

1 **Recurrence rate after Loop Electrosurgical Excision Procedure (LEEP) and Laser Conization:**
2 **a 5-year follow-up study**

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76 **Highlights:**

- 77 - HPV persistence correlates with an increased risk of 5-year recurrence in women undergoing
78 cervical conization
- 79 - Patients having laser conization experience a slightly lower risk of cervical dysplasia
80 recurrence in comparison to LEEP
- 81 - Further evidence regarding fertility and obstetrical issues is necessary

82 **Abstract**

83 **Objective:** Conization aims to remove pre-neoplastic lesions of the uterine cervix. Several techniques
84 for conization have been compared, but evidence regarding the most effective therapeutic option is
85 scant. Here, we aimed to compare the recurrence rate following laser conization and loop
86 electrosurgical excision procedure (LEEP) in patients with high-grade cervical dysplasia
87 (HSIL/CIN2+).

88 **Methods:** This is a retrospective multi-institutional study. Medical records of consecutive patients
89 with HSIL/CIN2+ undergoing conization between 2010 and 2014 were retrieved. A propensity-score
90 matching (PSM) was applied in order to reduce allocation bias. The risk of developing recurrence
91 was estimated using Kaplan-Meier and Cox hazard models.

92 **Results:** Overall, 2,966 patients had conization over the study period, including 567 (20%) and 2,399
93 (80%) patients having laser conization and LEEP, respectively. Looking at predictors of recurrence,
94 diagnosis of CIN3 (HR:3.80 (95%CI:2.01,7.21); $p<0.001$) and HPV persistence (HR:1.81
95 (95%CI:1.11,2.96); $p<0.001$) correlated with an increased risk of recurrence. After applying a PSM
96 we selected 500 patients undergoing laser conization and 1,000 undergoing LEEP. Patients
97 undergoing LEEP were at higher risk of having positive surgical margins in comparison to patients
98 undergoing laser conization (11.2% vs. 4.2%). The risk of having persistence of HPV was similar
99 between the two groups (15.0% vs. 11.6%; $p=0.256$). Five-year recurrence rate was 8.1% and 4% after
100 LEEP and laser conization, respectively ($p=0.023$). HPV persistence was the only factor associated
101 with 5-year recurrence after both laser conization ($p=0.003$) and LEEP ($p=0.001$).

102 **Conclusions:** HPV persistence is the only factor associated with an increased risk of recurrence after
103 either laser conization or LEEP. Owing to the lack of data regarding obstetrical outcomes, we are not
104 able to assess the best therapeutic option for women with cervical dysplasia.

105 **Keywords:** Conization; HPV; laser conization; LEEP; cervical dysplasia.

106 **Introduction**

107 In the recent years, the widespread adoption of primary and secondary preventions has dramatically
108 reduced the incidence of cervical cancer in developed countries [1,2]. However, cervical cancer still
109 represents a major health concern, being the third most common malignancy among women aged <39
110 years, and the second most common cause of death for cancer among females between 20 and 39
111 years in the United States [1].

112 Cervical cancer is one of the most preventable types of cancer, since it develops over a long time and
113 the causative agent has been recognized [2]. Persistent infection from human papillomavirus (HPV)
114 is the main factor causing cervical cancer [3]. Generally, persistent HPV infection causes cervical
115 dysplasia (also known as cervical intraepithelial neoplasia), which potentially evolves in cancer.
116 Although the majority of women with HPV infection will never develop lesions, a relatively high
117 number of women is at risk of developing cervical dysplasia. Women with cervical dysplasia who
118 have appropriate follow-up and treatments are at low risk of developing cervical cancer [3]. However,
119 recurrent cervical dysplasia is a well-known risk factor for cervical cancer [3,4]. Additionally,
120 recurrent cervical dysplasia might be cause of morbidity since adjunctive surgical treatments are
121 associated with fertility and obstetrical issues in women who wish to preserve their childbearing
122 potential [5]. With this background, identifying the best treatment modality for patients with cervical
123 dysplasia is of paramount importance. Cervical conization allows to remove cervical lesions (that
124 might progress to cancers (in about 30% [4])) and provide a classification according to the histology
125 and depth of cervical invasion, thus potentially identifying patients who deserve further treatments.
126 Additionally, occult invasive cancer could be detected at the time of conization [6]. To date, few
127 researches have evaluated various excisional modalities for the management of cervical dysplasia [7-
128 10]. There are few studies comparing different techniques of cervical conization (including cold knife
129 conization, laser CO2 conization and loop electrosurgical procedure (LEEP)), reporting discordant
130 results [8-10]. The level of evidence is still scant since the majority of these studies are characterized

131 by a small sample size and a short-term follow-up [8-10]. In the present study, we aimed to evaluate
132 long-term (5-year) outcomes of patients with cervical dysplasia following laser conization and LEEP.
133 As secondary outcomes, we sought to identify specific risk factors for cervical dysplasia recurrence
134 in this subset of patients.

135 **Methods**

136 This is a retrospective multi-institutional study conducted in Italy. Institutional Review Board (IRB)
137 approval was obtained (IRB#6812). For the purpose of the present study, we collected chart of
138 patients with newly diagnosed high-grade cervical dysplasia (HSIL/CIN2+) treated in Italy from
139 01/01/2010 to 12/31/2014.

140 The inclusion criteria were: (i) newly diagnosed moderate /severe cervical dysplasia (HSIL/CIN2+);
141 (ii) squamous cell lesions; (iii) the execution of surgical excisional procedure (i.e., conization); (iv)
142 cervical conization performed with laser or LEEP; iv) conization performed between 2010 and 2014;
143 (vi) patients with available 5-year follow-up data (for non recurring patients; while, patients who
144 recurred were included even if they did not complete the five-year follow-up period). For the study
145 purpose only consecutive series of patients were accepted. Exclusion criteria were: (i) age <18 years;
146 (ii) consent withdraw; (iii) execution of ablative procedure; (iv) diagnosis of invasive cancer at the
147 time of conization; (v) execution of cold knife conization; (vi) glandular lesions; (vii) ongoing
148 pregnancy; and (viii) history of hysterectomy. The main outcome measure of this research was to
149 estimate the recurrence rate of women with cervical dysplasia undergoing either laser conization or
150 LEEP. Secondary outcome measure was to identify predictors of recurrence after laser conization and
151 LEEP. Generally, patients were treated on an outpatient basis using local anesthesia. Procedures were
152 performed under colposcopic guidance, using either laser or LEEP technique.

153 Demographic details, data about HPV type(s) detected, as well as data on treatment for the occurrence
154 of cervical dysplasia were retrospectively reviewed. HPV types were considered as high-risk in

155 according to the data of the International Agency for Research on Cancer (IARC) [11]. During the
156 study period, different surgeons perform all the procedures across the participant centers. However,
157 no differences in the facilities available for patients' care and in the referral patterns of various service
158 were present. Conization aimed to remove a cone-shaped section of the cervix surrounding the
159 endocervical canal, which includes the entire transformation zone. The technique for laser conization
160 and LEEP were standardized [12, 13]. Details about surgical treatments are reported elsewhere [12,
161 13]. The execution of laser conization instead of LEEP basically were basically depending on
162 available facilities of the participant centers and on surgeons' preferences. Laser conization was
163 performed using laser CO₂. Laser allows simultaneous photo-thermal ablation and coagulation.
164 Details of follow-up schedule and examination were reported elsewhere [11, 12]. According to
165 institutional protocols, patients were evaluated colposcopically in outpatients' clinic at 3 (in case of
166 positive margins) – 6 (in case of negative margins) months after conization. Briefly, patients had a
167 follow-up scheduled including Pap-smear, colposcopy and colposcopic-guided biopsy if clinically
168 indicated, every 6 months for the first 2 years, and annually thereafter (until 5 years). A dedicated
169 team of gynecologists performed all gynecological and colposcopic examinations. Generally, HPV
170 testing was performed at the first examination after conization in patients with documented HPV
171 infections. Persistence of HPV infection was defined as the persistence of HPV detected at the first
172 clinical examination following conization (generally at 6 months). Persistence / recurrence after
173 conization was defined as the diagnosis of a new HSIL/CIN2+ requiring secondary conization or
174 hysterectomy. Patients who did not have a secondary conization were considered free of recurrence.
175 Persistence of cervical dysplasia was defined by the diagnosis of HSIL/CIN2+ at the first evaluation
176 following conization; conversely, patients with recurrent cervical dysplasia had at least one negative
177 examination between conization and the diagnosis of HSIL/CIN2+. Low grade cervical lesions
178 (LSIL/CIN1) were not considered as recurrent disease.

179 **Statistical methods**

180 Data are summarized using basic descriptive statistics. Since this is a retrospective comparison
181 between two groups, possible allocation biases might impair the quality of the results reporting.
182 Therefore, we performed a propensity score analysis. Propensity-score analysis aims to reduce biases
183 arising from different covariates. In order to perform this analysis, we developed a multivariable
184 logistic regression model. Age, type of lesion (CIN2 vs. CIN3), menopausal status, execution of HPV
185 testing before conization (yes vs. no). Detailed description of PSM is described elsewhere [14].
186 Patients who had laser conization were matched 1:2 to a group of patients who had LEEP. PSM
187 analysis attempts to estimate the effect of a treatment by accounting for possible factors (e.g.,
188 constitutional variables) that predicts receiving the treatment, thus reducing possible inherent
189 selection biases of a retrospective study. We used a caliper width ≤ 0.1 standard deviations (SDs) of
190 the logit odds of the estimated propensity score. Basic descriptive statistics were used to describe the
191 two populations (patients undergoing LEEP and laser conization). Differences in categorical variables
192 were analyzed using the Fisher exact test. Odds ratio (OR) and 95% confidence intervals (95%CI)
193 were calculated for each comparison. T-test and Mann-Whitney test were used to compare continuous
194 variables as appropriate. Recurrence-free survival was estimated using Kaplan-Meier and Cox
195 models. The log-rank test was used to compare the risk of developing recurrence and the risk of death
196 between the two groups over the time. Hazard ratio (HR) and 95%CI were calculated for each
197 comparison. Univariate and multivariate analyses were performed when appropriate. All covariates
198 with a p value less than 0.10, based on univariate analysis were included in the multivariate model.
199 Disease-free survival and overall survivals were calculated starting from the date of primary surgery.
200 P values < 0.05 were considered statistically significant. Statistical analysis was performed with
201 GraphPad Prism version 6.0 (GraphPad Software, San Diego CA) and IBM-Microsoft SPSS version
202 20.0 (SPSS Statistics. International Business Machines Corporation IBM 2013 Armonk, USA) for
203 Mac.

204 **Results**

205 Overall, the medical records of 2,966 women undergoing conization for newly diagnosed
206 HSIL/CIN2+ in the years 2011-2014 were retrieved. The study population included 567 (20%) and
207 2,399 (80%) patients undergoing laser conization and LEEP, respectively. Figure 1 shows the flow
208 of patients through the study design. Baseline characteristics of the whole population are reported in
209 Table 1. Median (range) patients' age was 40 (range, 18-89) years. Reason for conization was CIN2
210 and CIN3 in 969 (32.7) and 1,984 (66.9%) patients. For the remaining 13 (0.4%) patients, surgical
211 indication was classified as HSIL. Overall, 175 women received a second conization within the first
212 5 years after primary treatment, with a recurrence rate of 6%. Median time to recurrence was 18
213 (range, 5-52) months. Most patients (5%) developed recurrence within the first 2 years; while only
214 1% of patients recurred between 24 and 60 months of follow-up. Secondary conization was
215 performed in 155 (6.4%) and 20 (3.5%) patients included in the LEEP and laser conization groups,
216 respectively (p=0.007). Looking at factors predicting HSIL/CIN2+ recurrence, we observed that type
217 of cervical dysplasia (HR: 1.68 (95%CI: 1.21, 2.33) per CIN3; p=0.002), preoperative detection of a
218 high-risk HPV types (HR: 2.69 (95%CI: 1.25, 5.81); p=0.011), positive surgical margins (HR: 2.44
219 (95% CI: 1.68, 3.55); p<0.001) HPV persistence (HR: 2.34 (95%CI: 1.64, 3.33); p<0.001) and type
220 of surgical approach (HR: 2.50 (95%CI: 1.99, 3.16); p=0.007) impacted on 5-year recurrence free
221 survival at univariate analysis (Table 2). Via multivariate analysis, only diagnosis of CIN3 (HR: 3.80
222 (95%CI: 2.01, 7.21); p<0.001) and HPV persistence (HR: 1.81 (95%CI: 1.11, 2.96); p<0.001)
223 correlated with an increased risk of recurrence. Although it did not reach statistically, significance at
224 multivariate analysis, type of surgical approach was slightly associated with the risk of recurrence
225 (HR: 1.28 (95%CI: 0.78, 2.69); p=0.071).

226 **Propensity-matched cohort**

227 In order to evaluate the role of laser conization and LEEP in a balanced analysis, we adopted a PSM.
228 Through PSM we selected a study population of 1,500 patients, matching (1:2) 500 undergoing laser
229 conization and 1,000 undergoing LEEP. Baseline patients' characteristics of patients included in the

230 PSM analysis are reported in Supplemental Table 1. As the results of PSM, patients undergoing laser
231 conization had similar characteristics than those undergoing LEEP. The prevalence of positive
232 surgical margins was higher among patients undergoing LEEP compared to patients in the laser
233 conization group (11.2% vs. 4.2%). Considering available specific data, positive endocervical margin
234 rate was 6.1% and 2.2% after LEEP and laser conization, respectively ($p<0.001$). Considering patients
235 with specific data available, persistence of HPV was similar between the two groups. Persistent HPV
236 infection was observed in 71 out of 472 patients included in the LEEP group, and 28 out of 241
237 patients included in the laser conization group (15.0% vs. 11.6%; $p=0.256$). Looking at the crude
238 number of recurrence within the first 5 years, secondary conization was performed in 8.1% and 4%
239 of women in the LEEP and laser conization groups, respectively ($p=0.003$). Figure 2 shows recurrence
240 free survival. Patients undergoing LEEP were at high risk of recurrence over the 5-year follow-up
241 compared to patients undergoing laser conization ($p=0.023$, log-rank test). Supplemental Figure 1
242 shows the 5-year recurrence rate according to positive margins and HPV persistence. Factors
243 predicting 5-year recurrence after laser conization and LEEP are reported in Supplemental Table 2
244 and 3. Among patients undergoing laser conization, HPV persistence was associate to a higher risk
245 of 5-year recurrence (HR: 15.0 (95%CI: 2.47, 91.1); $p=0.003$). Among patients undergoing LEEP,
246 factors associated with 5-year recurrence were: CIN3 (HR: 3.48 (95%CI: 1.67, 7.26); $p=0.001$),
247 positive margins (HR:1.76 (95%CI: 1.09, 2.45); $p=0.001$) and HPV persistence (HR: 1.6 (95%CI:
248 1.01, 2.86); $p=0.001$).

249 **Discussion**

250 The present study reports the recurrence rate after LEEP and laser conization in women diagnosed
251 with cervical dysplasia (HSIL/CIN2+). This retrospective multi-institutional study has collected data
252 of ~3000 women undergoing conization for whom 5-year follow-up is available. This is the largest
253 study investigating the impact of different surgical techniques for conization in patients with cervical
254 dysplasia. In order to reduce possible allocation biases, two propensity score algorithms were applied.

255 We observed a number of noteworthy findings. First, HPV persistence is the only factor associate
256 with an increased risk of recurrence, regardless type of surgical approach. Second, women undergoing
257 laser conization are at low risk of developing recurrent HSIL/CIN2+ in comparison to women
258 undergoing LEEP. Third, prevalence of positive margins (in particular endocervical ones) is lower in
259 patients having laser conization than LEEP; while HPV persistence rate is not influenced by the type
260 of surgical approach. Fourth, having positive margins is a significant risk factor for recurrence only
261 in patients who had LEEP; no association between positive margins and recurrence rate is observed
262 in the laser conization group.

263 Several investigations evaluated the role of various surgical techniques for conization with discordant
264 results [7-10]. Basically, most of these studies are characterized by a small sample size and short term
265 follow-up. Few randomized studies comparing laser conization and LEEP suggested the oncology
266 equivalence of these two techniques, but they were underpowered to demonstrate a significant
267 difference in recurrence rate [9, 10]. Mathevet et al., reported data of a randomized controlled trial of
268 86 patients, followed-up for at least 3 years [9]. Among those patients, 28, 29 and 29 women had cold
269 knife, LEEP and laser conization, respectively. The authors suggested that all procedures were
270 characterized by a similar recurrence rate. Cervical stenosis occurred in 0, 1, and 4 cases after laser
271 conization, LEEP, and cold knife group, respectively. Additionally, long-term obstetrical outcomes
272 were similar among the three techniques [9]. Other researches corroborated these findings, suggesting
273 that type of conization does not impact on obstetrics outcomes [15, 16]. Interestingly, Sadler et al.,
274 examining data of 1,078 women evaluated at colposcopy clinics serving Auckland, New Zealand
275 between 1988 and 2000, suggested that laser conization does not increase the risk of preterm delivery
276 in comparison to LEEP [15]. Recently, a systematic review and meta-analysis compared the efficacy
277 and safety of various ablative and excisional treatment (including cryotherapy, cold knife or
278 thermocoagulation and LEEP) for the treatment of cervical dysplasia [17]. They observed that cold
279 knife conization reduces the risk of residual disease in comparison to LEEP. Women undergoing

280 LEEP experienced an approximately 2-fold increase in positive margins rate in comparison to cold
281 knife conization. This finding is similar to what we observed in the present study comparing laser
282 conization and LEEP [17]. Accumulating evidence underlines that LEEP appears to be faster, less
283 costly, and requires less expertise than laser conization [9].

284 In the present paper laser conization is associated with a lower recurrence rate in comparison to LEEP.
285 Two main reasons might explain this finding. First, similar to what we did with cold knife with
286 conization, using laser we can perform a step-by-step conization, following the geometry of the
287 lesions, thus tailoring the radicality of the procedure. Second, the extensive vaporization of the
288 surgical margins performed using laser CO2 might provide a higher local control, than those achieved
289 via diathermocoagulation. The inherent biases of the retrospective study design are the main
290 weakness of the present paper. Other limitations included: (i) Selective reporting bias. The selective
291 reporting of some outcomes but not others, depending on the nature of the study. It is possible that
292 women developing CIN2+/HSIL recurrence had treatments in other centers and were not captured in
293 the present report, thus underestimating the risk of recurrence; (ii) Several potentially useful variables
294 are missing, including smoking history and immunosuppression. We can suppose that HIV positive
295 women (who are at high risk of developing recurrent CIN2+/HSIL) were more likely to be managed
296 with LEEP; (iii) The lack of data regarding size and deep of the cone as well as fragmentation of the
297 specimen (that generally are more likely to occur during LEEP) might impact the value of results'
298 reporting; (iv) Similarly, we were not able to correct our data on the expertise of the surgeons
299 (attending vs. residents); (v) More important, we did not evaluate short term complication rate
300 (including postoperative bleeding, cervical stenosis) as well as long-term fertility and obstetrical
301 issues (risk of pPROM (preterm premature rupture of membrane) and premature delivery). Our paper
302 only focuses on the oncology outcomes after conization, thus our results should be interpreted with
303 caution and cannot be generalized. In fact, the lack of data on surgery-related complications and
304 obstetrical outcomes would be necessary to assess the best therapeutic option for women with cervical

305 dysplasia. Another point deserving attention is the non-negligible potential regression rate of CIN2
306 [18-20]. In fact, we have to take into account that a watch-and-wait approach could be a valuable
307 option for young patients affected by CIN2. In fact, observation could be proposed in young women
308 without suspicious lesions and when the following colposcopic criteria were present: the entire lesion
309 is visible, the squamocolumnar junction is visible, and the lesion does not cover more than 75% of
310 the ectocervix [19]. Moreover, testing patients for E6/E7 mRNA and p16/ki67 might help in identify
311 those patients at low risk of progression, thus potentially avoiding further treatments [21].

312 More important, patients should be counseled about pros and cons of surgical and conservative
313 approaches. The main strengths of the present paper include: (i) the wide sample size, (ii) the multi-
314 institutional study design, (iii) the long-term follow-up (only women having 5 years of follow-up
315 were included in the study), and (iv) the use of sophisticated statistical method (ie, PSM) aiming to
316 reduce biases of the study design.

317 In conclusion, the present paper evaluated a large group of women undergoing conization for cervical
318 dysplasia. Only patients with a follow-up of at least 5 years were included. We observed that laser
319 conization was associated with a lower risk of positive surgical margins in comparison to LEEP. The
320 type of surgical approach did not influence HPV persistence. HPV persistence increase the risk of
321 recurrence either after LEEP and laser conization. According to our PSM model, patients undergoing
322 LEEP experienced a slightly increased risk of recurrence compared to patients undergoing laser
323 conization. Laser conization allows an personalized excisional procedure, that is tailored on the basis
324 of the geometry of the lesion. Our paper can not support any conclusion on the best therapeutic option
325 for women with cervical dysplasia, but it seeds for further researches. At this moment, we can not
326 suggested the adoption of laser conization instead of LEEP. Several biases impacted our investigation,
327 starting from its retrospective nature and the lack of data on fertility and obstetrics outcomes. Further
328 randomized studies comparing laser conization and LEEP are needed to assess the impact of surgical
329 techniques on long-term recurrence rates and on fertility and obstetrical issues. Furthermore, we

330 auspicate that in the next future the adoption of therapeutic vaccines might overcome concerns on the
331 execution of excisional procedures in young women.

332

333 **Author contribution:**

334 Conceptualization: GB, VD, FS, FR., Methodology: All authors.; Project administration: FR.;

335 Supervision: FR.; writing – original draft: All authors; writing – review & editing: All authors.

336

337 **Conflicts of interest:**

338 The Authors declare no conflicts of interest.

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340

341 **Legend to Figure:**

342 **Figure 1:** Study design

343 **Figure 2:** Recurrence free survival

344 **Supplemental Figure 1:** Recurrence free survival according to margin status and HPV persistence

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1 **Recurrence rate after Loop Electrosurgical Excision Procedure (LEEP) and Laser Conization:**
2 **a 5-year follow-up study**

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76 **Highlights:**

- 77 - HPV persistence correlates with an increased risk of 5-year recurrence in women undergoing
78 cervical conization
- 79 - Patients having laser conization experience a slightly lower risk of cervical dysplasia
80 recurrence in comparison to LEEP
- 81 - Further evidence regarding fertility and obstetrical issues is necessary

82 **Abstract**

83 **Objective:** Conization aims to remove pre-neoplastic lesions of the uterine cervix. Several techniques
84 for conization have been compared, but evidence regarding the most effective therapeutic option is
85 scant. Here, we aimed to compare the recurrence rate following laser conization and loop
86 electrosurgical excision procedure (LEEP) in patients with high-grade cervical dysplasia
87 (HSIL/CIN2+).

88 **Methods:** This is a retrospective multi-institutional study. Medical records of consecutive patients
89 with HSIL/CIN2+ undergoing conization between 2010 and 2014 were retrieved. A propensity-score
90 matching (PSM) was applied in order to reduce allocation bias. The risk of developing recurrence
91 was estimated using Kaplan-Meier and Cox hazard models.

92 **Results:** Overall, 2,966 patients had conization over the study period, including 567 (20%) and 2,399
93 (80%) patients having laser conization and LEEP, respectively. Looking at predictors of recurrence,
94 diagnosis of CIN3 (HR:3.80 (95%CI:2.01,7.21); $p<0.001$) and HPV persistence (HR:1.81
95 (95%CI:1.11,2.96); $p<0.001$) correlated with an increased risk of recurrence. After applying a PSM
96 we selected 500 patients undergoing laser conization and 1,000 undergoing LEEP. Patients
97 undergoing LEEP were at higher risk of having positive surgical margins in comparison to patients
98 undergoing laser conization (11.2% vs. 4.2%). The risk of having persistence of HPV was similar
99 between the two groups (15.0% vs. 11.6%; $p=0.256$). Five-year recurrence rate was 8.1% and 4% after
100 LEEP and laser conization, respectively ($p=0.023$). HPV persistence was the only factor associated
101 with 5-year recurrence after both laser conization ($p=0.003$) and LEEP ($p=0.001$).

102 **Conclusions:** HPV persistence is the only factor associated with an increased risk of recurrence after
103 either laser conization or LEEP. Owing to the lack of data regarding obstetrical outcomes, we are not
104 able to assess the best therapeutic option for women with cervical dysplasia.

105 **Keywords:** Conization; HPV; laser conization; LEEP; cervical dysplasia.

106 **Introduction**

107 In the recent years, the widespread adoption of primary and secondary preventions has dramatically
108 reduced the incidence of cervical cancer in developed countries [1,2]. However, cervical cancer still
109 represents a major health concern, being the third most common malignancy among women aged <39
110 years, and the second most common cause of death for cancer among females between 20 and 39
111 years in the United States [1].

112 Cervical cancer is one of the most preventable types of cancer, since it develops over a long time and
113 the causative agent has been recognized [2]. Persistent infection from human papillomavirus (HPV)
114 is the main factor causing cervical cancer [3]. Generally, persistent HPV infection causes cervical
115 dysplasia (also known as cervical intraepithelial neoplasia), which potentially evolves in cancer.
116 Although the majority of women with HPV infection will never develop lesions, a relatively high
117 number of women is at risk of developing cervical dysplasia. Women with cervical dysplasia who
118 have appropriate follow-up and treatments are at low risk of developing cervical cancer [3]. However,
119 recurrent cervical dysplasia is a well-known risk factor for cervical cancer [3,4]. Additionally,
120 recurrent cervical dysplasia might be cause of morbidity since adjunctive surgical treatments are
121 associated with fertility and obstetrical issues in women who wish to preserve their childbearing
122 potential [5]. With this background, identifying the best treatment modality for patients with cervical
123 dysplasia is of paramount importance. Cervical conization allows to remove cervical lesions (that
124 might progress to cancers (in about 30% [4])) and provide a classification according to the histology
125 and depth of cervical invasion, thus potentially identifying patients who deserve further treatments.
126 Additionally, occult invasive cancer could be detected at the time of conization [6]. To date, few
127 researches have evaluated various excisional modalities for the management of cervical dysplasia [7-
128 10]. There are few studies comparing different techniques of cervical conization (including cold knife
129 conization, laser CO2 conization and loop electrosurgical procedure (LEEP)), reporting discordant
130 results [8-10]. The level of evidence is still scant since the majority of these studies are characterized

131 by a small sample size and a short-term follow-up [8-10]. In the present study, we aimed to evaluate
132 long-term (5-year) outcomes of patients with cervical dysplasia following laser conization and LEEP.
133 As secondary outcomes, we sought to identify specific risk factors for cervical dysplasia recurrence
134 in this subset of patients.

135 **Methods**

136 This is a retrospective multi-institutional study conducted in Italy. Institutional Review Board (IRB)
137 approval was obtained (IRB#6812). **The list of participant centers is reported in supplemental material**
138 **1**. For the purpose of the present study, we collected chart of patients with newly diagnosed high-
139 grade cervical dysplasia (HSIL/CIN2+) treated in Italy from 01/01/2010 to 12/31/2014.

140 The inclusion criteria were: (i) newly diagnosed moderate /severe cervical dysplasia (HSIL/CIN2+);
141 (ii) squamous cell lesions; (iii) the execution of surgical excisional procedure (i.e., conization); (iv)
142 cervical conization performed with laser or LEEP; iv) conization performed between 2010 and 2014;
143 (vi) patients with available 5-year follow-up data (for non recurring patients; while, patients who
144 recurred were included even if they did not complete the five-year follow-up period). For the study
145 purpose only consecutive series of patients were accepted. Exclusion criteria were: (i) age <18 years;
146 (ii) consent withdraw; (iii) execution of ablative procedure; (iv) diagnosis of invasive cancer at the
147 time of conization; (v) execution of cold knife conization; (vi) glandular lesions; (vii) ongoing
148 pregnancy; and (viii) history of hysterectomy. The main outcome measure of this research was to
149 estimate the recurrence rate of women with cervical dysplasia undergoing either laser conization or
150 LEEP. Secondary outcome measure was to identify predictors of recurrence after laser conization and
151 LEEP. Generally, patients were treated on an outpatient basis using local anesthesia. Procedures were
152 performed under colposcopic guidance, using either laser or LEEP technique.

153 Demographic details, data about HPV type(s) detected, as well as data on treatment for the occurrence
154 of cervical dysplasia were retrospectively reviewed. HPV types were considered as high-risk in

155 according to the data of the International Agency for Research on Cancer (IARC) [11]. During the
156 study period, different surgeons perform all the procedures across the participant centers. However,
157 no differences in the facilities available for patients' care and in the referral patterns of various service
158 were present. Conization aimed to remove a cone-shaped section of the cervix surrounding the
159 endocervical canal, which includes the entire transformation zone. The technique for laser conization
160 and LEEP were standardized [12, 13]. Details about surgical treatments are reported elsewhere [12,
161 13]. The execution of laser conization instead of LEEP basically were basically depending on
162 available facilities of the participant centers and on surgeons' preferences. Laser conization was
163 performed using laser CO₂. Laser allows simultaneous photo-thermal ablation and coagulation.
164 ~~Combination between power of laser (i.e., watts) and spot diameter determines the effect between~~
165 ~~laser and cervical tissues. Power density (i.e., intensity) is expressed in watts/cm². During laser~~
166 ~~procedures, power density might range between 18,000 to 20,000 (having a power of 35-50 watts and~~
167 ~~a spot diameter of 0.2-0.5mm) watts/cm². Wavelength of 10,600 nm, depth of penetration of 0.1-~~
168 ~~0.5 mm and 0.5 mm of lateral thermal damage characterize laser CO₂.~~

169 Details of follow-up schedule and examination were reported elsewhere [11, 12]. According to
170 institutional protocols, patients were evaluated colposcopically in outpatients' clinic at 3 (in case of
171 positive margins) – 6 (in case of negative margins) months after conization. Briefly, patients had a
172 follow-up scheduled including Pap-smear, colposcopy and colposcopic-guided biopsy if clinically
173 indicated, every 6 months for the first 2 years, and annually thereafter (until 5 years). A dedicated
174 team of gynecologists performed all gynecological and colposcopic examinations. Generally, HPV
175 testing was performed at the first examination after conization in patients with documented HPV
176 infections. Persistence of HPV infection was defined as the persistence of HPV detected at the first
177 clinical examination following conization (generally at 6 months). ~~Persistence / recurrence after~~
178 ~~conization was defined as the diagnosis of a new HSIL/CIN2+ requiring secondary conization or~~
179 ~~hysterectomy.~~ Patients who did not have a secondary conization were considered free of recurrence.

180 Persistence of cervical dysplasia was defined by the diagnosis of HSIL/CIN2+ at the first evaluation
181 following conization; conversely, patients with recurrent cervical dysplasia had at least one negative
182 examination between conization and the diagnosis of HSIL/CIN2+. Low grade cervical lesions
183 (LSIL/CIN1) were not considered as recurrent disease.

184 **Statistical methods**

185 Data are summarized using basic descriptive statistics. Since this is a retrospective comparison
186 between two groups, possible allocation biases might impair the quality of the results reporting.
187 Therefore, we performed a propensity score analysis. Propensity-score analysis aims to reduce biases
188 arising from different covariates. In order to perform this analysis, we developed a multivariable
189 logistic regression model. Age, type of lesion (CIN2 vs. CIN3), menopausal status, execution of HPV
190 testing before conization (yes vs. no). Detailed description of PSM is described elsewhere [14].
191 Patients who had laser conization were matched 1:2 to a group of patients who had LEEP. PSM
192 analysis attempts to estimate the effect of a treatment by accounting for possible factors (e.g.,
193 constitutional variables) that predicts receiving the treatment, thus reducing possible inherent
194 selection biases of a retrospective study. We used a caliper width ≤ 0.1 standard deviations (SDs) of
195 the logit odds of the estimated propensity score. Basic descriptive statistics were used to describe the
196 two populations (patients undergoing LEEP and laser conization). Differences in categorical variables
197 were analyzed using the Fisher exact test. Odds ratio (OR) and 95% confidence intervals (95%CI)
198 were calculated for each comparison. T-test and Mann-Whitney test were used to compare continuous
199 variables as appropriate. Recurrence-free survival was estimated using Kaplan-Meier and Cox
200 models. The log-rank test was used to compare the risk of developing recurrence and the risk of death
201 between the two groups over the time. Hazard ratio (HR) and 95%CI were calculated for each
202 comparison. Univariate and multivariate analyses were performed when appropriate. All covariates
203 with a p value less than 0.10, based on univariate analysis were included in the multivariate model.
204 Disease-free survival and overall survivals were calculated starting from the date of primary surgery.

205 P values <0.05 were considered statistically significant. Statistical analysis was performed with
206 GraphPad Prism version 6.0 (GraphPad Software, San Diego CA) and IBM-Microsoft SPSS version
207 20.0 (SPSS Statistics. International Business Machines Corporation IBM 2013 Armonk, USA) for
208 Mac.

209 **Results**

210 Overall, the medical records of 2,966 women undergoing conization for newly diagnosed
211 HSIL/CIN2+ in the years 2011-2014 were retrieved. The study population included 567 (20%) and
212 2,399 (80%) patients undergoing laser conization and LEEP, respectively. Figure 1 shows the flow
213 of patients through the study design. Baseline characteristics of the whole population are reported in
214 Table 1. Median (range) patients' age was 40 (range, 18-89) years. Reason for conization was CIN2
215 and CIN3 in 969 (32.7) and 1,984 (66.9%) patients. For the remaining 13 (0.4%) patients, surgical
216 indication was classified as HSIL. Overall, 175 women received a second conization within the first
217 5 years after primary treatment, with a recurrence rate of 6%. Median time to recurrence was 18
218 (range, 5-52) months. Most patients (5%) developed recurrence within the first 2 years; while only
219 1% of patients recurred between 24 and 60 months of follow-up. Secondary conization was
220 performed in 155 (6.4%) and 20 (3.5%) patients included in the LEEP and laser conization groups,
221 respectively (p=0.007). Looking at factors predicting HSIL/CIN2+ recurrence, we observed that type
222 of cervical dysplasia (HR: 1.68 (95%CI: 1.21, 2.33) per CIN3; p=0.002), preoperative detection of a
223 high-risk HPV types (HR: 2.69 (95%CI: 1.25, 5.81); p=0.011), positive surgical margins (HR: 2.44
224 (95% CI: 1.68, 3.55); p<0.001) HPV persistence (HR: 2.34 (95%CI: 1.64, 3.33); p<0.001) and type
225 of surgical approach (HR: 2.50 (95%CI: 1.99, 3.16); p=0.007) impacted on 5-year recurrence free
226 survival at univariate analysis (Table 2). Via multivariate analysis, only diagnosis of CIN3 (HR: 3.80
227 (95%CI: 2.01, 7.21); p<0.001) and HPV persistence (HR: 1.81 (95%CI: 1.11, 2.96); p<0.001)
228 correlated with an increased risk of recurrence. Although it did not reach statistically, significance at

229 multivariate analysis, type of surgical approach was slightly associated with the risk of recurrence
230 (HR: 1.28 (95%CI: 0.78, 2.69); p=0.071).

231 **Propensity-matched cohort**

232 In order to evaluate the role of laser conization and LEEP in a balanced analysis, we adopted a PSM.
233 Through PSM we selected a study population of 1,500 patients, matching (1:2) 500 undergoing laser
234 conization and 1,000 undergoing LEEP. Baseline patients' characteristics of patients included in the
235 PSM analysis are reported in **Supplemental Table 1**. As the results of PSM, patients undergoing laser
236 conization had similar characteristics than those undergoing LEEP. The prevalence of positive
237 surgical margins was higher among patients undergoing LEEP compared to patients in the laser
238 conization group (11.2% vs. 4.2%). Considering available specific data, positive endocervical margin
239 rate was 6.1% and 2.2% after LEEP and laser conization, respectively (p<0.001). Considering patients
240 with specific data available, persistence of HPV was similar between the two groups. Persistent HPV
241 infection was observed in 71 out of 472 patients included in the LEEP group, and 28 out of 241
242 patients included in the laser conization group (15.0% vs. 11.6%; p=0.256). Looking at the crude
243 number of recurrence within the first 5 years, secondary conization was performed in 8.1% and 4%
244 of women in the LEEP and laser conization groups, respectively (p=0.003). Figure 2 shows recurrence
245 free survival. Patients undergoing LEEP were at high risk of recurrence over the 5-year follow-up
246 compared to patients undergoing laser conization (p=0.023, log-rank test). **Supplemental Figure 1**
247 shows the 5-year recurrence rate according to positive margins and HPV persistence. Factors
248 predicting 5-year recurrence after laser conization and LEEP are reported in **Supplemental Table 2**
249 and **3**. Among patients undergoing laser conization, HPV persistence was associate to a higher risk
250 of 5-year recurrence (HR: 15.0 (95%CI: 2.47, 91.1); p=0.003). Among patients undergoing LEEP,
251 factors associated with 5-year recurrence were: CIN3 (HR: 3.48 (95%CI: 1.67, 7.26); p=0.001),
252 positive margins (HR:1.76 (95%CI: 1.09, 2.45); p=0.001) and HPV persistence (HR: 1.6 (95%CI:
253 1.01, 2.86); p=0.001).

254 **Discussion**

255 The present study reports the recurrence rate after LEEP and laser conization in women diagnosed
256 with cervical dysplasia (HSIL/CIN2+). This retrospective multi-institutional study has collected data
257 of ~3000 women undergoing conization for whom 5-year follow-up is available. This is the largest
258 study investigating the impact of different surgical techniques for conization in patients with cervical
259 dysplasia. In order to reduce possible allocation biases, two propensity score algorithms were applied.
260 We observed a number of noteworthy findings. First, HPV persistence is the only factor associate
261 with an increased risk of recurrence, regardless type of surgical approach. Second, women undergoing
262 laser conization are at low risk of developing recurrent HSIL/CIN2+ in comparison to women
263 undergoing LEEP. Third, prevalence of positive margins (in particular endocervical ones) is lower in
264 patients having laser conization than LEEP; while HPV persistence rate is not influenced by the type
265 of surgical approach. Fourth, having positive margins is a significant risk factor for recurrence only
266 in patients who had LEEP; no association between positive margins and recurrence rate is observed
267 in the laser conization group.

268 Several investigations evaluated the role of various surgical techniques for conization with discordant
269 results [7-10]. Basically, most of these studies are characterized by a small sample size and short term
270 follow-up. Few randomized studies comparing laser conization and LEEP suggested the oncology
271 equivalence of these two techniques, but they were underpowered to demonstrate a significant
272 difference in recurrence rate [9, 10]. Mathevet et al., reported data of a randomized controlled trial of
273 86 patients, followed-up for at least 3 years [9]. Among those patients, 28, 29 and 29 women had cold
274 knife, LEEP and laser conization, respectively. The authors suggested that all procedures were
275 characterized by a similar recurrence rate. Cervical stenosis occurred in 0, 1, and 4 cases after laser
276 conization, LEEP, and cold knife group, respectively. Additionally, long-term obstetrical outcomes
277 were similar among the three techniques [9]. Other researches corroborated these findings, suggesting
278 that type of conization does not impact on obstetrics outcomes [15, 16]. Interestingly, Sadler et al.,

279 examining data of 1,078 women evaluated at colposcopy clinics serving Auckland, New Zealand
280 between 1988 and 2000, suggested that laser conization does not increase the risk of preterm delivery
281 in comparison to LEEP [15]. Recently, a systematic review and meta-analysis compared the efficacy
282 and safety of various ablative and excisional treatment (including cryotherapy, cold knife or
283 thermocoagulation and LEEP) for the treatment of cervical dysplasia [17]. They observed that cold
284 knife conization reduces the risk of residual disease in comparison to LEEP. Women undergoing
285 LEEP experienced an approximately 2-fold increase in positive margins rate in comparison to cold
286 knife conization. This finding is similar to what we observed in the present study comparing laser
287 conization and LEEP [17]. Accumulating evidence underlines that LEEP appears to be faster, less
288 costly, and requires less expertise than laser conization [9].

289 In the present paper laser conization is associated with a lower recurrence rate in comparison to LEEP.
290 Two main reasons might explain this finding. First, similar to what we did with cold knife with
291 conization, using laser we can perform a step-by-step conization, following the geometry of the
292 lesions, thus tailoring the radicality of the procedure. Second, the extensive vaporization of the
293 surgical margins performed using laser CO2 might provide a higher local control, than those achieved
294 via diathermocoagulation. The inherent biases of the retrospective study design are the main
295 weakness of the present paper. Other limitations included: (i) Selective reporting bias. The selective
296 reporting of some outcomes but not others, depending on the nature of the study. It is possible that
297 women developing CIN2+/HSIL recurrence had treatments in other centers and were not captured in
298 the present report, thus underestimating the risk of recurrence; (ii) Several potentially useful variables
299 are missing, including smoking history and immunosuppression. We can suppose that HIV positive
300 women (who are at high risk of developing recurrent CIN2+/HSIL) were more likely to be managed
301 with LEEP; (iii) The lack of data regarding size and deep of the cone as well as fragmentation of the
302 specimen (that generally are more likely to occur during LEEP) might impact the value of results'
303 reporting; (iv) Similarly, we were not able to correct our data on the expertise of the surgeons

304 (attending vs. residents); (v) More important, we did not evaluate short term complication rate
305 (including postoperative bleeding, cervical stenosis) as well as long-term fertility and obstetrical
306 issues (risk of pPROM (preterm premature rupture of membrane) and premature delivery). Our paper
307 only focuses on the oncology outcomes after conization, thus our results should be interpreted with
308 caution and cannot be generalized. In fact, the lack of data on surgery-related complications and
309 obstetrical outcomes would be necessary to assess the best therapeutic option for women with cervical
310 dysplasia. Another point deserving attention is the non-negligible potential regression rate of CIN2
311 [18-20]. In fact, we have to take into account that a watch-and-wait approach could be a valuable
312 option for young patients affected by CIN2. In fact, observation could be proposed in young women
313 without suspicious lesions and when the following colposcopic criteria were present: the entire lesion
314 is visible, the squamocolumnar junction is visible, and the lesion does not cover more than 75% of
315 the ectocervix [19]. Moreover, testing patients for E6/E7 mRNA and p16/ki67 might help in identify
316 those patients at low risk of progression, thus potentially avoiding further treatments [21].

317 More important, patients should be counseled about pros and cons of surgical and conservative
318 approaches. The main strengths of the present paper include: (i) the wide sample size, (ii) the multi-
319 institutional study design, (iii) the long-term follow-up (only women having 5 years of follow-up
320 were included in the study), and (iv) the use of sophisticated statistical method (ie, PSM) aiming to
321 reduce biases of the study design.

322 In conclusion, the present paper evaluated a large group of women undergoing conization for cervical
323 dysplasia. Only patients with a follow-up of at least 5 years were included. We observed that laser
324 conization was associated with a lower risk of positive surgical margins in comparison to LEEP. The
325 type of surgical approach did not influence HPV persistence. HPV persistence increase the risk of
326 recurrence either after LEEP and laser conization. According to our PSM model, patients undergoing
327 LEEP experienced a slightly increased risk of recurrence compared to patients undergoing laser
328 conization. Laser conization allows an personalized excisional procedure, that is tailored on the basis

329 of the geometry of the lesion. Our paper can not support any conclusion on the best therapeutic option
330 for women with cervical dysplasia, but it seeds for further researches. At this moment, we can not
331 suggested the adoption of laser conization instead of LEEP. Several biases impacted our investigation,
332 starting from its retrospective nature and the lack of data on fertility and obstetrics outcomes. Further
333 randomized studies comparing laser conization and LEEP are needed to assess the impact of surgical
334 techniques on long-term recurrence rates and on fertility and obstetrical issues. Furthermore, we
335 auspicate that in the next future the adoption of therapeutic vaccines might overcome concerns on the
336 execution of excisional procedures in young women.

337

338 **Author contribution:**

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

341

342 **Conflicts of interest:**

343 The Authors declare no conflicts of interest.

344 No funding sources supported this investigation.

345

346 **Legend to Figure:**

347 **Figure 1:** Study design

348 **Figure 2:** Recurrence free survival

349 **Supplemental Figure 1:** Recurrence free survival according to margin status and HPV persistence

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414

415

1 **Table 1: Baseline characteristics of the population**

	Whole population undergoing conization (n=2,966)	Patients undergoing laser conization (n=567)	Patients undergoing LEEP (n=2,399)
Age, years	40 (18, 89)	38 (23, 68)	41 (18, 89)
BMI	24 (14.4, 44.0)	23.8 (16, 40)	24 (14.4, 44.0)
Menopause			
No	2,373 (80.1%)	520 (91.7%)	1,853 (77.3%)
Yes	593 (19.9%)	47 (8.3%)	546 (22.7%)
Reason for conization			
CIN2	969 (32.7%)	143 (25.2%)	826 (34.4%)
CIN3	1,984 (66.9%)	424 (74.6%)	1,560 (65.0%)
HSIL	13 (0.4%)	0	13 (0.6%)
HR HPV involved*			
No	106 (6.6%)	29 (19.5%)	77 (5.3%)
Yes	1491 (93.4%)	120 (80.5%)	1,371 (94.7%)
Positive margins			
Endocervical	224 (7.5%)	13 (2.3%)	211 (8.8%)
Esocervical	112 (3.8%)	20 (3.5%)	92 (3.8%)
Vaccination after conization			
No	2,848 (96%)	565 (99.6%)	2,283 (95%)
Yes	118 (4%)	2 (0.4%)	116 (5%)
HPV persistence **			
No	1,320 (87.1%)	250 (89.3%)	874 (84.0%)
Yes	196 (1.9%)	30 (10.7%)	166 (6.0%)

2

3 Data are reported as median (range) and number (%); Abbreviation: BMI, body mass index; CIN,
4 cervical intraepithelial neoplasia; HSIL; high-grade squamous intraepithelial lesion; HPV, human
5 papillomavirus; *, Data on HPV involved in HSIL/CIN2+ were calculated on the basis of 1,597
6 patients undergoing HPV testing before conization; **, Data on HPV persistence were calculated on
7 1,516 patients undergoing HPV testing after conization.

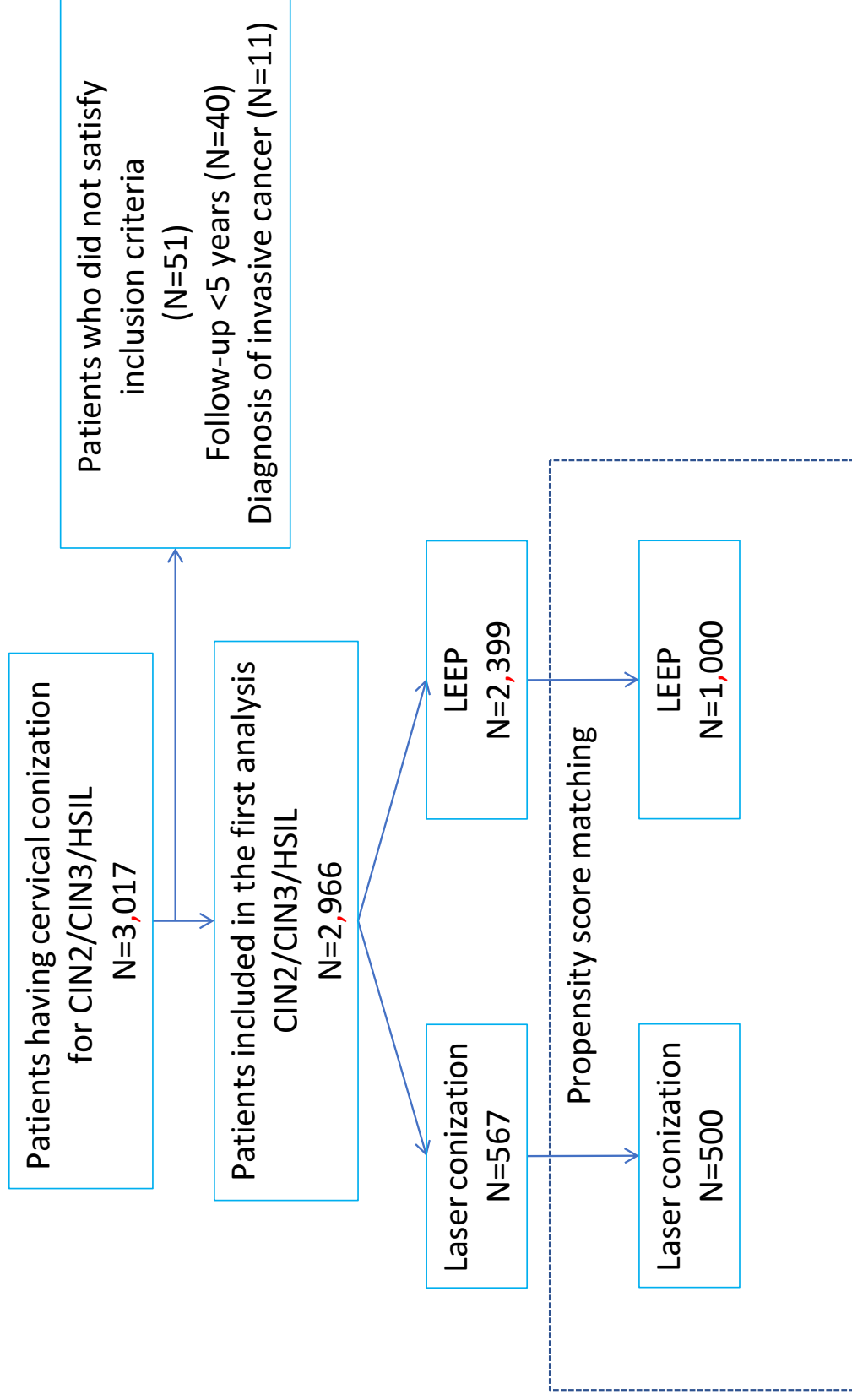
8

9 **Table 2: Factors predicting recurrence in women having cervical conization**

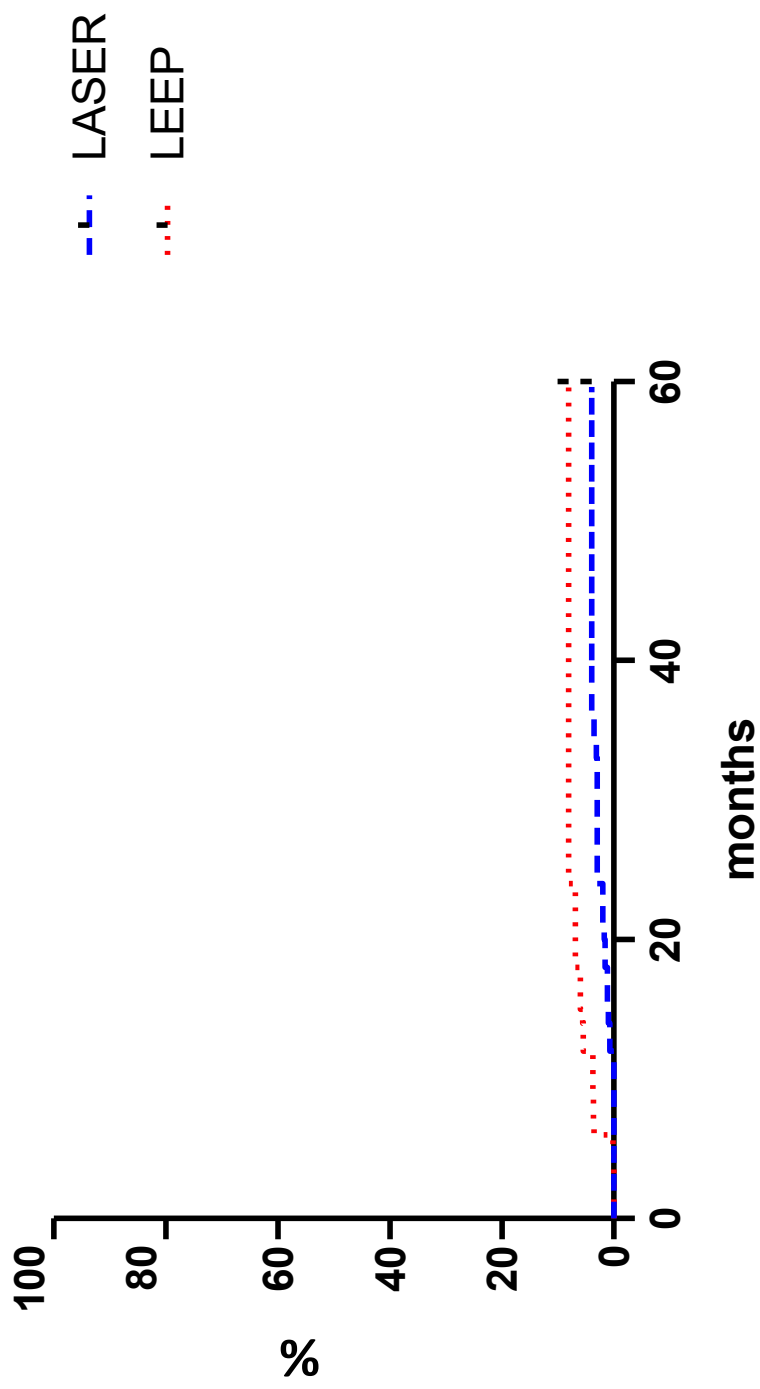
	Univariate		Multivariate	
	HR (95%CI)	P value	HR (95%CI)	P value
Age, years	0.98 (0.97, 1.00)	0.136	-	-
BMI, kg/mq	1.02 (0.97, 1.06)	0.337	-	-
Type of cervical dysplasia*		0.002		<0.001
CIN2	Reference		Reference	
CIN3	1.68 (1.21, 2.33)		3.80 (2.01, 7.21)	
Menopause		0.234		-
No	Reference		-	
Yes	0.97 (0.72, 1.21)		-	
HPV involved		0.178		-
Negative or HR other than HPV16/18	Reference		-	
HPV16/18	1.34 (0.87, 2.08)		-	
HPV involved		0.011		0.244
No HR	Reference		Reference	
HPV16/18 and other HR	2.69 (1.25, 5.81)		1.65 (0.70, 3.88)	
Multiple HR infections		0.651		-
No	References		-	
Yes	1.05 (0.84, 1.36)		-	
Margin status		<0.001		0.793
Negative	Reference		Reference	
Positive	2.44 (1.68, 3.55)		1.08 (0.59, 1.95)	
Type of involved margin				
Endocervical positive	2.70 (1.65, 4.39)	<0.001	1.01 (0.71, 1.67)	0.201
Esocervcial positive	1.52 (0.92, 2.52)	0.102	-	-
Surgical technique		0.007		0.071
Laser conization	Reference		Reference	
LEEP	2.50 (1.99, 3.16)		1.28 (0.78, 2.69)	
Vaccination after conization		0.124		-
No	Reference		-	
Yes	0.33 (0.08, 1.35)		-	
HPV persistence		<0.001		<0.001
No	Reference		Reference	
Yes	2.34 (1.64, 3.33)		1.81 (1.11, 2.96)	

10

11 Abbreviation: BMI, body mass index; CIN, cervical intraepithelial neoplasia; HSIL; high-grade
 12 squamous intraepithelial lesion; HPV, human papillomavirus; LEEP, Loop Electrosurgical Excision
 13 Procedure; *, For patients with HSIL, the type of cervical dysplasia was considered unknown.



Recurrence-free survival

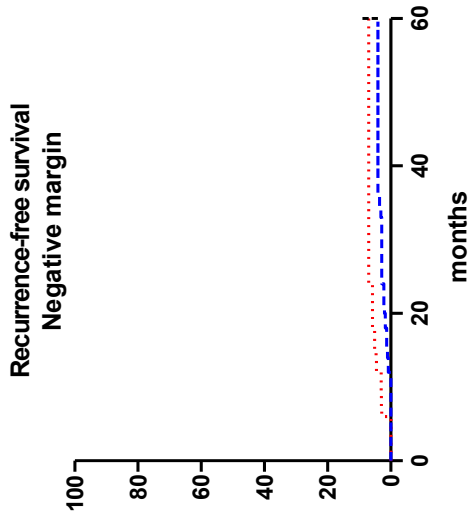




— LASER

... LEEP

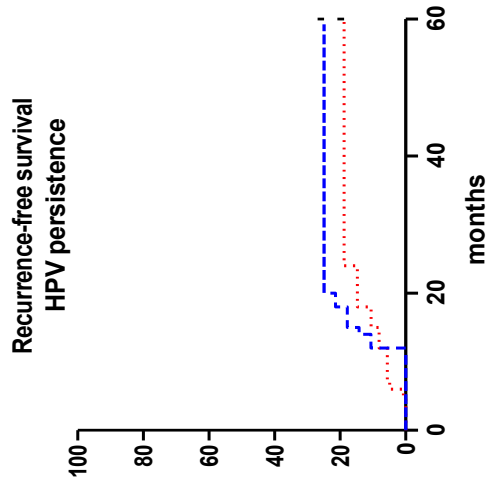
p=0.370



— LASER

... LEEP

p=0.003

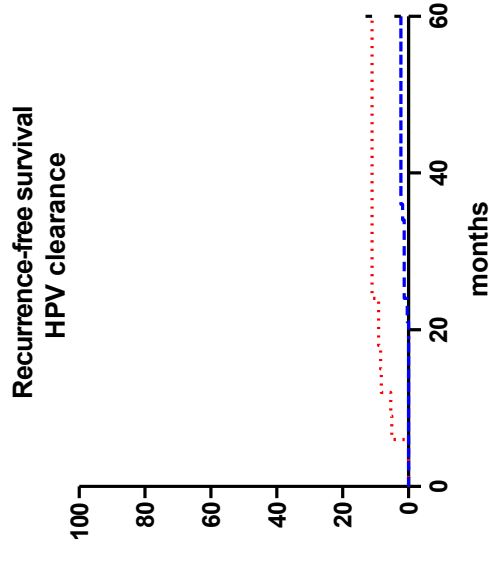


LASER

— LASER

... LEEP

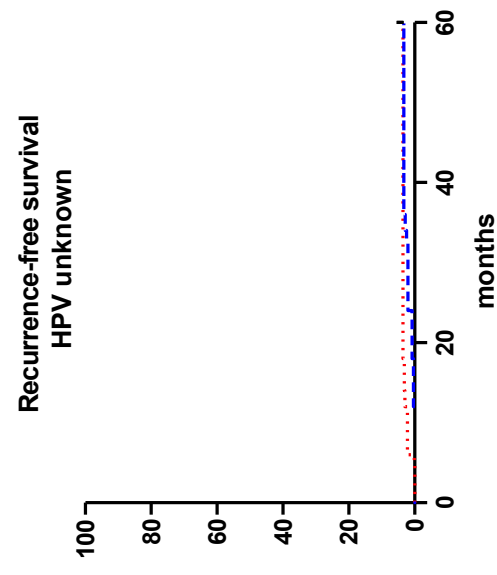
p=0.454



— LASER

... LEEP

P<0.001



— LASER

... LEEP

p=0.849

1 **Supplemental Table 1: Baseline characteristics of the population included in the propensity**
 2 **score matching**

3

	Patients undergoing laser conization (N=500)	Patients undergoing LEEP (N=1000)	P value
Age, years	38 (23-68)	38 (22-68)	0.891
BMI, Kg/mq	20 (16.4-40.0)	20 (16.6-40.0)	0.872
Menopause			1.00
No	436 (87.2%)	872 (87.2%)	
Yes	64 (12.8%)	128 (12.8%)	
Type of cervical dysplasia			0.853
CIN2	133 (26.6%)	272 (27.2%)	
CIN3	367 (73.4%)	728 (72.8%)	
Positive margins			<0.001
No	479 (95.8%)	888 (88.8%)	
Yes	21 (4.2%)	112 (11.2%)	
Type of involved margins			
Endocervical	11 (2.2%)	61 (6.1%)	<0.001
Esocervical	14 (2.8%)	53 (5.3%)	0.033
HPV persistence **			0.256
No	213 (88.4%)	401 (85%)	
Yes	28 (11.6%)	71 (15%)	
Recurrence			0.003
No	480 (96%)	919 (91.9%)	
Yes	20 (4%)	81 (8.1%)	

4

5 Data are reported as median (range) and number (%); Abbreviation: BMI, body mass index; CIN,
 6 cervical intraepithelial neoplasia; *, Data on HPV persistence were calculated on 713 patients
 7 undergoing HPV testing after conization.

9 **Supplemental Table 2: Factors predicting 5-year recurrence in women having laser conization**
 10

	Univariate		Multivariate	
	HR (95%CI)	P value	HR (95%CI)	P value
Age, years	1.03 (0.99, 1.08)	0.116	-	-
BMI, kg/mq	0.89 (0.68, 1.16)	0.403	-	-
Type of cervical dysplasia*		0.064		0.981
CIN2	Reference		Reference	
CIN3	0.43 (0.18, 1.05)		0.87 (0.61, 1.11)	
Menopause		0.434		-
No	Reference		-	
Yes	0.87 (0.65, 1.11)		-	
HPV involved		0.402		-
Negative or HR other than HPV16/18	Reference		-	
HPV16/18	1.26 (0.81, 16.5)		-	
HPV involved		0.013		0.151
No HR	Reference		Reference	
HPV16/18 and other HR	6.11 (1.46, 25.5)		39.6 (0.26, >100)	
Margin status		0.254		-
Negative	Reference		-	
Positive	2.34 (0.54, 10.0)		-	
Type of involved margin				
Endocervical positive	4.68 (1.08, 20.1)	0.038	2.42 (0.30, 19.0)	0.400
Esocervical positive	0.04 (0.00, 724.1)	0.526	-	-
Vaccination after conization		0.324		-
No	Reference		-	
Yes	0.85 (0.78, 1.24)		-	
HPV persistence		<0.001		0.003
No	Reference		Reference	

Yes	12.7 (4.02, 40.1)		15.0 (2.47, 91.1)	
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11

12 Abbreviation: BMI, body mass index; CIN, cervical intraepithelial neoplasia; HSIL; high-grade
13 squamous intraepithelial lesion; HPV, human papillomavirus; LEEP, Loop Electrosurgical Excision
14 Procedure; *, For patients with HIS, the type of cervical dysplasia was considered unknown.

15 **Supplemental Table 3: Factors predicting 5-year recurrence in women having LEEP**

	Univariate		Multivariate	
	HR (95%CI)	P value	HR (95%CI)	P value
Age, years	1.01 (0.99, 1.03)	0.288	-	-
BMI, kg/mq	0.96 (0.90, 1.02)	0.216	-	-
Type of cervical dysplasia		<0.001		0.001
CIN2	Reference		Reference	
CIN3	2.36 (1.75, 3.17)		3.48 (1.67, 7.26)	
Menopause		0.234		-
No	Reference		-	
Yes	0.97 (0.72, 1.21)		-	
HPV involved		0.004		0.818
Negative or HR other than HPV16/18	Reference		Reference	
HPV16/18	1.22 (1.01, 1.75)		1.08 (0.53, 2.20)	
HPV involved		0.356		-
No HR	Reference		-	
HPV16/18 and other HR	21.1 (0.032, >100)		-	
Margin status		<0.001		0.001
Negative	Reference		Reference	
Positive	2.55 (1.53, 4.27)		1.76 (1.09, 2.45)	
Type of involved margin				
Endocervical positive	3.90 (2.16, 7.09)	<0.001	1.69 (0.87, 3.01)	0.121
Esocervcial positive	1.48 (0.68, 3.22)	0.318	-	-
Vaccination after conization		0.365		-
No	Reference		-	
Yes	0.48 (0.01, 34.5)		-	
HPV persistence		0.034		0.001
No	Reference		Reference	
Yes	1.74 (1.04, 2.92)		1.6 (1.01, 2.86)	

17 Abbreviation: BMI, body mass index; CIN, cervical intraepithelial neoplasia; HSIL; high-grade
18 squamous intraepithelial lesion; HPV, human papillomavirus; LEEP, Loop Electrosurgical Excision
19 Procedure; *, For patients with HIS, the type of cervical dysplasia was considered unknown.

20

21

Highlights:

- HPV persistence correlates with an increased risk of 5-year recurrence in women undergoing cervical conization
- Patients undergoing laser conization experience a slightly lower risk of recurrence in comparison to LEEP
- Further evidence regarding fertility and obstetrical issues is necessary

Recurrence rate after Loop Electrosurgical Excision Procedure (LEEP) and Laser Conization: a 5-year follow-up study

Bogani G, Di Donato V, Sopracordevole F et al.

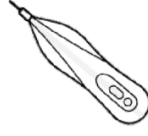
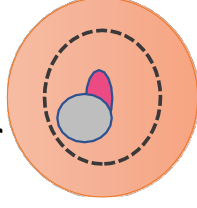
Study population

Women with newly diagnosed moderate /severe cervical dysplasia (HSIL/CIN2+)

- 2,966 conization with 5-year follow-up
- 567 laser conization
- 2,399 LEEP

Propensity-matched analysis

Study cohort

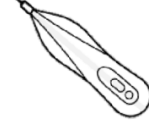
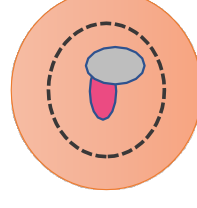


Laser conization
N=500



LEEP conization
N=1,000

5-year recurrence rate



4%
LEEP doubled recurrence rate



8.1%

At multivariate analysis **HPV persistence** increased 5-year recurrence rate either after laser conization or LEEP