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

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Highlights:

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

Abstract

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

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

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

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

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

Introduction

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

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

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

Methods

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

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

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

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

Statistical methods

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

Results

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

Propensity-matched cohort

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

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

Discussion

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

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

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

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

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

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

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

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

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

Author contribution:

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

Conflicts of interest:

The Authors declare no conflicts of interest.

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Legend to Figure:

Figure 1: Study design

Figure 2: Recurrence free survival

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

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

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Highlights:

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

Abstract

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

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

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

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

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

Introduction

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

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

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

Methods

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

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

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

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

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

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

Statistical methods

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

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

Results

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

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

Propensity-matched cohort

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

Discussion

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

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

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

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

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

Author contribution:

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

Conflicts of interest:

The Authors declare no conflicts of interest.

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Legend to Figure:

Figure 1: Study design

Figure 2: Recurrence free survival

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

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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%)

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

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)	

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

5. Figure

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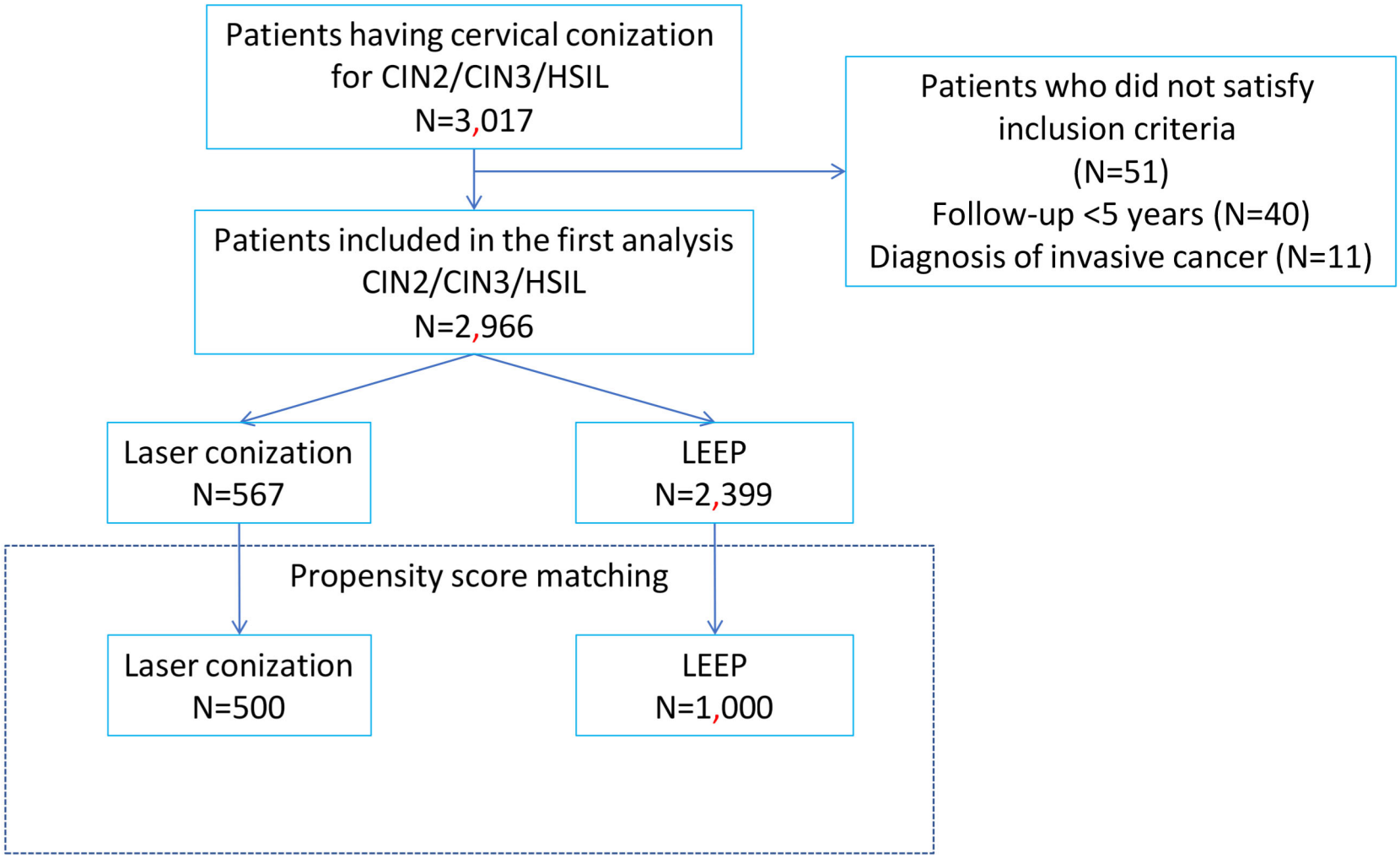
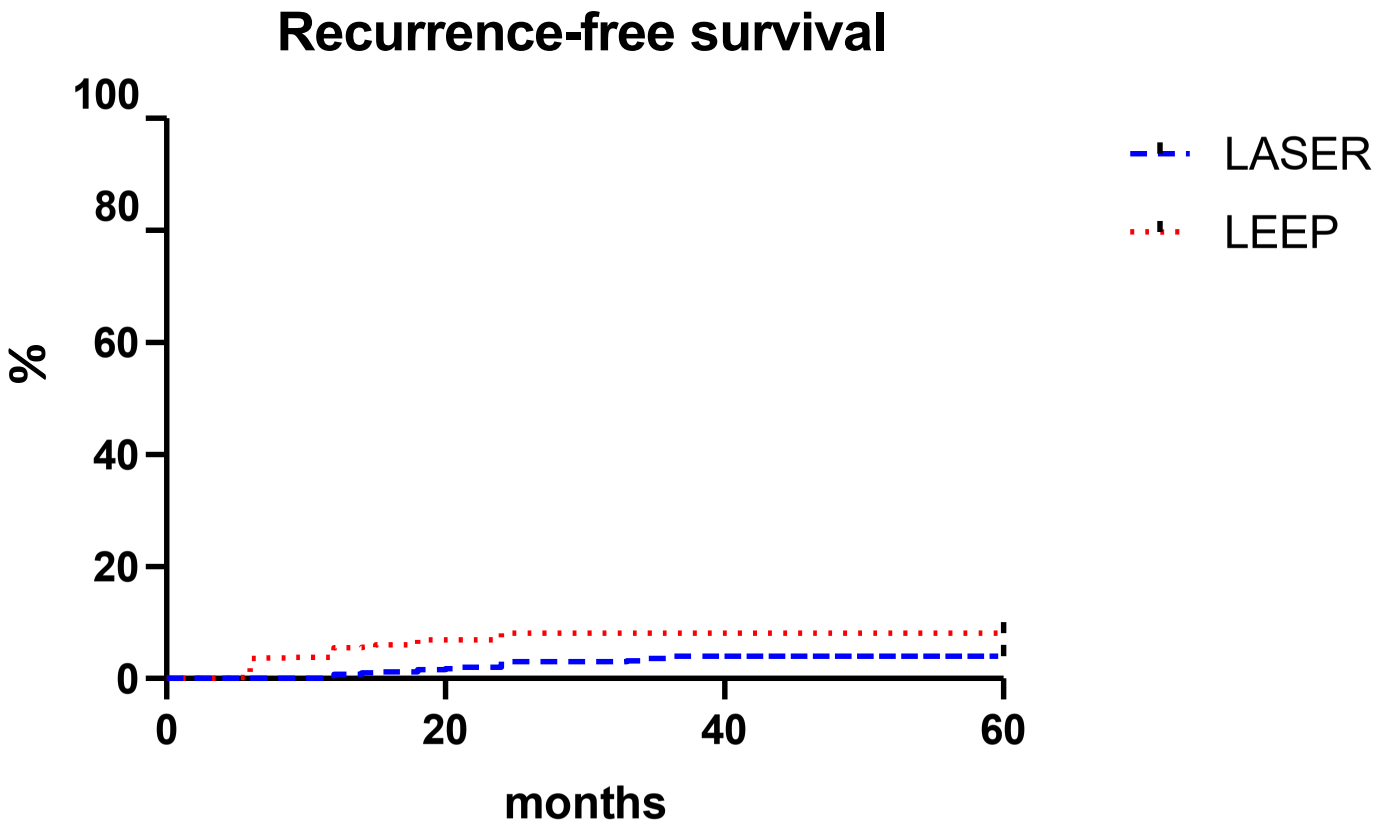
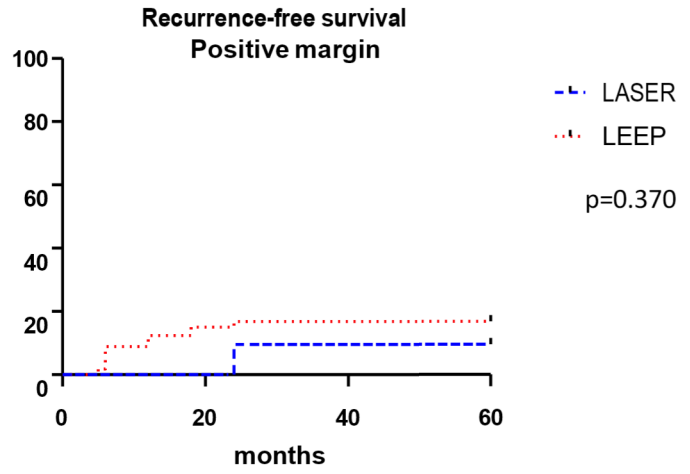
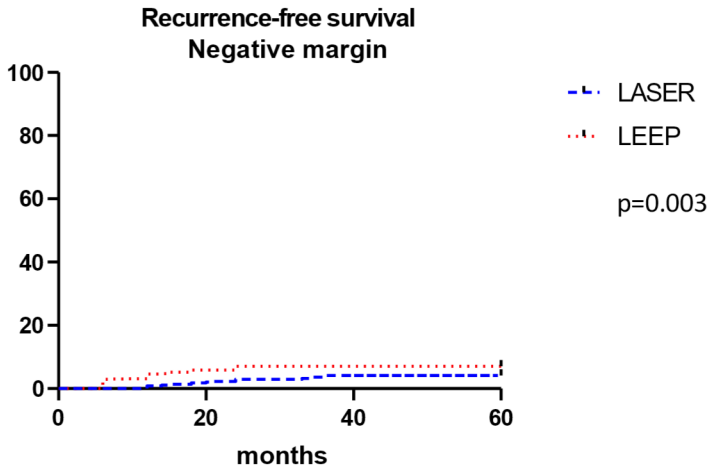


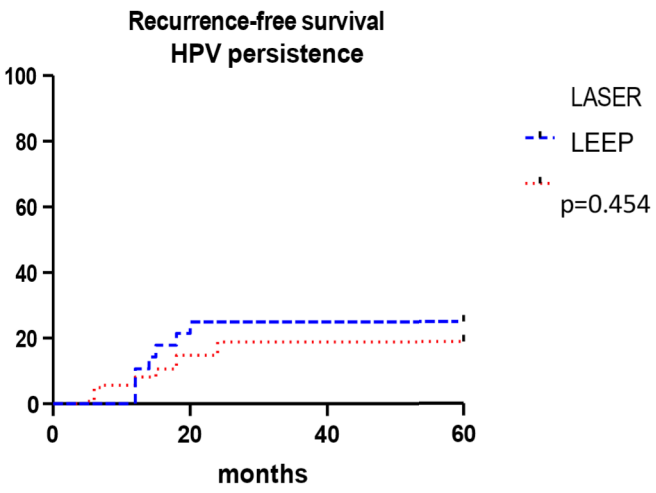

Recurrence-free survival
100
80
60
40
20
0
%
0
20
40
60
months
LASER
LEEP

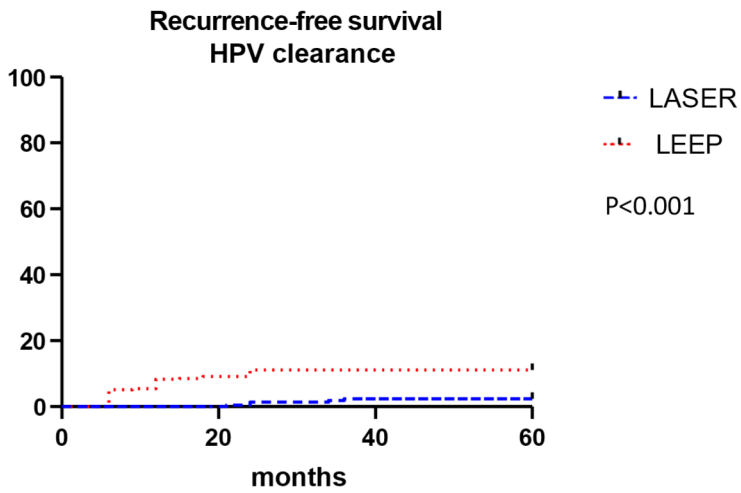
6. Supplementary Material

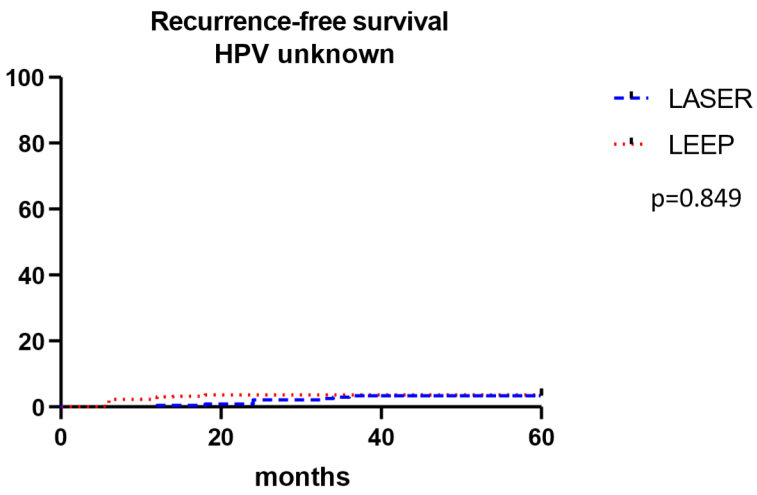
Click here to access/download;6. Supplementary Figure 1.pptx











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

	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%)	

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

8

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

	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
Esocervcial 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)	

Abbreviation: BMI, body mass index; CIN, cervical intraepithelial neoplasia; HSIL; high-grade squamous intraepithelial lesion; HPV, human papillomavirus; LEEP, Loop Electrosurgical Excision 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)	

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