

# Multiple targeting CO2 laser ablation for the treatment of vaginal intraepithelial neoplasia: a pilot study

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## Abstract

**Objective** To investigate the detailed procedure and efficacy of multiple targeting CO2 laser ablation (MTLA-CO2) for the treatment of vaginal intraepithelial neoplasia (VaIN) and to obtain a stably high cure rate and low complication incidence. **Design** Patients with VaIN were recruited and received MTLA. After follow-up, we aimed to find the risk factors affecting the efficacy. **Setting** Obstetrics & Gynecological outpatients' department of Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology. **Population** Fifty-seven cases of LSIL(VaIN1) and 160 cases of HSIL(VaIN2,3). **Methods** Patients with VaIN were recruited and received MTLA. The detailed clinical data were recorded and the risk factors were analyzed. **Main Outcome Measures** The pathological cure and HPV clearance. **Results** The cumulative pathological cure rate was 92.0% and 96.0%, and the cumulative HPV negative rate was 77.5% and 75.5%, for the HSIL(VaIN2,3) and LSIL(VaIN1) groups respectively. Larger areas of lesions ( $p = 0.083$ ) and history of hysterectomy ( $p = 0.037$ ) were independent risk factors for pathological persistence. Menopause ( $p = 0.006$ ) and immunosuppression ( $p = 0.059$ ) were independent risk factors for HR-HPV persistent infection. Condemn use ( $p = 0.002$ ) was a protective factor against HR-HPV infection. It was proposed 3~5 times of laser ablation for HSIL(VaIN2,3) and 2~3 times of laser ablation for LSIL(VaIN1). **Conclusions** The MTLA for VaIN was effective and well-tolerated. The schematization of MTLA was decided according to the characteristics of VaIN lesions, high-risk factors and surveillance. **Funding** The National Natural Science Foundation of China (81974410, 81572571, 81630060, 82002769, 81230038). **Keywords** Laser ablation, targeting, VaIN

## Introduction

VaIN is the dysplasia of the vaginal squamous epithelium, mostly associated with persistent HR-HPV infection. VaIN is classified as low-grade squamous intraepithelial lesion (LSIL) including VaIN1 (mild dysplasia in the past classification) and high-grade intraepithelial lesion (HSIL) including VaIN2,3 (moderate and severe dysplasia respectively) according to the 2014 WHO classification of tumors of female reproductive organs [1]. The natural history of VaIN is similar to that of cervical intraepithelial neoplasia (CIN), but the incidence of VaIN is less than 1% of CIN. The annual morbidity of VaIN was about 0.2~2 per million [2], accounting for approximately 0.4% of all squamous neoplastic lesions of the lower genital tract [3]. The incidence of VaIN was severely underestimated due to missed diagnosis. It was reported that VaIN consumed 8.09%, 12.45% and 13.08% of squamous lesions of the lower genital tract from 2013 to 2015 in a hospital in China [4]. The yearly increasing detection rates of VaIN were attributable to the increased awareness of the condition in our peers.

The management for LSIL(VaIN1) is conservative because most LSIL(VaIN1) can regress spontaneously [5~7]. Otherwise, the rate of HSIL(VaIN2,3) progressing to cancer was about 2~12% [8~10]. Thus, HSIL(VaIN2,3)

are usually treated aggressively. The treatment modalities for HSIL(VaIN2,3) generally include conservative observation, topical agents, laser ablation, excision and so on [3]. Because of the specialty of vaginal anatomy and sexual function, the excisional procedure is technically difficult and adversely affects the quality-of-life. Observation or topical agents for VaIN show uncertain effects or evident side effects. Thus, laser ablation has become a dominant choice for VaIN with the advantages of considerable efficacy, easy training for practitioners and few severe complications [11]. There was obvious fluctuation in the literature about the efficacy of laser ablation for VaIN with effective rate varying from 47% to 100% and relapse rate varying from 20% to 57% [8,12~18]. The samples of literature on laser ablation for VaIN were usually small, and the essays lacked detailed description of treatment modalities and standard follow-up schedule.

To achieve a standard method of CO<sub>2</sub> laser ablation for the treatment of VaIN with stably high cure rate and low complication incidence, this study discussed the details of MTLA procedure and related high-risk factors.

## Methods

The patients with biopsy-proven VaIN were recruited in the study from April 2016 to April 2021, and followed-up until October 2021 in the gynecological out-patient department at Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology in China. The patients were eligible for inclusion if the following criteria were fulfilled: more than two pathologists diagnosed the biopsy of the vagina as VaIN; and the patients accepted MTLA procedures and were compliant with regular follow-up. The exclusion criteria were as follows: 1. patients suspicious of malignant tumors of the lower anogenital tract; 2. patients suffering unhealing malignant tumors of other systems; 3. patients received radical hysterectomy with after-loading radiotherapy; 4. patients with insufficient exposure of VaIN lesions; and 5. pregnant women. Those patients diagnosed with LSIL(VaIN1) should suffer at least one of the following risk factors: the cytologic test suggesting HSIL, persistent HR-HPV infection, concomitant or previous diseases of cervical or vulvar intraepithelial neoplasia (VIN), multifocal lesions, large areas of lesions, immunosuppression and so on. Immunosuppression was defined as suffering the situations of autoimmune diseases, history of chemotherapy and/or radiotherapy for cancers, using immunosuppressing drugs after organ transplants and so on. Suitable subjects were enrolled in the study with written informed content. This study was approved by the Institutional Review Board at Tongji Hospital (TJ-IRB20210134) and registered at ClinicalTrials.gov (NCT05170620).

All subjects were tested for cytology mostly with Thin-prep (Hologic Inc, USA) and tested HPV-DNA mostly with Cervista (Hologic Inc, USA) or Cobas 4800 (Roche Group, Switzerland). The colposcopic examinations were completed with Leisgang colposcope (3ML, Germany). The terminology and interpretation of colposcopy was implemented according to the 2017 criteria of the International Federation of Cervical Pathology and Colposcopy (IFCPC). Laser ablation was performed with a CO<sub>2</sub> laser treatment machine (IC25, Yuhua Medical Instruments Company in China).

The laser treatment for VaIN in the study was named multiple targeting laser ablation (MTLA) including therapeutic laser ablation (TLA) and strengthened laser ablation (SLA). All patients received colposcopic examinations before initial treatment by the same gynecologist with more than ten years' experience to locate the lesions. The procedure of laser ablation required targeting at the lesions in continuous mode, energy 10~20W, vaporizing 1~2mm deep and outside 3~5mm of the lesions' margins. TLA was defined as MTLA with the intervals of 1~6 months until pathological regression and HR-HPV clearance. SLA was then arranged for some patients with high-risk factors of relapse with 1~2 number of laser ablation. The intervals between MTLA were decided according to the wound healing state. The contents of surveillance in therapeutic phase included cytologic tests, HR-HPV tests, colposcopic examinations and biopsies if necessary, every 3~6 months. If suspicious VaIN lesions were found by colposcopy or pathological examinations, repeat laser ablation was preferred. But for a few patients suspicious of invasive lesions, surgical excisions were performed. After accomplishing the treatment, the patients were followed-up every 6 months in 2 years, then every 3 years after three consecutive normal results.

The primary outcome measure was the pathological cure. Pathological cure meant no VaIN found by colposcopy and biopsy. The secondary outcome measure was HR-HPV negative rate. Data were collected including age, menopause status, gravidity, parity, contraceptive methods, history of past illness, cytologic and HR-HPV tests, colposcopic examinations, areas of VaIN lesions, pathological examinations, parameters of laser ablation and so on.

Meta-analysis of relevant studies was also carried out through a search of PubMed, Cochrane Library, Web of Science, Wanfang, China Science and Technology Journal Database, China National Knowledge Infrastructure with the key words of “laser” and “vaginal intraepithelial neoplasia”. The last time of searching was August 24<sup>th</sup>, 2021. Researches published in English or Chinese were included in the meta-analysis. The screening process was accessed by two independent researchers. Researches with samples of laser ablation for VaIN more than 15 cases from 2000 to date were included, and efficacy and 95% confidence interval (CI) were calculated.

The data of the study were calculated and analyzed by SPSS version 27. Univariate analysis was performed by bivariate correlation analysis, and multivariate analysis was performed by multivariate logistic regression analysis with two-sided  $P < 0.05$  as statistically different. Meta-analysis was performed by Stata 15SE. Heterogeneity was assessed by Q statistical test with  $P < 0.1$  as statistically different. According to the heterogeneity between studies, the random effect model was used to calculate and combine the laser ablation efficacy.

## Results

Two hundred seventeen patients were recruited in the study including 57 LSIL(VaIN1) and 160 HSIL(VaIN2,3) patients. The general information of the patients is shown in Table 1. There was no significant difference in age, menopause status, gravidity, parity, abortion, condom use status, cervical contact bleeding or cytologic results before laser between the LSIL(VaIN1) and HSIL(VaIN2,3) groups. There was a significant difference in HR-HPV test results before laser ablation between the two groups, and the HSIL(VaIN2,3) group showed a higher HR-HPV infection rate than the LSIL(VaIN1) group (89.4% vs 78.9%,  $p = 0.047$ ).

Information about concomitant or previous diseases of CIN or cervical cancer (CC) and the corresponding previous treatments are listed in Table 2. Of all the patients, 61.9% had the illness of CIN/CC.

The characteristics of VaIN lesions are listed in Table 3. The average area of VaIN lesions was 1.17cm<sup>2</sup>. Areas of 63.1% of lesions were less than 1cm<sup>2</sup>. The areas of lesions in the HSIL(VaIN2,3) group were larger than that in the LSIL(VaIN1) group with a significant difference (1.31cm<sup>2</sup> vs 0.79cm<sup>2</sup>,  $p = 0.055$ ). A history of CIN/VIN in the LSIL(VaIN1) group was more common than that in the HSIL(VaIN2,3) group with a significant difference (75.4% vs 58.1%,  $p = 0.020$ ).

The average laser number for all the patients was 2.94. The average number of therapeutic laser ablation was 2.32 and that of strengthened laser ablation was 0.62 (Fig. 1). There was a significant difference in the total number, therapeutic number and strengthened number of laser ablation between the HSIL(VaIN2,3) and LSIL(VaIN1) groups (3.15 vs 2.35,  $p = 0.000$ ; 2.47 vs 1.91,  $p = 0.002$ ; 0.68 vs 0.44,  $p = 0.060$ ; respectively).

Seventeen patients lost follow-up for the assessment of pathological cure and 22 patients lost for the assessment of HPV clearance. The pathological cure rate increased along with increasing laser times in all the patients (Fig. S1). The cumulative pathological cure rate was 93.0% (186/200) and the cumulative HR-HPV negative rate was 76.9% (150/195). Multivariate logistic regression analysis showed that larger areas of VaIN lesions ( $p = 0.048$ ) and history of hysterectomy ( $p = 0.017$ ) were independent risk factors for the pathological persistence (Table S1), and that menopause ( $p = 0.042$ ) and non-use of condoms ( $p = 0.068$ ) were independent risk factors for persistent HR-HPV infection (Table S2).

In the HSIL(VaIN2,3) group, the cumulative pathological cure rate was 92.0% (138/150) and the cumulative HR-HPV negative rate was 77.5% (110/142). Three to five times of laser ablation could obtain a relatively high pathological cure rate (90.4~92.0%) and HR-HPV negative rate (68.5~77.5%) (Fig. S2). Multivariate logistic regression analysis showed that larger areas of VaIN lesions ( $p = 0.083$ ) and history of hysterectomy

( $p=0.037$ ) were independent risk factors for pathological persistence, and that menopause ( $p =0.006$ ) and immunosuppression ( $p =0.095$ ) were risk factors for persistent HR-HPV infection (Table S3 and Table S4).

In the LSIL(VaIN1) group, the cumulative pathological cure rate was 96.0% (48/50) and the cumulative HR-HPV negative rate was 75.5% (40/53). Two to three times of laser ablation could obtain a relatively high pathological cure rate (92.0~93.5%) and HR-HPV negative rate (65.5~73.6%) (Fig. S3). Multivariate logistic regression analysis showed there was no risk factor for pathological persistence, and that condemn use ( $p =0.002$ ) was a protective factor against HR-HPV infection (Table S5 and Table S6).

The main complication of laser ablation for VaIN was the vaginal adhesions (21/217, 9.68%). There was another case of surgical site bleeding. The complication incidence was significantly higher in the HSIL group than that in the LSIL group (13.13% vs 1.75%,  $p=0.014$ ). Bivariate correlation analysis (Table S7) showed that complication incidence was related to vaginitis ( $p =0.004$ ), VaIN grade ( $p =0.014$ ) and laser times ( $p =0.046$ ). Multivariate logistic regression analysis showed that the upper three factors were independent risk factors for complication incidence ( $p =0.015, p =0.029$  and  $p =0.046$ , respectively).

There were 455 published studies searched out in the meta-analysis and 21 eligible articles [14, 18-37] were chosen for analysis. The overall combined effect was 0.78 (95%CI 0.72-0.84,  $P<0.05$ ) for the assessment of pathological cure of laser ablation for VaIN (Fig. S4). The combined effect was 0.84 (95%CI 0.75~0.92,  $P<0.05$ ) of laser ablation for both the HSIL(VaIN2,3) and LSIL(VaIN1) patients.

## Discussion

### Main Findings

The cumulative pathological cure rate of laser ablation for the treatment of VaIN in this study was 93.0%, which was higher than that in most studies reported. The high efficacy was attributed to the MTLA method. We proposed 3~5 circles of laser ablation for the HSIL(VaIN2,3) and 2~3 circles for the LSIL(VaIN1) patients to obtain a high pathological cure rate and HR-HPV clearance rate in our study.

### Strengths and Limitations

The most obvious strength of this study was newly proposed principle of laser ablation for the old problem of the treatment for VaIN. Because of the rarity of VaIN, small sample of patients remained the limitation of the study. Though the pathological cure rate was high in the study, the HPV clearance rate remained to be elevated. Also, the follow-up time was not long enough to observe the relapse rate of the disease. Maybe a large sample of randomized trial from multiple centers in the future could provide more evidence for the efficacy of MTLA for the treatment of VaIN.

### Interpretation

The literature about laser ablation for the treatment of VaIN lacked detailed description of the procedure and led to large fluctuation of effective rate and relapse rate of the disease. MTLA was proposed in this study and we obtained high pathological cure rate under this treatment mode. We expected to maintain the high efficacy in the long term.

Because laser ablation is a destructive procedure with no histopathological examination, malignancy should be excluded if we choose laser ablation for VaIN. The punch biopsy-proven VaIN could not exclude microinvasion, and the incidence of coexisting malignancy was 1.0~30.3% as the literature reported [10,14,15,38]. All patients in our study received colposcopic and pathological examinations. Patients were excluded if the lesions observed by colposcopy were suspicious of malignancy with the indications such as widespread or bulging lesions, or if the pathological examinations suspected of invasive lesions. The incidence of coexisting cancer rises in widespread VaIN3 or carcinoma in situ.

The MTLA method was decided according to the overall assessment of patients' status during the treatment. Firstly, colposcopic examination played a key role. Colposcopic examination could not only assess the severity

of the lesions, but also guide multiple biopsies to exclude invasive lesions and evaluate the efficacy in follow-up period. The accuracy rate of colposcopic examination diagnosing VaIN was 52.17% and the risk rate of HSIL(VaIN2,3) missed diagnosis was about 35.71% [39]. Experienced practitioners predominantly elevated the accuracy rate. Secondly, MTLA method emphasized sufficient exposure of lesions and precise targeting at the lesions when ablating. VaIN was usually detected in the upper third of the vagina and sheltered by the cervix which might lead to missed diagnosis and treatment. For patients received hysterectomy, VaIN usually arose in the mucosal folds especially the bilateral apex of vagina. MTLA required flattening wrinkles and overlapping ablation scanning. Thirdly, surveillance was important for the individual decision-making of MTLA. The results of cytologic tests, HR-HPV tests, colposcopic and pathological examinations were key factors to decide whether to repeat laser ablation in follow-up phase. Persistent HPV infection was a high-risk factor for relapse of VaIN [14,16]. Repeat laser ablation was preferred if any of below appeared: abnormal results of cytologic tests, positive results of HPV tests, proliferative lesions found by colposcopic examinations indicating VaIN, or biopsy-proven VaIN. Excisional procedure was preferred if any signs indicated malignancy. The incidence of VaIN progressing to malignant tumors after treatment was 3.4~12% [5,8,10,15,18].

The high-risk factors for VaIN included menopause, HR-HPV infection, CIN/CC history, vaginal intracavity radiotherapy, immunosuppression and so on as reported [40~44]. Larger VaIN areas and hysterectomy were independent risk factors for pathological persistence in this study. Patients with larger areas of VaIN might have a higher rate of suffering high-grade lesions, multifocal lesions, CIN/VIN and other risk factors, which increased the difficulty of laser procedures. The indications of hysterectomy in this study were mainly past diseases of CIN/CC. The patients with the history of hysterectomy experienced more adverse factors like older age, a more complicated history of HPV-associated diseases, vaginal atrophy and local immunosuppression. In a report, 2/3 VaIN patients had the history of hysterectomy mainly indicated by cervical HSIL [45]. It was known that VaIN might share the same etiology with CIN. And VaIN might have occurred but missed diagnosis when hysterectomy was done. Menopause and no-use of condoms were independent risk factors for HPV persistence in the study. For postmenopausal females, insufficient estrogen leads to vaginal atrophy and local immunosuppression which result in a low capacity to eliminate viruses. Doctors can publicize the benefit of condom use to protect our patients from HPV infection. HPV vaccine could also prevent HPV-related VaIN [46,47].

## Conclusion

Laser ablation for the treatment of VaIN is effective and well-tolerated, requiring exclusion of malignant lesions of the lower anogenital tract. The MTLA method was individually carried out according to the comprehensive status including characteristics of lesions, high-risk factors and results of surveillance. It was recommended 3~5 number of laser ablation for the HSIL(VaIN2,3) and 2~3 number for the LSIL(VaIN1) patients to obtain a high pathological cure rate and HR-HPV clearance rate in our study. Larger areas of lesions and hysterectomy were independent risk factors for pathological persistence. It was harder for postmenopausal females to eliminate HR-HPV, and condom use could protect patients from HR-HPV infection. Preventing vaginitis was important to reduce the complication incidence of laser ablation.

## Declaration of Interest statement

All authors declared no conflict of interest.

## Authors' contributions are listed as follows.

Dongli Kong: Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation, Visualization

Ruqi Chen: Methodology, Software, Data curation, Writing-Original draft preparation

Silu Meng: Methodology, Software, Data curation

Xinran Fan: Methodology, Software

Ruoqi Ning: Data curation, validation

Haiying Sun: Data curation, Visualization

Yao Jia: Data curation, Investigation

Jianwei Zhang: Resources

Bing Xiao: Resources

Xiangqin Li: Resources

Shuang Li: Conceptualization, Supervision, Reviewing and Editing, Project administration

### Details of Ethics Approval

The study was approved by the Institutional Review Board at Tongji Hospital (TJ-IRB20210134) on January 19, 2021 and registered at ClinicalTrials.gov (NCT05170620).

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Table 3 The characteristics of VaIN lesions.docx available at <https://authorea.com/users/474122/articles/564071-multiple-targeting-co2-laser-ablation-for-the-treatment-of-vaginal-intraepithelial-neoplasia-a-pilot-study>

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