

Vloga spremenjene mikrobiote nožnice na pojavnost pooperativne krvavitve po eksciziji transformacijske cone z električno zanko (LLETZ)

The role of modified vaginal microbiota on postoperative bleeding following large loop excision of the transformation zone (LLETZ)

Avtor / Author

Darja Arko^{1,2}, Tomaž Rojko³, Andrej Cokan¹, Ivan Žebeljan⁴, Iztok Takač^{1,2}

Ustanova / Institute

¹Univerzitetni klinični center Maribor, Klinika za ginekologijo in perinatologijo, Oddelek za ginekološko onkologijo in onkologijo dojk, Maribor, Slovenija; ²Univerza v Mariboru, Medicinska fakulteta, Katedra za ginekologijo in porodništvo, Maribor, Slovenija; ³Univerzitetni klinični center Maribor, Oddelek za patologijo, Maribor, Slovenija; ⁴Zdravstveni dom Lenart, Dispanzer za ženske, Lenart, Slovenija.

¹University Medical Centre Maribor, Clinic for Gynaecology and Obstetrics, Department for Gynaecological Oncology and Breast Oncology, Maribor, Slovenia; ²University of Maribor, Faculty of Medicine, Department for Gynaecology and Obstetrics, Maribor, Slovenia; ³University Medical Centre Maribor, Department for Pathology, Maribor, Slovenia; ⁴Health Centre Lenart, Clinic for Women, Lenart, Slovenia;

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Key words:

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Izvleček

Namen: Z raziskavo smo želeli ugotoviti, ali spremenjena mikrobiota nožnice s simultano prisotnimi različnimi genitalnimi patogeni in klamidijo trahomatis vpliva na incidenco in jakost pooperativne krvavitve po široki eksciziji transformacijske cone materničnega vratu z diatermijsko zanko (angl. large loop excision of the transformation zone - LLETZ).

Metode: Izvedli smo retrospektivno analizo bolnic, pri katerih smo na Kliniki za ginekologijo in perinatologijo Univerzitetnega kliničnega centra Maribor v letih od 1993 do 2015 naredili LLETZ. Brisi na patogene bakterije in

Abstract

Purpose: To evaluate the effect of a modified vaginal microbiota containing various vaginal pathogens and Chlamydia trachomatis on postoperative bleeding following large loop excision of the transformation zone (LLETZ) of the uterine cervix.

Methods: We performed a retrospective analysis of patients with cervical intraepithelial neoplasia (CIN) who underwent LLETZ at the Clinic for Gynaecology and Obstetrics, UMC Maribor, between 1993 and 2005. Cervical swabs were collected before the procedure to evaluate the presence of Chlamydia trachomatis and other genital pathogens. After the

Naslov za dopisovanje / Correspondence

Asist. Andrej Cokan, dr. med.,
Univerzitetni klinični center Maribor,
Klinika za ginekologijo in perinatologijo,
Oddelek za ginekološko onkologijo in
onkologijo dojk, Ljubljanska ulica 5,
2000 Maribor, Slovenija
Telefon: +386 23212195
E-pošta: cokan.andrej@gmail.com

klamidijo trahomatis so bili odvzeti pred posegom. Po operativnem posegu so bolnice izpolnile vprašalnik, v katerem so ocenjevale trajanje in intenziteto krvavitve iz nožnice.

Rezultati: V raziskavo smo vključili 192 bolnic. Spremenjeno mikrobioto s simultano prisotnimi genitalnimi patogeni in klamidijo trahomatis je imelo 5,2 % bolnic. Med genitalnimi patogeni smo največkrat izolirali streptokok skupine B in kandido. Pojavnost pooperativne krvavitve po LLETZ ni bila različna med skupinama z ali brez spremenjene mikrobiote s simultano prisotnimi genitalnimi patogeni in klamidijo trahomatis (50,0 % proti 37,9 %, p -vrednost = 0,512).

Zaključek: Spremenjena vaginalna mikrobiota s simultano prisotnimi različnimi genitalnimi patogeni in klamidijo trahomatis je pogosta pri bolnicah s cervikalno intraepiteljsko neoplazijo (CIN), vendar ne povzroča povečane pojavnosti pooperativne vaginalne krvavitve po LLETZ.

procedure, the occurrence and severity of postoperative bleeding was evaluated.

Results: A total of 192 patients were recruited. A modified vaginal microbiota containing various vaginal pathogens and Chlamydia trachomatis was present in 5.2% of patients. The most frequently isolated groups of vaginal pathogens were group B streptococcus and Candida species. The occurrence of postoperative bleeding after LLETZ did not differ significantly when compared between groups with or without a modified vaginal microbiota containing various vaginal pathogens and Chlamydia trachomatis (50.0% vs. 37.9%, $p = 0.512$).

Conclusion: Patients with cervical intraepithelial neoplasia (CIN) commonly exhibit a modified vaginal microbiota containing various vaginal pathogens and Chlamydia trachomatis. However, this is not an important risk factor for postoperative bleeding after LLETZ.

INTRODUCTION

Cervical intraepithelial neoplasia (CIN) is the most common premalignant condition of the uterine cervix. This condition involves atypical squamous changes of the transformation zone, located between the endocervix, covered by the glandular epithelium, and the ectocervix that is covered by squamous epithelium. The Lower Anogenital Squamous Terminology (LAST) Project published in 2012, divided premalignant conditions into low-grade (LSIL) and high-grade squamous intraepithelial lesions (HSILs) (1, 2). The major etiological agent underlying cervical precancer and cancer is human papillomavirus (HPV) (3, 4). Neither the collection of data on new cases of HPV infection, nor changes in the proportion of people infected with HPV, are incorporated in the epidemiological monitoring of

sexually transmitted infections in Slovenia. However, data from 2010 showed that at least 59.2% of women had been infected with HPV once in their lifetime with at least one of the high-risk HPV genotypes (5). On the other hand, we also know that 80 - 90% of vaginal infections are caused by various pathogens and that the most commonly reported sexually transmitted bacterial infection in Slovenia in 2016 was Chlamydia trachomatis (246 cases; 11.9/100.000 inhabitants) (5, 6). Therefore, we suspected that a proportion of women with CIN will also be infected either by vaginal pathological microorganisms or by Chlamydia trachomatis. Studies have shown that local excisional treatment appears to alter the cervical microbiota towards a less diverse microbiota and also that women

with CIN have a more diverse cervical microbiota than women with normal cervical cytology (7). The most common surgical method for treating CIN at present is large loop excision of the transformation zone (LLETZ). However, this procedure is associated with a range of postoperative complications, including pain, discharge, and bleeding. In the present study, we analyzed the effects of a modified vaginal microbiota containing various vaginal pathogens and *Chlamydia trachomatis* on the prevalence of postoperative bleeding after LLETZ.

MATERIALS AND METHODS

This was a retrospective study of patients with CIN who underwent LLETZ between 1993 and 2015 at the Department for Gynaecological Oncology and Breast Oncology, University Medical Centre (UMC) Maribor, Slovenia. All patients had been histologically diagnosed with CIN via the analysis of specimens obtained by punch biopsy.

Prior to LLETZ, all patients were asymptomatic for vaginal infection. To evaluate the presence of *Chlamydia trachomatis* and other genital pathogens (anaerobes, *Streptococcus* groups A–G, coliforms, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Candida* species) we took microbiological swabs from the vagina and uterine cervix using cotton-tipped swabs inserted in medium (UNI-TER AMIES CLR, Piove di Sacco, Italy) and rayon sterile dry swabs with a regular tip (162C, Copan, Brescia, Italy), respectively. First, the patients were given a local anesthetic (1% lidocaine). Then, the LLETZ procedure was carried out with an Elektrotom 400 Unit (Berchtold, Berlin, Germany) with loops ranging in size from 10 mm to 20 mm. The excision was performed using a bipolar current with a cut frequency set to 40 W. In addition, the wound surface was coagulated with a coagulation frequency from 20 W to 40 W.

Following the procedure, we monitored patients for the occurrence and severity of postoperative bleeding. Patients were asked to record any bleeding on a daily basis for the first 90 days following the procedure. Daily

bleeding was subjectively scored as none (0), mild (1), moderate (2), or severe (3). Patients were instructed to mark the occurrence of postoperative bleeding in a questionnaire that was given to them when they were discharged from the hospital (Figure 1). Each questionnaire related to a thirty-day period; thus, patients received three questionnaires each. Three months after the procedure, each patient was invited for a checkup during which gynecological examination was performed and a cervical smear was taken.

Fisher's exact test was used to compare categorical data between groups of patients with modified vaginal microbiota containing various vaginal pathogens and *Chlamydia trachomatis* and patients without this modification. A statistically significant difference in the occurrence of postoperative bleeding between groups was defined as when $p < 0.05$. We did not perform a sample size calculation because this was a retrospective study and because parameters were provided by post-hoc analysis.

RESULTS

Our analysis included 192 patients. Modified vaginal microbiota containing various pathogens and *Chlamydia trachomatis* was present in 5.2% (10/192) and absent in 94.8% (182/192) of patients. In patients without such changes in the vaginal microbiota, 3.7% (7/182) of patients only showed *Chlamydia trachomatis*; in 45.3% (87/182) of cases, only other genital pathogens were present. In 45.8% (88/182) of patients, the cervical swabs were negative. The most frequently isolated groups of genital pathogens were α -hemolytic and group B β -hemolytic streptococcus and *Candida* species (Table 1). The incidence of postoperative bleeding among our study cohort is shown in Table 2, while the severity of bleeding (mild, moderate or severe) is shown in Table 3. Combined postoperative bleeding (subjectively scored as mild, moderate or severe) was present in 50.0% (5/10) of the patients with a modified vaginal microbiota containing various pathogens and *Chlamydia trachomatis*, and in 37.9% (69/182) of patients who did not show these changes. The difference between these two groups was not statistically significant (50% vs.

37.9%; $p = 0.512$), although the absolute difference in the occurrence of postoperative bleeding exceeded 10%.

DISCUSSION

In this retrospective study, patients who had modified vaginal microbiota containing various pathogens and Chlamydia trachomatis did not experience significantly higher levels of postoperative bleeding after LLETZ than patients without such changes in their vaginal microbiota (50% vs. 37.9%; $p = 0.512$). However, this finding should be interpreted with caution, since the number of patients in the first subgroup was very low and in the latter group, there were only 45.8% of patients with negative cervical swabs. Furthermore, the period used to record postoperative bleeding was

quite long (90 days). From this dataset, were unable to ascertain whether all of the patients recorded just postoperative bleeding; it is possible that some patients also recorded menstrual bleeding, especially after the 30 day timepoint post-LLETZ.

The severity and distribution of bleeding was interesting; although 50% of patients with a modified vaginal microbiota containing various pathogens and Chlamydia trachomatis reported postoperative bleeding, this was minimal in all cases. However, in the group who did not show such changes in their vaginal microbiota, 12.1% and 7.1% of patients experienced moderate and severe postoperative bleeding, respectively.

In a previous study, we investigated the role of genital pathogens in morbidity following LLETZ and found that that genital pathogens or Chlamydia trachomatis alone did not have a significant effect on postoperative morbidity, including postoperative bleeding (8-10). Sarkar et al. also came to the same conclusion and found no statistically significant difference in the mean duration of bleeding after LLETZ in two study groups

KONIZACIJA

PRIIMEK IN IME

BOLEČINE
0 ne
1 minimalna
2 zmerna
3 huda

ISČEDEK
0 ne
1 minimalen
2 zmern
3 močan

KRVAVITVE
0 ne
1 minimalna
2 izobna
3 močna

1	31	61
2	32	62
3	33	63
4	34	64
5	35	65
6	36	66
7	37	67
8	38	68
9	39	69
10	40	70
11	41	71
12	42	72
13	43	73
14	44	74
15	45	75
16	46	76
17	47	77
18	48	78
19	49	79
20	50	80
21	51	81
22	52	82
23	53	83
24	54	84
25	55	85
26	56	86
27	57	87
28	58	88
29	59	89
30	60	90

1	31	61
2	32	62
3	33	63
4	34	64
5	35	65
6	36	66
7	37	67
8	38	68
9	39	69
10	40	70
11	41	71
12	42	72
13	43	73
14	44	74
15	45	75
16	46	76
17	47	77
18	48	78
19	49	79
20	50	80
21	51	81
22	52	82
23	53	83
24	54	84
25	55	85
26	56	86
27	57	87
28	58	88
29	59	89
30	60	90

1	31	61
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12	42	72
13	43	73
14	44	74
15	45	75
16	46	76
17	47	77
18	48	78
19	49	79
20	50	80
21	51	81
22	52	82
23	53	83
24	54	84
25	55	85
26	56	86
27	57	87
28	58	88
29	59	89
30	60	90

Navodilo: vsak dan vpilji v ustrezno rúbiko oceno bolečin, izcedka iz nožnice in krvavitve. Uporabi številke od 0 do 3 iz legende. Pri oceni 3 se posvetuj z ginekologom. Dan 1 je prvi dan po operaciji.

Pri kontrolnem pregledu 3 mesece po operaciji obvezno prinesai:
- ta list
- fotokopijo edspustnice z vpisanim histološkim izvidom.

Figure 1. The questionnaire used by patients for marking the occurrence of postoperative bleeding after LLETZ.

Table 1. Types of genital pathogens in patients with cervical intraepithelial neoplasia (n = 143)

Pathogen	No. (%)
Group B β-hemolytic Streptococcus	24 (16.8)
α-hemolytic Streptococcus	20 (14.0)
Candida	18 (12.6)
Chlamydia trachomatis	17 (11.8)
Gardnerella vaginalis	15 (10.5)
Enterococcus species	12 (8.4)
Diphtheroids	11 (7.7)
Coagulase-negative Staphylococcus	11 (7.7)
Escherichia coli	10 (7.0)
Atropine (mg)	10 (7.0)
Staphylococcus aureus	3 (2.1)
Haemophilus species	2 (1.4)

Table 2. The incidence of postoperative bleeding among a group of patients with modified vaginal microbiota containing various vaginal pathogens and *Chlamydia trachomatis* and a group of patients without such changes in the vaginal microbiota following LLETZ (n = 192).

Postoperative bleeding	Various vaginal pathogens and <i>Chlamydia trachomatis</i> present [No. (%)]	Various vaginal pathogens and <i>Chlamydia trachomatis</i> not present [No. (%)]	Total [No. (%)]
Absent	5 (50.0)	113 (62.1)	118 (61.5)
Present	5 (50.0)	69 (37.9)	74 (38.5)
Total	10 (100.0)	182 (100.0)	192 (100.0)

Large loop excision of the transformation zone (LLETZ); $p = 0.512$

Table 3. The severity of postoperative bleeding in a group of patients with modified vaginal microbiota containing various vaginal pathogens and *Chlamydia trachomatis* and a group of patients without such changes in the vaginal microbiota following LLETZ (n = 192).

Severity	Vaginal pathogens and <i>Chlamydia trachomatis</i> present [No. (%)]	Vaginal pathogens and <i>Chlamydia trachomatis</i> not present [No. (%)]	Total [No. (%)]
Absent	5 (50.0)	113 (62.1)	118 (61.5)
Minimal	5 (50.0)	34 (18.7)	39 (20.3)
Moderate	0 (0.0)	22 (12.1)	22 (11.5)
Severe	0 (0.0)	13 (7.1)	13 (6.8)
Total	10 (100.0)	182 (100.0)	192 (100.0)

Large loop excision of the transformation zone (LLETZ)

(genital pathogens present or absent), although the mean duration of bleeding, graded by the patient as being moderate to severe, did differ significantly between the two groups, thus, suggesting that prophylactic antibiotic therapy may be useful for such cases (11). On the other hand, Chan et al. concluded that the use of routine prophylactic topical antibiotics should not be recommended for the general population since it is not possible to identify subgroups of women with positive vaginal or endocervical swabs prior to the procedure because swabs are not routinely taken (12).

Nevertheless, genital pathogens are very common among patients with CIN and could also represent an important co-factor that prevents the spontaneous clearance of HPV and contributes to the progression towards neoplasia, although their specific role in cervical carcinogenesis has yet to be elucidated (13-15). In our study, 50.1% of patients tested positive for genital pathogens, although it is important to note that inflammatory changes and symptoms do not necessarily occur in women who are asymptomatic carriers or have modified vaginal microbiota. This means that these symptoms are not good indicators for a modified vaginal microbiota (16). Data from the literature report a relatively high incidence and prevalence of asymptomatic chlamydial infection (17). However, there are also data to suggest that chlamydial infection is not an important risk factor for the development or promotion of CIN; indeed, chlamydial infection is not commonly detected in patients with CIN (17-20). In our study, chlamydial infection was present in only 8.9% of patients with CIN.

The major limitations of this report are the retrospective nature of the study, the small number of patients included, and the subjective assessment of the occurrence and severity of postoperative bleeding. Furthermore, the number of cases with simultaneous infection was much smaller than those without infection; this may have affected the validity of our results. It is possible that these limitations may have restricted the statistical analysis. Further analyses, involving larger sample sizes from prospective studies, are now needed to confirm our findings.

CONCLUSIONS

A modified vaginal microbiota containing genital pathogens and *Chlamydia trachomatis* does not play a specific role in the postoperative bleeding experienced after LLETZ and it is not an important risk factor for such bleeding. Our results suggest that routine testing with microbiological cervical swabs prior to LLETZ, or the routine use of prophylactic topical antibiotics, should not be recommended for the general population.

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DECLARATION OF CONFLICTING INTERESTS

None of the authors have any conflicts of interest to declare.

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