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Number of colposcopic cervical biopsies and diagnosis of cervical intraepithelial neoplasia: a prospective study

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Objective: To define the relationship between the number of cervical colposcopic biopsies performed on a patient and the diagnosis of each grade of cervical intraepithelial neoplasia (CIN). *Methods*: Patients who underwent a colposcopy and biopsy between January and June 2018 in an Italian second-level check-point for cervical cancer screening were prospectively enrolled in the study. Cervical punch biopsies were performed on abnormal acetowhite areas that were identified by colposcopy and endocervical sampling was performed if needed. The number of cervical biopsies per patient was recorded along with the following parameters: type of transforming zone, colposcopic grading, Pap smear result, the patient's age, and endocervical sampling. All parameters were included in multivariable models. The dependent variable was a diagnosis of CIN-0/1, CIN-2, or CIN-3. *Results*: Independently of other variables, a Pap test result of atypical squamous cells—cannot be excluded H-SIL (ASC-H), atypical glandular cells, not otherwise specified (AGC-NOS), or high grade squamous intraepithelial lesion (H-SIL) is associated with reduced odds of a CIN-0 or CIN-1 diagnosis. More than one cervical biopsy per patient is associated with reduced odds of a CIN-0 or CIN-1 diagnosis whereas three or four biopsies is associated with increased odds of a CIN-2 diagnosis. A Pap test result of HSIL, ASC-H, or AGC-NOS is the only variable that increased the odds of a CIN-3 diagnosis. *Discussion*: A greater number of cervical biopsies performed on a patient increases the likelihood of diagnosing a CIN-2 but has no effect on the diagnoses of CIN-0/1 or CIN-3.

Keywords

Cervical biopsy; Colposcopy; Cervical intraepithelial neoplasia

1. Introduction

Since the introduction of regular Pap smear screening, the number of patients diagnosed with cervical cancers has decreased. In the most recent decades, additional tools have been added to conventional cytology slides that improve the accuracy of Pap smears. Tools such as liquid-based cytology and the human papillomavirus (HPV) DNA test increase the sensitivity of Pap smears in detecting HPV related lesions while also reducing the frequency of false-negative test results and unclear cytologic patterns. For these reasons, liquid-based cytology and the HPV DNA test are included in the current cervical screening plans in many countries [1].

Colposcopy is the second step of cervical cancer screening. International guidelines recommend that all cases of abnormal cytology, including both those without a HPV test and those with a positive HPV test for high-risk HPV viruses, are referred for a colposcopy. If co-testing (a HPV test and Pap smear cytology) of a patient reveals HPV-negative Log grade squamous intraepithelial lesion (LSIL), physicians may choose to repeat co-testing of the patient the following year, although a colposcopy with biopsies of the abnormal areas are preferred under most guidelines [2–6]. When histological cervical intraepithelial neoplasias (CINs) are identified, loop excision is usually recommended for severe lesions (CIN-2+) [2–4, 6].

While CINs introduce a small risk of preterm delivery, a history of previous ablative and excisional treatments on the cervix for any grade of CIN increases that risk [7]. As such, there are concerns about the extension of a treatment for CIN in younger women [2]. A minimum excision treatment may prevent premature delivery [8, 9]. However, there is a greater risk of CIN recurrence if not all of the abnormal tissue is removed within the cone margins involved in the dysplasia [10–12]. When no obvious transforming zone is detected during the colposcopy, a deeper excision is needed [3], while the discovery of a wide abnormal transforming zone justifies a wide excision. Additionally, due to the poor concordance in diagnosing abnormal colposcopic areas [13], there is a risk that severe lesions may not be removed in minimal cervical excisions. According to Tainio *et al.* [14], the rate of spontaneous remission for histologically confirmed CIN-2 is high (about 50% after 12 and 24 months) while the progression to CIN-3 or a worse grade is approximately 14% after 12 months and 18% after 24 months. Additionally, vaccinating patients with the quadrivalent HPV vaccine after they receive treatment for CIN-2/3 has been reported to prevent recurrences [15, 16]. Therefore, this vaccine may be considered as a precautionary measure to prevent recurrences of CIN-2/3.

An accurate diagnosis of CIN grade is necessary to minimize the risk of CIN recurrence after a minimal excision treatment. The subjectivity of colposcopies can easily result

in a misdiagnosis of CIN-2 [13]. On the other hand, performing several cervical biopsies on a patient increases the likelihood of a CIN-2 diagnosis [13], resulting in more surgical treatments according to some guidelines [2–4, 6]. In Italy, caregivers legally must follow the CIN treatment guidelines established by their regional government even if they disagree with the treatment plan for ethical reasons. With no treatment, half of CIN-2 lesions regress within 12 to 24 months and there is a minimal risk of the lesion progressing to a worse grade of CIN [14]. Therefore, missing a diagnosis of CIN-2 may be beneficial as invasive treatments could be avoided at a minimal risk to the patient. Additionally, patients would have less anxiety without the CIN-2 diagnosis and caregivers would not have legal issues with their Italian regional law establishments.

The aim of this study is to determine whether there is an association between the number of cervical colposcopic biopsies per patient and the number of diagnoses for each grade of CIN, independently of other factors.

2. Patients and methods

This study has been conducted in compliance with the Helsinki declaration and did not interfere with the screening and follow-up protocol approved by the Emilia Romagna legislation for CINs and cervical cancer. All patients who underwent colposcopic examinations at the colposcopic unit of Ferrara (Emilia-Romagna, Italy) between January 2018 and June 2018 were included in the study. Colposcopic examinations were performed either as part of the regional screening program for cervical cancer or as follow-up after treatment for CINs. During this period, 624 patients evaluations were recorded. Patients with only vaginal biopsies were excluded, as were patients for whom colposcopic assessments were not performed.

The colposcopic examinations were performed by applying a 5% acetic acid solution swab to the cervix and vagina, followed by Lugol's solution. The colposcopic assessment followed the acknowledged terminology for colposcopy [17]. Pap smear international terminology [18] was used to diagnose cervical lesions. Pap smears were sampled both from the external cervix and within the cervical channel and stored in a liquid-based box (ThinPrep LBC, Cytyc Corp., Boxborough, MA, USA) for reading.

Cervical punch biopsies were performed on acetowhite areas as identified by colposcopic patterns. Endocervical curettage were performed as required in cases of transformation zone type 2 and 3. The regional flow-charts for cervical cancer screening and intracervical neoplasia treatment are reported at the reference number [19].

The number of cervical biopsies (sum of exocervical biopsies and curettage) per patient was recorded along with the following parameters: type of transforming zone (type 1, 2, 3), grade of colposcopy (normal or iodine-negative acetic mute area), reason for admission to colposcopy (screening Pap smear resulting in LSIL, ASC-US, ASC-H, HSIL, AGC-NOS, positive HPV test, follow-up after treatment for CIN), the patient's age, and endocervical sampling (yes/no). These parameters were considered as independent variables in our logistic regression models and were chosen based on our hypothesis that they will affect the proportion of CIN diagnoses. The dependent variables were the diagnosis of any grade of CIN (CIN-1: yes/no; CIN-2: yes/no; CIN-3: yes/no). In cases where multiple biopsies of the same patient resulted in different grades of CINs, the most severe diagnosis was recorded for that patient. The logistic regression (Backward Stepwise Wald) models were built by introducing the variables resulting in a p level $\leq$0.250 at univariate analyses. SPSS 16.0 (IBM®, Armonk, NY, USA),was used for calculations.

3. Results

Between January 2018 and June 2018, 270 biopsies were collected. Among these biopsies, 244 were included in this study. Twenty-six biopsies were excluded from this study including 23 cases of vaginal biopsies, 1 case of a clinically diagnosed cervical cancer, 1 case of a bleeding ectropion with a negative Pap smear, and 1 case of a cervical wart and no screening Pap smear. The descriptive statistics of the biopsy samples are reported in Table 1 according to the diagnosis of CIN.

Sensitivities for detecting CIN-1 were 63.8% (95/149) with only one biopsy, 27.5% (41/149) with two biopsies, 6.7% (10/149) with three biopsies and 2.0% (3/149) with four biopsies. Sensitivities for detecting CIN-2 were 33.3% (10/30) with only one biopsy, 36.7% (11/30) with two biopsies, 26.7% (8/30) with three biopsies and 3.3% (1/30) with four biopsies. Sensitivities for detecting CIN-3 were 28.6% (6/21) with only one biopsy, 38.1% (8/21) with two biopsies, 14.3% (3/21) with three biopsies and 19.0% (4/21) with four biopsies.

The results from the univariate analysis for an outcome of CIN-0 or CIN-1 are reported in Table 2. Among the variables with a $p \leq 0.250$ from the univariate analysis, only the number of biopsies and the Pap test result were associated with a diagnosis of CIN-0/1 with the multivariable analysis (Table 3). A Pap test result of ASC-H, AGC-NOS or HSIL reduced the odds ratio of diagnosing a CIN-0 or CIN-1 while more than a single biopsy reduced the odds ratio of diagnosing a CIN-0 or CIN-1 at multivariable assessment (Table 3).

The results from the univariate analysis for an outcome of CIN-2 are reported in Table 4. On univariate analysis, the number of cervical biopsies per patient was the only parameter that was associated with a higher odds ratio of diagnosing a CIN-2 with $p \leq 0.250$. Therefore, a multivariate analysis was not needed.

The results from the univariate analysis for an outcome of CIN-3 are reported in Table 5. On univariate analysis, the Pap smear result was the only parameter that was associated with a higher odds ratio of diagnosing CIN-3 with $p \leq 0.250$. Specifically, a Pap test result of H-SIL, ASC-H, AGC-NOS ($p < 0.001$) increased the odds ratio of diagnosing a CIN-3 (a multivariate analysis was not needed).

Table 1. Descriptive statistics.

	CIN-1 or no CINs	CIN-2	CIN-3
	193	30	21
Admission to colposcopy			
L-SIL/ASC-US*/HPV+	157 (81.3%)	21 (70%)	3 (14.3%)
ASC-H/AGC-NOS/H-SIL	18 (9.3%)	8 (26.7%)	16 (76.2%)
Follow-up after treatment	13 (6.7%)	1 (3.3%)	2 (9.5%)
Colposcopic pattern			
Normal or iodine negative acetic-mute area	31 (16.1%)	2 (6.7%)	2 (9.5%)
Grade 1 colposcopy	134 (69.4%)	22 (73.3%)	8 (38.1%)
Grade 2 colposcopy	28 (14.5%)	6 (20.0%)	11 (52.4%)
Number of biopsies			
1	132 (68.4%)	10 (33.3%)	6 (28.6%)
2	46 (23.8%)	11 (36.7%)	8 (38.1%)
3 or 4	15 (7.8%)	9 (30.0%)	7 (33.3%)
Endocervical sampling			
Yes	63 (32.6%)	12 (40.0%)	11 (52.4%)
No	130 (67.4%)	18 (60.0%)	10 (47.6%)
Transforming zone type			
3	53 (27.5%)	6 (20.0%)	6 (28.6%)
2	41 (21.2%)	4 (13.3%)	3 (14.3%)
1	99 (51.3%)	20 (66.7%)	12 (57.1%)
Mean age	42.74 ± 14.0	39.3 ± 9.8	39.1 ± 9.5

*ASC-US: atypical squamous cells of undetermined significance.

Table 2. First model. Dependent variable: CIN-0/CIN-1 univariate results.

	Odds ratio	95% confidence intervals	p
Type of transforming zone			
3	1		
2	1.534	0.543–4.332	0.419
1	2.525	0.820–7.776	0.107
Grade of colposcopy			
Normal or iodine negative acetic-mute area	1		
1	1.071	0.270–4.247	0.922
2	1.032	0.210–5.076	0.969
Number of biopsies			
1	1		
2	0.388	0.157–0.962	0.041
3 or 4	0.196	0.068–0.563	0.002
Endocervical sampling	0.663	0.253–1.753	0.403
Admission to colposcopy			
-Screening pap L-SIL or ASC-US, or HPV test +	1		
-H-SIL, ASC-H, AGC-NOS	0.167	0.072–0.389	<0.001
-Follow-up after treatment	1.075	0.270–4.281	0.919
Age	1.017	0.982–1.054	0.343

Hosmer-Lemeshow test: $p = 0.277$.

4. Discussion

The results of this study are consistent with the existing literature [20–29]. The main aim of previous studies was to detect all cervical lesions with a grade of CIN-2 or greater to treat the lesions. However, conservative management of CIN-2 in younger women has also been discussed and assessed [30]. Instead of excising CIN-2 lesions in young patients, Silver *et al.* [30] chose a strict follow-up protocol to monitor the lesions for progression. More research is needed to determine whether such prolonged management is necessary following a negative co-test.

Our study demonstrates that the number of biopsies per patient is independently associated only with the diagnosis of CIN-2 and not with the diagnosis of CIN-3. The odds ratio of diagnosing a CIN-2 is directly related to the number of colposcopic biopsies taken from a patient's cervix. More-

Table 3. First model. Dependent variable: CIN-0 / CIN-1 multivariate results.

	Odds ratio	95% confidence intervals	*p*
Number of biopsies			
1	1		
2	0.365	0.167–0.794	0.011
3 or 4	0.164	0.065–0.417	<0.001
Admission to colposcopy			
-Screening pap L-SIL or ASC-US, or HPV test +	1		
-H-SIL, ASC-H, AGC-NOS	0.174	0.081–0.376	<0.001
-Follow-up after treatment	1.066	0.275–4.133	0.927

Hosmer and Lemeshow: $p = 0.857$.

Table 4. Second model. Dependent variable: CIN-2 univariate results.

	Odds ratio	95% confidence intervals	*p*
Type of transforming zone			
3	1		
2	0.502	0.147–1.711	0.271
1	0.441	0.122–1.597	0.212
Grade of colposcopy			
Normal or iodine negative acetic-mute area	1		
1	1.359	0.230–8.040	0.735
2	0.628	0.080–4.948	0.658
Number of biopsies			
1	1		
2	2.950	1.043–8.348	0.041
3 or 4	6.076	1.830–20.168	0.003
Endocervical sampling	1.550	0.514–4.674	0.436
Admission to colposcopy			
-Screening pap L-SIL or ASC-US, or HPV test +	1		
-H-SIL, ASC-H, AGC-NOS.	1.254	0.449–3.501	0.666
-Follow-up after treatment	0.345	0.041–2.935	0.330
Age	0.996	0.955–1.038	0.842

Hosmer and Lemeshow test: $p = 0.751$.

Table 5. Third model. Dependent variable: CIN-3 univariate results.

	Odds ratio	95% confidence intervals	*p*
Type of transforming zone			
3	1		
2	0.994	0.238–4.150	0.929
1	0.525	0.104–2.643	0.435
Grade of colposcopy			
Normal or iodine negative acetic-mute area	1		
1	0.637	0.081–4.984	0.667
2	1.948	0.223–17.035	0.547
Number of biopsies			
1	1		
2	1.526	0.352–6.616	0.572
3 or 4	1.534	0.306–7.690	0.603
Endocervical sampling	1.010	0.273–3.737	0.988
Admission to colposcopy			
-Screening pap L-SIL or ASC-US, or HPV test +	1		
-H-SIL, ASC-H, AGC-NOS	29.465	7.052–123.102	<0.001
-Follow-up after treatment	5.724	0.838–39.079	0.075
Age	0.966	0.912–1.023	0.240

Hosmer and Lemeshow test: $p = 0.668$.

over, the degree of severity of the colposcopy is not associated with a higher odds ratio of diagnosing a CIN-2 or CIN-3 which suggests that the specificity of colposcopies is poor, as has been previously suggested [13, 24]. Therefore, it should be assessed whether missed detection of CIN-2 lesions result in a worse prognosis if they are treated more conservatively as lower grade cervical lesions. To that end, Skorstengaard *et al.* [31] suggested that half of women with CIN-2 can be managed conservatively with minimal risk to the patient. Missing some CIN-2 lesions by performing only one biopsy instead of several per patient can also reduce patients' anxiety along with practitioners' medical liability in the context of Italian law.

The behavior of colposcopists when performing cervical biopsies and endocercervical curettage is inconsistent, with various techniques, number of biopsies, and rationale disclosed by colposcopists in a British survey [32]. This inconsistent behaviour would affect the number of diagnoses of CIN-2, explaining the heterogeneity found by Tainio *et al.* [14] in their meta-analysis on the proportion of CIN-2 remission. Therefore, is hard to generalize the finding of the present study. Moreover, the present study has a low number of CIN-2 cases (12.3%). It would be of interest to compare the number of CIN-2 lesions detected with the first biopsy in patients with multiple biopsies to the number with CIN-2 lesions detected in patients with a single biopsy. This analysis may provide a reliable estimation for the number of missed CIN-2 lesions. Unfortunately, we were unable to perform such an analysis in the present study as the specimens sent for pathological examination were not marked in the order of collection. Therefore, the pathologist was not able to determine which biopsy was collected first for patients with multiple biopsies.

Additional studies that compare longer term outcomes for patients with single biopsies to patients with multiple biopsies with a diagnosis of CIN-2 are needed to determine whether multiple biopsies improve health outcomes. Based on our results, we would expect that, with no invasive treatment, the rate of disease progression would be the same in patients with either one biopsy or several biopsies.

5. Conclusions

The number of cervical biopsies per patient is independently associated with the diagnosis of CIN-2. An approach of waiting for CIN-2 remission may be suggested for some younger patients instead of the invasive treatment that is currently required by Italian guidelines. Further studies, including a randomized, controlled trial that compares patient outcomes after single cervical biopsies versus multiple cervical biopsies, are needed to demonstrate whether missing some diagnoses of CIN-2 with fewer biopsies has any effect on the number of CIN-2 progressions and treatment rates over time.

Author contributions

UI designed the study, analyzed statistically and wrote the article; ES, PI, CB collected the data; PG supervised and interpreted this study. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study has been conducted in compliance with the Helsinki declaration and with no interference of the screening and follow-up protocol approved by the Emilia Romagna legislation for CINs and cervical cancer. Patients, at the time of colposcopic assessment, provided their signed consent to use their health data for scientific research.

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Conflict of interest

The authors declare no conflict of interest.

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