HPV infection is a sine qua non condition for the development and recurrence of CIN2+. Factors predisposing for HPV persistence include: smoking, older age (>50 years), and menopause.[9] Contributing factors for recurrence or persistence of CIN2+, after treatment, include: CIN3 diagnosis in comparison with CIN2, margins' involvement (endocervical margin posing a greater risk), and HIV infection.[6,10,11]

The available data—mostly derived from observational studies, but also from meta-analysis—suggest that adding HPV vaccination to ETZ leads to reduction of the recurrence risk, especially for high-grade disease related to HPV16 or 18.[12] Several large-scale randomized controlled trials (RCTs) are ongoing and are expected to provide a definite answer to questions such as: what should be the ideal time to vaccinate (pretreatment or posttreatment), whether it is worth revaccinating women previously vaccinated, and up to what age is it worth vaccinating.[13–15]

In the present study, our primary objective was to evaluate the performance of different follow-up strategies, including isolated HPV detection, cytology, or colposcopy, as well as their combinations, in the follow-up after treatment of HSIL. In addition, we aimed to compare the influence of the persistence of HPV 16/18 versus that of other HR-HPV genotypes during follow-up in the recurrence risk.

METHODS

Study Population

This was an observational, descriptive, retrospective, longitudinal study of women treated with ETZ for a diagnosis of CIN2 or CIN3, between January 2011 and December 2022, at the Lower Genital Tract Unit of the Centro Hospitalar de São João, Porto, Portugal. Ethical approval was granted by the local institutional review board (IRB) of Centro Hospitalar de São João (approval 96/2023; May 3, 2023).

The following criteria were applied for selection of cases for this study:

- Inclusion criteria: histologically confirmed diagnosis of high-grade cervical dysplasia (CIN2 or CIN3), in a biopsy and/or at the ETZ specimen.

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- Exclusion criteria: age younger than 25 years; adenocarcinoma in situ (AIS); invasive disease (squamous cell carcinoma (SCC) or adenocarcinoma); unknown margin status; repeated ETZ during the specified time frame; previous diagnosis of HSIL.

We performed a review of the electronic medical records of the patients included in the study. Collected data included age at the time of the ETZ, menopausal status, smoking, comorbidity (diabetes, HIV, and immunosuppression), HPV vaccination status, baseline HPV status, histological diagnosis (biopsies and ETZ specimen), excisional specimen margin status, post-ETZ HR-HPV/cytology/biopsy/colposcopy results from follow-up visits, time of first follow-up visit, follow-up time, and time until recurrent/persistent disease.

Outcome

The outcome assessed was histopathological recurrence/persistence of CIN2+ after ETZ. It was defined as a diagnosis of CIN2+, histologically confirmed with a biopsy, in a new ETZ or hysterectomy specimen during follow-up.

We analyzed the HR-HPV, cytology and colposcopy results from follow-up visits. The protocol of follow-up in our institution includes HPV, cytology, and colposcopy 6–12 months after treatment. Currently, according to our institution's protocol, the first visit is performed at 6 months; previously, it used to be performed at 12 months if the margins were negative and at 6 months if positive.

The HPV test in use at our institution is the Roche cobas HPV (Roche Diagnostics, Indianapolis, IN), which includes detection of HPV 16, 18, and others (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68). Cytology was classified according to the Bethesda criteria[16] and was considered positive if it was reported as atypical squamous cervical – undetermined significance (ASC-US) or worse. Colposcopy was considered positive if clinically described as "abnormal," including the findings of minor or major changes (grade 1 or 2, respectively, according to the International Federation for Cervical Pathology and Colposcopy nomenclature).[17] If changes are encountered during a follow-up colposcopy, biopsies are recommended. To minimize information bias, all results (apart from colposcopy) were confirmed directly from the laboratory reports, in addition to the follow-up visit medical notes.

Statistical Analysis

Characteristics of the population were summarized using descriptive statistics. Patients lost to follow-up were excluded from the analyses. To focus on the study aim of evaluating the utility of different follow-up strategies, including HPV detection, cytology, and colposcopy, as well as their combinations, in predicting recurrent/persistent disease after ETZ, we calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with exact binomial 95% CIs. The gold standard was the histopathological confirmation of CIN2+, as previously stated.

We defined a positive cotesting if: 1) the HPV test was positive and the cytology was ASC-US or worse, or 2) the HPV test was positive for HPV16/18, regardless of the cytology result.

We plotted 2 cumulative recurrence-free survival functions, 1 for patients with isolated HPV 16/18 infection at baseline and another for infection with other high-risk genotypes. For this analysis, patients whose HPV tests, from at least 2 follow-up visits, did not yield results were excluded to minimize verification bias. This bias arises because, typically, only patients with positive test results are verified with a gold standard test, so having 2 negative HPV tests could reduce the risk of missing a recurrence case. With this, we aimed to analyze the influence of the persistent positivity/clearance of different HPV genotypes during follow-up, in the recurrence rate of CIN2+. Baseline was defined as the date of the surgical treatment. Hazard ratio (HR) and corresponding 95% CI were estimated by Cox proportional hazards regression. All statistical analyses were performed using IBM SPSS Statistics version 29.0.0 (IBM, Armonk, NY). For all tests, the level of significance was set at 0.05.

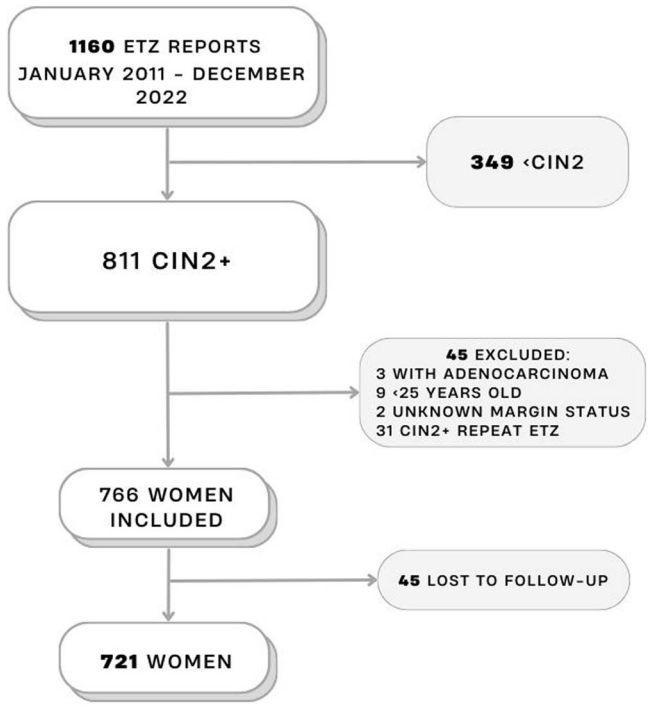

FIGURE 1. Patient selection.

RESULTS

During the specified time frame, 1,160 ETZs were performed, of which, 766 fulfilled the selection criteria. Forty-five (5.9%) of these were lost to follow-up (Figure 1).

The median age of all patients was 39 years (interquartile range [IQR] = 33.0–47.0) and ranged between 25 and 81 years. The median follow-up time was 24 months (IQR = 13.0–34.0).

At baseline, 93.2% (672/721) of the patients were positive for HR-HPV, 2.2% (16/721) were negative, and in 4.6% (33/721), that information was not available.

Recurrence/persistence of CIN2+ occurred in 6.8% (49/721) of cases. The median time to diagnosis of HSIL after the ETZ was 16 months (IQR = 7.0–26.3). Of the 49 women who developed posttreatment CIN2+, 39 were HPV positive during follow-up, and 1 was negative (with HSIL cytology); 9 cases were not considered because the ETZ was followed by a hysterectomy (18.4%). Human papillomavirus 16 was the most commonly identified HPV genotype during the follow-up in women with posttreatment CIN2+ (23/40 [57.5%]), and at their baseline as well (24/44 [54.5%]). In 12.5% (5/40), there was an acquisition of a new HPV16 infection, with clearance of a previous non-HPV16/18 infection in 1 case (1/40), representing a genotype switch. Epidemiological data, as well as data related with histopathological findings, are summarized in Table 1.

The sensitivity, specificity, PPV and NPV for different follow-up approaches (isolated tests or combination of tests) to predict recurrent disease after treatment are listed in Table 2. The use of isolated HPV test sensitivity and specificity was 97.4% and 80%, respectively, whereas for cotesting these figures were of 86.8% and 90.1%. The first strategy leads to the referral for colposcopy of 24.3% of the patients, whereas cotesting decreases this figure to 14.2%. In a scenario in which the referral threshold is a positive HPV test or an abnormal cytology, the sensitivity is the same as for isolated HPV test, but the specificity was lower (72.9%).

While all strategies had a very high NPV (>95%), the PPV was more variable, being higher for cotesting and colposcopy (34.4 and 32.0%, respectively).

The rate of recurrence was 42.6% (23/54), 7.7% (1/13), and 10.2% (15/147) for women who were positive for HPV16, 18, or others, respectively, during follow-up.

In Figure 2, the plots revealed that patients who tested positive for HPV16/18 during follow-up have a shorter recurrence-free survival time than those positive for other HR genotypes or HPV negative. Compared with women who tested positive only for HR-HPV genotypes other than HPV16/18 during baseline and follow-up, those who were initially positive for non-16/18 HR genotypes at baseline, and who became HPV16/18 positive during follow-up, had a statistically significant increased risk of CIN2+ (hazard ratio = 4.98; 95% CI = 1.66–14.91) (Figure 2A).

DISCUSSION

Our data confirm that HPV test is a fundamental part of the follow-up strategy after treatment of cervical HSIL. Adding cytology (cotesting) can decrease the referral rate for colposcopy by more than 40%, at the cost of a 10% loss of sensitivity. Colposcopy can be safely avoided at follow-up if the HPV test is negative, as long as pretreatment HPV status is known. Our data confirm current knowledge that HPV16/18 before treatment of HSIL is a risk factor for persistence, but also add that a new HPV16/18 infection after ETZ is associated with an increased risk, even in the short term.

In the present study, 6.8% of patients had persistent/recurrent disease in the follow-up period, which is in line with other studies.[4–8] In addition, 46.9% of recurrent cases tested positive for HPV16 during the follow-up period, emphasizing the well-known role of this genotype in HSIL recurrence and cervical cancer.[18] Interestingly, we found that women positive for HR-HPV, excluding HPV16/18 at baseline, and who later became positive for HPV16/18 during follow-up, were nearly 5 times more likely to develop CIN2+ when compared with women who remained positive only for other HPV genotypes during baseline and follow-up. The explanation of such finding is not straightforward, given that persistence is needed for the development of HSIL. We theorize that a woman who already had an HSIL is probably more susceptible to the development of new lesions, there may be a persistence of risk factors (i.e., smoking), or that the persistence of an unfavorable cervicovaginal milieu (including the microbiome) may contribute for the rapid development of new lesions.[19] This finding may be an additional argument to recommend HPV vaccination in women with HSIL and previously unvaccinated, regardless of the involved genotypes. The ongoing trials will likely provide a definite answer to this question.

Because the risk of developing CIN2+ is higher in patients treated for CIN2 or CIN3 than in the general population, it is of great importance to use a very sensitive test in the follow-up to detect recurrences early. Current American Society for Colposcopy and Cervical Pathology guidelines recommend that the follow-up after treatment for CIN2 or CIN3 should be HPV-based. In our clinical setting, as in many others, the preferred test of cure has been cotesting. There are still many questions regarding the reliability of isolated HPV test. However, studies have shown that the diagnostic performance of HPV test alone is equivalent to cotesting with cytology and HPV.[5,20,21] Our analyses showed that HR-HPV testing had a slightly higher sensitivity than cotesting for predicting recurrence (97.4% vs 86.8%), but the HPV test specificity was slightly lower (90.1% vs 80.0%).

Combining colposcopy and cotesting increased the sensitivity to 100%. Nevertheless, it implies a longer examination duration and potentially more discomfort, the need for specialized personnel (colposcopists and trained nurses), equipment and facilities.

A strategy of referring all cases with a positive HPV test or an abnormal cytology showed a reduced gain in sensitivity, at the expense of a loss of specificity and, consequently, a lower PPV. Therefore, isolated HPV testing or triage of the HPV-positive cases with cytology (cotesting) are superior strategies for follow-up after treatment of CIN2 or CIN3. This strategy could minimize the number of performed colposcopies without missing cases of HSIL because those that are HPV positive will be kept under tight follow-up and will be submitted to colposcopy if they are again positive, regardless of the cytology results.

In well-organized screening programs, the follow-up test can be performed out of the colposcopy units. Nevertheless, it must be kept in mind that these women remain at increased risk not only of cervical disease, but also of other anogenital HPV-related malignancies.[22–24] To consider such option, more training in vulvovaginal and anal diseases must be implemented.[25]

Long-term follow-up data are crucial to evaluate the long-term outcome of the different follow-up strategies. This landscape is constantly evolving, and different cohorts will coexist: women vaccinated during childhood, vaccinated at later age, vaccinated in the context of an HSIL diagnosis, etc. This may translate in different efficacy and needs in terms of follow-up. Moreover, the role of the new biomarkers (i.e., p16/Ki67 and methylation) deserves further investigation in this context.

In our series, in 9 cases, hysterectomy was performed after an EZT. These were cases in which the margins were positive or in which the EZT was performed in women who had another indication for hysterectomy, and invasive cervical disease needed to be excluded. The former indication is no longer acceptable according to current knowledge and protocols in use—if needed and feasible, a second EZT is preferred.[26]

TABLE 1. Characteristics of the Women Enrolled in the Study, According to the Development of Recurrence/Persistence of Cervical High-Grade Squamous Intraepithelial Lesions (HSIL) After Excisional Treatment

	Total (n = 721)	No recurrent/persistent residual CIN2+ (n = 672)	Recurrent/persistent residual CIN2+ (n = 49)
Age at excision of the transformation zone, y			
Age range: 25–81			
Age (median ± IQR)	39.0 (33.0–47.0)	39.0 (33.0–46.0)	48.0 (37.0–55.5)
25–30	93 (12.9)	92 (13.7)	1 (2.0)
30–50	507 (70.3)	479 (71.3)	28 (57.1)
50–65	113 (15.7)	98 (14.6)	15 (30.6)
65+	8 (1.1)	4 (0.6)	4 (8.2)
Time for first follow-up visit (n = 706) (median ± IQR)	12.0 (6.0–12.0)	12.0 (6.0–12.0)	6.0 (6.0–12.0)
Menopausal status			
Nonmenopausal	593 (82.2)	564 (83.9)	29 (59.2)
Menopausal	123 (17.1)	103 (15.5)	20 (38.8)
NA	5 (0.7)	5 (0.7)	0 (0.0)
Smoking			
Nonsmoker	447 (62.0)	418 (62.2)	29 (59.2)
Current smoker	256 (35.5)	238 (35.4)	18 (36.7)
Former smoker	13 (1.8)	12 (1.8)	1 (2.0)
NA	5 (0.7)	5 (0.7)	0 (0.0)
Comorbidity			
None	669 (92.8)	626 (93.2)	43 (87.8)
HIV infection	16 (2.2)	13 (1.9)	3 (6.1)
Diabetes	17 (2.4)	17 (2.5)	0 (0.0)
Immunocompromised	15 (2.1)	12 (1.8)	3 (6.1)
NA	5 (0.7)	5 (0.7)	0 (0.0)
Pretreatment HR-HPV			
Negative	16 (2.2)	15 (2.0)	1 (2.0)
Positive	672 (93.2)	629 (82.1)	43 (87.8)
HPV 16/18	350 (48.5)	181 (23.6)	15 (30.6)
HR-HPV others	476 (66.0)	448 (66.7)	28 (57.1)
HPV 16/18 only	196 (27.2)	181 (26.9)	15 (30.6)
HR-HPV other than HPV16/18 only	322 (44.7)	304 (45.2)	18 (36.7)
Unknown	33 (4.6)	28 (3.7)	5 (10.2)
Pretreatment histology (biopsy)			
Normal	13 (1.8)	12 (1.8)	1 (2.0)
HPV cytopathic effect	6 (0.8)	6 (0.9)	0 (0.0)
CIN1	55 (7.6)	51 (7.6)	4 (8.2)
CIN2	264 (36.6)	252 (37.5)	12 (24.5)
CIN2/3	83 (11.5)	81 (12.1)	2 (4.1)
CIN3	151 (20.9)	137 (20.4)	14 (28.6)
Inconclusive	6 (0.8)	5 (0.7)	1 (2.0)
NA	143 (19.8)	128 (19.0)	15 (30.6)
HPV Vaccination			
Nonvaccinated	528 (73.2)	496 (73.8)	32 (65.3)
Vaccinated before treatment	40 (5.5)	39 (5.8)	1 (2.0)
Vaccinated after treatment	148 (20.5)	132 (19.6)	16 (32.7)
NA	5 (0.7)	5 (0.7)	0 (0.0)
Histology (ETZ)			
CIN2	294 (40.8)	278 (41.4)	16 (32.7)
CIN2/3	85 (11.8)	80 (11.9)	5 (10.2)
CIN3	342 (47.4)	314 (46.7)	28 (57.1)
Margins			
Negative	608 (84.3)	583 (86.8)	25 (51.0)
Positive	113 (15.7)	89 (13.2)	24 (49.0)

CIN indicates cervical intraepithelial neoplasia, ETZ, excision of the transformation zone; IQR, interquartile range; NA, not available.

TABLE 2. Performance of Different Follow-Up Strategies for the Diagnosis of Persistent/Recurrent HSIL After ETZ at First Follow-Up Visit for 706 Women, Being Cervical Intraepithelial Neoplasia (CIN) 2 or Worse at Any Point of Follow-Up the Endpoint

	% Of positive tests	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive predictive value, % (95% CI)	Negative predictive value, % (95% CI)
HR-HPV	24.3% (166/682)	97.4% (88.9–99.8)	80.0% (76.8–82.9)	22.3% (16.4–29.0)	99.8% (99.1–100.0)
Cytology	18.0% (126/698)	71.4% (56.8–83.5)	85.4% (82.5–87.9)	23.8% (16.9–31.7)	97.9% (96.5–98.9)
Colposcopy	8.3% (25/302)	40.0% (20.7–61.7)	94.0% (90.8–96.4)	32.0% (16.1–51.4)	95.7% (92.8–97.7)
HR-HPV/cytology	31.1% (210/676)	97.4% (88.9–99.8)	72.9% (69.3–76.2)	17.6% (12.9–23.1)	99.8% (99.1–100.0)
HPV/cytology/colposcopy	32.9% (97/295)	100.0%	72.0% (66.5–77.1)	20.6% (13.4–29.4)	100.0%
HR-HPV/colposcopy	28.3% (84/297)	100.0%	76.9% (71.7–81.6)	23.8% (15.6–33.6)	100.0%
Cytology/colposcopy	24.0% (72/300)	85.0% (65.6–96.0)	80.4% (75.4–84.7)	23.6% (14.8–34.2)	98.7% (96.6–99.7)
HR-HPV + cytology (cotesting)[a]	14.2% (96/676)	86.8% (73.8–95.1)	90.1% (87.7–92.3)	34.4% (25.4–44.2)	99.1% (98.2–99.7)

Test1/test2 (at least 1 positive).

[a]HR-HPV positive and cytology ≥ASC-US or HPV16/18 and any cytology result.

The main limitation of our study is its retrospective nature. This may have caused a selection bias because we were restricted to patients who had their follow-up at our institution. In addition, the follow-up protocols have had changes along the years. For instance, in recent years, there is a trend to discharge patients after 1 negative HPV test at 6 months (referred to their primary care physician to repeat it within 1 year), whereas before, 2 or even 3 tests were requested before discharge. This may have limited our ability to assess for persistence/recurrence after treatment because late recurrences are virtually not considered.

Missing data is another limitation that comes from our study design; the reasons for this could not always be fully understood, and thus neither the level of bias it may have introduced. For instance, colposcopy was performed and recorded in less than half of the women during follow-up. For diagnostic performance analyses, 89% of our sample was tested for HPV on the first visit, and 91.1% had a cytology. However, missing data did not exceed 15%–20%.[27] Another concern, which was briefly introduced in the Methods section, was verification bias because only patients with positive HPV or cytology and/or "abnormal" colposcopy findings are verified with a gold standard test (histology). This could lead to an underestimation of false negatives in our study.

However, overall, our study is representative of our population, and the results can be extrapolated to similar populations

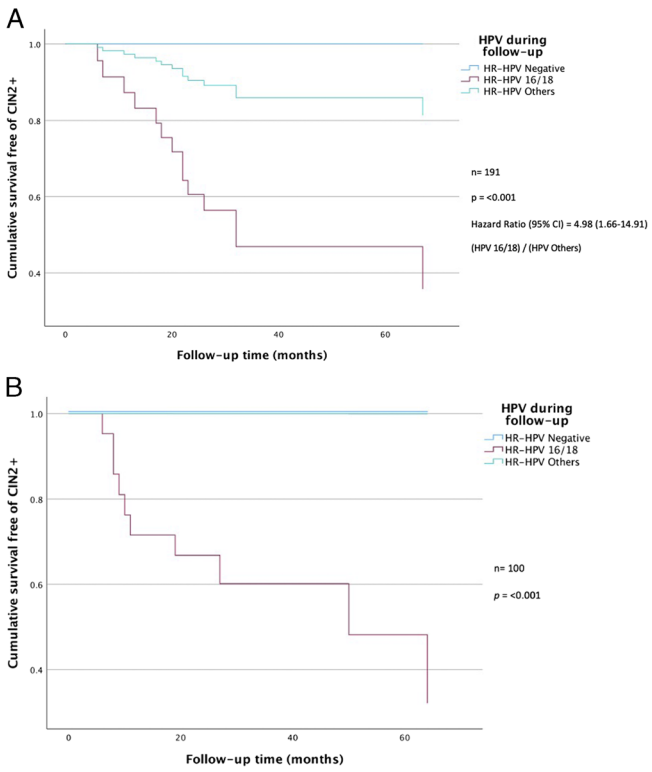

FIGURE 2. Recurrence-free survival: A, Baseline isolated other HR-HPV positive. B, Baseline isolated HR-HPV 16/18 positive. HPV, human papillomavirus; HR, high risk.

and comparable follow-up settings. The percentage of patients lost to follow-up was small (5.9%). Another strength of our study is that many women had colposcopy as part of their routine care during follow-up, allowing to evaluate the weight of its possible added value. These colposcopies, however, were performed "blindly" for the result of the cytology and HPV test because they were performed at the time of sampling.

CONCLUSION

Human papillomavirus testing is the best strategy for follow-up after treatment of cervical HSIL. The addition of cytology triage of the positive cases may halve the referrals for colposcopy without significantly missing cases of recurrence or persistence.

Human papillomavirus 16/18 in the follow-up, regardless of being previously positive, is associated with higher risk of recurrence/persistence of HSIL.

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