Original Research

Topical Imiquimod for the Treatment of High-Grade Squamous Intraepithelial Lesions of the Cervix

A Randomized Controlled Trial

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OBJECTIVE: To evaluate the histologic response rate of high-grade squamous intraepithelial lesions (HSIL) of the cervix after topical application of 5% imiquimod cream.

METHODS: In this phase II trial, women with cervical HSIL (cervical intraepithelial neoplasia [CIN] 2–3) were randomly assigned to 250 mg of 5% imiquimod cream applied to the cervix weekly for 12 weeks, followed by loop electrosurgical excision procedure (LEEP) without preceding treatment. The sample size was calculated based on the HSIL regression rates previously reported by Grimm et al. The primary outcome was rate of histologic regression (to CIN 1 or less) in LEEP specimens. Prespecified secondary endpoints included surgical margin status and adverse events. Outcomes were stratified by human papillomavirus type and lesion grade (CIN 2 or CIN 3). Results were reported according to per protocol (PP) and intention-to-treat (ITT) analyses.

RESULTS: Ninety women were enrolled: 49 in the experimental group and 41 in the control group. In the PP population, histologic regression was observed in 23 of 38 participants (61%) in the experimental group compared with 9 of 40 (23%) in the control group (P=.001). Surgical margins were negative for HSIL in 36 of 38 participants (95%) in the experimental group and 28 of 40 (70%) in the control group (P=.004). In the ITT population, rates of histologic regression also were significantly higher in the experimental group. Rates of adverse events in the experimental group were 74% (28/38) in the PP population and 78% (35/45) in the ITT population. Adverse events were mild, with abdominal pain being the most common. Three patients in the experimental group had grade 2 adverse events, including vaginal ulcer, vaginal pruritus with local edema, and moderate pelvic pain.

CONCLUSION: Weekly topical treatment with imiquimod is effective in promoting regression of cervical HSIL.

CLINICAL TRIAL REGISTRATION: ClinicalTrials.gov, NCT03233412.

Histologically confirmed high-grade squamous intraepithelial lesions (HSIL) of the cervix is induced by human papillomavirus (HPV)[1] and is a precursor of cervical cancer. Surgical excision by either cold knife conization, laser conization, or loop electrosurgical excision procedure (LEEP) is the gold

standard treatment.[2] However, surgical excision is associated with obstetric complications such as preterm delivery, premature rupture of amniotic membranes, chorioamnionitis, low birth weight, admission of the newborn to the intensive care unit, and an increase in perinatal morbidity.[3–6]

Imiquimod is an imidazoquinoline amine that binds to Toll-like receptors 7 and 8 of macrophages, producing cytokines and interferons (IFNs), specifically IFN-alpha and IFN-beta, that limit viral replication and stimulate natural killer cells.[7] Additionally, these cytokines and IFNs stimulate dendritic cells, thereby generating proliferation of CD4 T lymphocytes, IFN-gamma, cytokines and activation of CD8 T lymphocytes, all of which are toxic to HPV.[8,9]

The aim of this study was to evaluate the histologic response of cervical HSIL after topical application of 5% imiquimod cream, with histologic response defined as histologic regression to cervical intraepithelial neoplasia (CIN) 1 or less in the LEEP specimen. Secondary objectives included the effect of imiquimod treatment on LEEP margin status, adverse events, and tolerance of treatment.

METHODS

Patients aged 25–50 years with a confirmed diagnosis of CIN 2–3 were prospectively enrolled at Barretos Cancer Hospital in Barretos, Brazil, from August 2017 through April 2019. All patients had had participated in our screening program for cervical cancer prevention and had abnormal cervical cytology. Colposcopy was performed by a gynecologist using acetic acid 5%, followed by Lugol's solution 1% at magnification increments from 6× to 40× with directed cervical biopsy. Findings were classified according to the 2011 Colposcopic Terminology of the International Federation for Cervical Pathology and Colposcopy.[10] In cases of histologically confirmed CIN 2 or CIN 3, women were invited to participate in this study. Exclusion criteria included: 1) suspected or confirmed invasive squamous carcinoma or in situ or invasive
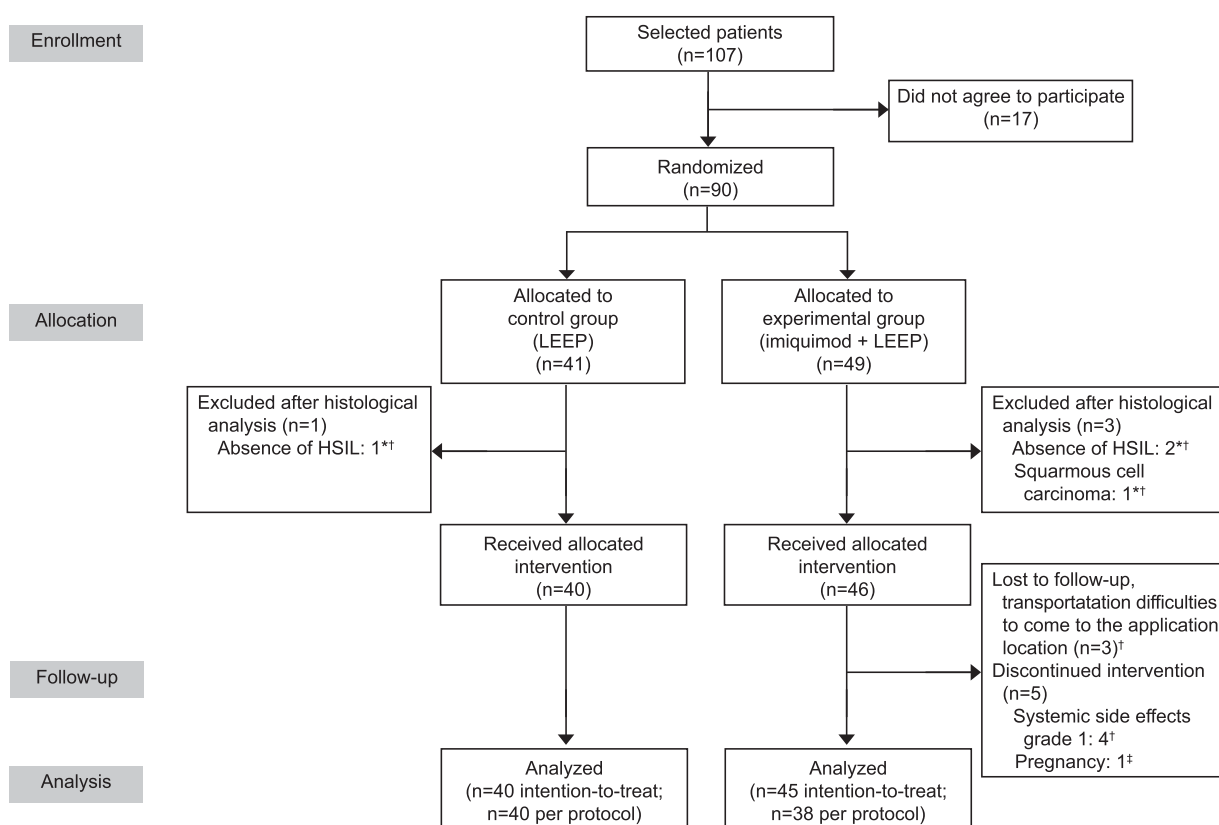
Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flow chart of randomization and clinical trial progress. *Patients redrawn from intention-to-treat analysis. †Patients redrawn from per protocol analysis. ‡Excluded from per protocol and intention-to-treat analysis because they became pregnant during treatment and had not yet undergone loop electrosurgical excision procedure (LEEP). HSIL, high-grade squamous intraepithelial lesion.

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Video 1. Imiquimod application in the cervix. Application of 5% imiquimod cream on the cervix with the aid of a disposable brush. Video created by Bruno de Oliveira Fonseca, MD. Used with permission.

adenocarcinoma by colposcopy, biopsy, or cytology; 2) current pregnancy or lactation; 3) immunosuppression due to HIV or organ transplantation; and 4) previous treatment for HSIL. Eligible patients who agreed to participate in the study provided informed consent. The CONSORT (Consolidated Standards of Reporting Trials) flow diagram[11] is shown in Figure 1.

The study was a randomized phase II trial, without blinding and with parallel groups (Clinical-Trials.gov Identifier: NCT03233412). The study was approved by the Barretos Cancer Hospital Research Ethics Committee (No. 2,133,654). Patients were randomly assigned to two parallel groups: 1) imiquimod followed by LEEP and 2) LEEP without preceding treatment. After randomization, all pathology samples were reanalyzed by two pathologists with specialized training in gynecologic cancers to confirm HSIL.

The randomization sequence was in blocks of eight using the R software 3.4.3 by function sample. This list was loaded on the REDCap[12] platform where a simple random allocation.

Patients in both groups underwent molecular testing for high-risk HPV (COBAS 4800 test). The Brazilian cervical cancer screening program is based

only on cervical cytology. These patients underwent the COBAS HPV test only on the date of entry into the study, that is, 3 months after the collection of cytology in the screening program and 1 month after the initial colposcopy and confirmatory HSIL biopsy.

Patients in the experimental group underwent application of imiquimod directly to the cervix (Video 1) once a week for 12 weeks followed by LEEP. The women underwent a weekly gynecologic examination during which a speculum was used to visualize the cervix, and 250 mg of 5% imiquimod cream was applied using a disposable brush (Viba-Brush) (Fig. 2). The entire transformation zone of the cervix was covered during the application (Fig. 3). Sexual abstinence was advised for at least 72 hours after application. Before the seventh week of treatment, the women underwent a repeat colposcopic examination to assess clinical response by a doctor different from the one responsible for the application of imiquimod.

In both the experimental and control groups, patients underwent LEEP with local anesthesia (with mepivacaine) performed by the same surgeon (B.O.F.), per local guidelines. Our patient follow-up schedule was identical for the control group and the experimental group: cytology, high-risk HPV testing, and colposcopy every 6 months for at least 2 years.

All pathology slides from biopsy and LEEP were evaluated by two pathologists with specialized training in gynecologic cancers. Consensus was achieved for discordant cases. Histologic diagnosis categories included cervicitis–benign, CIN 1, CIN 2, CIN 3, and invasive cancer. If HSIL could not be precisely graded as CIN 2 or 3, it was defined as high-grade CIN. In cases of uncertain diagnosis, complementary immunohistochemical examination was performed. In cases positive for p16, the final diagnosis was high-grade CIN. When p16 staining was inconclusive, Ki-67 staining was performed. The size of the LEEP
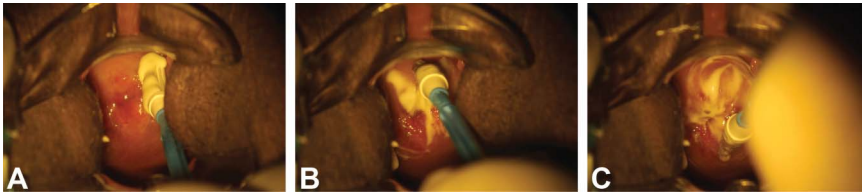

Fig. 2. Disposable brush.
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Scan this image to view Video 1 on your smartphone.

Fig. 3. Application of the imiquimod immunomodulator to the cervix. Beginning of the application (**A**); imiquimod reaching the entire transformation zone (**B**); imiquimod covering all lesion and transformation zone (**C**).

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specimens and the status of the surgical margins (endocervical, ectocervical, or both) were recorded. Response was categorized as: regression, defined as CIN 1 or cervicitis–benign; persistence, defined as presence of HSIL; or progression, defined as presence of invasive cervical carcinoma.

Adverse events were documented weekly according to patient reports and findings on the gynecologic examination. They were graded according to the Common Terminology Criteria for Adverse Events guidelines v.4.03[13] from grade 0 (no symptoms) to grade 5 (death). In patients with grade 1 adverse events, treatment was continued as long as the patient was willing; treatment for symptoms was prescribed if necessary. In patients with grade 2 adverse events, treatment was suspended for 7 days and then reassessment was performed to determine if treatment could be restarted. In patients with grade 3 or 4 adverse events, treatment would be suspended and a LEEP scheduled as soon as possible.

Based on the HSIL regression rates previously reported by Grimm et al,[14] of a 34% response difference (39% and 73% in the placebo and imiquimod groups, respectively) with a significance level of 5% and a power of 85%, a sample size of 41 was estimated for each group (G-Power software 3.1.9.6). Assuming a rate of loss to follow-up of 20% for the experimental group and in an effort to ensure equal numbers of patients in the two groups at the end of the study, eight additional patients were included in the experimental group, for a total sample size of 90 patients.

Analyses were undertaken in two populations: the per protocol (PP) population, defined as patients who completed the entire study protocol, excluding patients with protocol violations, and the intention-to-treat (ITT) population, defined as patients who fully or partially completed the study protocol. A mandatory interim analysis for imiquimod efficacy was done in the middle of the study recruitment and showed that 60% (ITT population) to 80% (PP population) of patients treated with imiquimod had histologic regression of HSIL by analysis of the LEEP specimens.

Patient characteristics were summarized with mean and SD for quantitative variables and relative and absolute frequencies for qualitative variables. Normality of the data was verified using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The average number of patients who would need to receive the intervention for the outcome to occur, the number needed to treat (NNT), was calculated as the inverse of absolute risk reduction. Absolute risk reduction was defined as the percentage of patients with histologic regression in the control group subtracted from the percentage of patients with histologic

Table 1. Allocation of Cases Analyzed by Intention To Treat According to Sociodemographic and Clinical Characteristics

Variable	Control Group (n=40)	Experimental Group (n=45)
Age (y)	36±13	32±10
No. of pregnancies	2±3	2±2
Education[†]		
High	9 (22.5)	4 (8.9)
Medium	17 (42.5)	19 (42.2)
Low	14 (35.0)	22 (48.9)
Smoking		
No	28 (70.0)	32 (71.1)
Yes	12 (30.0)	13 (28.9)
HR HPV test result		
Positive	29 (72.5)	36 (80.0)
Negative	10 (25.0)	9 (20.0)
Invalid	1 (2.5)	0
HPV type		
16	12 (30.0)	18 (40.0)
18	3 (7.5)	2 (4.4)
16 and others	3 (7.5)	12 (26.7)
18 and others	3 (7.5)	2 (4.4)
Others	20 (50.0)	30 (66.7)
Initial biopsy[‡]		
CIN 3	21 (52.5)	30 (66.6)
CIN 2	8 (20.0)	12 (26.7)

HR HPV, high-risk human papillomavirus; CIN, cervical intraepithelial neoplasia.

Data are median±interquartile range or n (%).

[†] Education level according to the International Standard Classification of Education (ISCED) 2011: low—comprises incomplete primary and elementary education; medium—includes complete elementary education and technical–vocational education; high—includes incomplete higher education or above.

[‡] In some HSIL samples, it was not possible histologically to classify them as CIN 2 or CIN 3.

regression in the experimental group. The difference in response rates between the experimental and control groups was calculated with a 95% CI. For group comparisons, we used Pearson's χ^2 test, the Fisher exact test, or the Mann-Whitney U test. Characteristics with $P<.20$ in the aforementioned analyses were selected to fit the multiple logistic regression model, through which the odds ratio (OR) and its 95% CI were estimated. The final model was adjusted with variables with $P<.05$. The level of significance assumed for the other tests was 5%.

RESULTS

The study included 41 participants in the control group and 49 in the experimental group. As a result of findings on reevaluation of the pathology samples after randomization, one patient was excluded from the control group because HSIL was not confirmed, and three patients were excluded from the experimental group, two because HSIL was not confirmed and one because invasive squamous cell carcinoma was diagnosed. Therefore, we included for treatment allocation 40 patients in the control group and 46 patients in the experimental group.

In the experimental group, after treatment allocation, one patient was excluded from per protocol and ITT analysis because she became pregnant during treatment and she had not yet undergone LEEP. This patient had her treatment interrupted in the fifth week

of pregnancy when she had already undergone eight applications of imiquimod. She underwent regular prenatal care, and no teratogenic effects were observed during pregnancy. At the end of the study, the infant was 5 months old and the patient was being followed up at the colposcopy clinic according to Barretos Cancer Hospital's standard care protocol. Seven more women in the experimental group were excluded from per protocol analysis because of systemic side effects (four patients) and transportation problems making it difficult to come to the hospital (three patients). Therefore, in the control group, we included 40 patients in the per protocol and ITT analyses, and in the experimental group, we included 45 patients in the ITT analysis and 38 patients in the per protocol analysis (Fig. 1). All patients removed from the study analysis were treated at the Barretos Cancer Hospital in accordance with the standard institutional protocol.

Sociodemographic and clinical characteristics were balanced between the two groups in the ITT population (Table 1). Characteristics were also balanced between groups in the PP population. In 14 patients, HSIL could not be graded as CIN 2 or CIN 3, and these lesions were classified as high-grade CIN.

The rate of histologic regression was higher in the experimental group than in the control group in both populations analyzed (Table 2). In the PP population, histologic regression occurred in 22.5% of the LEEP specimens in the control group and in 60.5% of the

Table 2. Histologic Evolution After Evaluation of the LEEP Surgical Specimen

Variable	Control Group	Experimental Group	Response Difference (%) (95% CI)
Analysis by ITT			
Primary outcome			
Regression*	9 (22.5)	24 (53.3)	30.8 (11–50)[†]
Secondary outcome			
Persistence[‡]	30 (75)	20 (44.5)	28.3 (9–48)[§]
Progression[‖]	1 (2.5)	1 (2.2)	—
Total	40 (100)	45 (100)	—
Analysis by PP			
Primary outcome			
Regression*	9 (22.5)	23 (60.5)	38 (18–58)[†]
Secondary outcome			
Persistence[‡]	30 (75)	15 (39.5)	35.5 (15–56)[§]
Progression[‖]	1 (2.5)	0 (0)	—
Total	40 (100)	38 (100)	—

LEEP, loop electrosurgical excision procedure; ITT, intention to treat; PP, per protocol.
Data are n (%) unless otherwise specified.
* Regression: grade 1 cervical intraepithelial injury or complete remission.
[†] Calculated by the percent regression of the experimental group subtracted from the percent of the control group.
[‡] Persistence: grade 2 or 3 cervical intraepithelial lesion.
[§] Calculated by the percent persistence of the control group subtracted from the percent of the experimental group.
[‖] Progression: invasive cervical carcinoma.

specimens in the experimental group (P=.001), resulting in a NNT of 2.63 (95% CI 1.7–5.6). High-grade squamous intraepithelial lesions persisted in 75% of the specimens in the control group and in 39.5% of those in the experimental group (P=.002). In the ITT population, histologic regression occurred in 22.5% of the LEEP specimen in the control group and 53.3% of the specimens in the experimental group (P=.004), resulting in a NNT of 3.25 (95% CI 2.0–9.1). High-grade squamous intraepithelial lesions persisted in 75% of the specimens in the control group and in 44.5% of those in the experimental group (P=.008). The analysis of histologic regression only in CIN 3 is showed in Appendix 1, available online at http://links.lww.com/AOG/C298, and the comparison of the response in CIN 2 and CIN 3 is showed in Appendix 2, also available online at http://links.lww.com/AOG/C298.

One patient in the control group had progression of the lesion in the LEEP specimen. The diagnosis was superficially invasive squamous cell carcinoma, stage IA1. She underwent laparoscopic hysterectomy and bilateral salpingectomy. The pathology report showed HSIL (CIN 3) without residual invasive neoplasia, with negative vaginal margins. In the ITT population, one patient in the experimental group had progression of the lesion. This patient underwent nine applications of imiquimod, after which she discontinued treatment. She subsequently underwent LEEP. The pathology report showed stage IA1 invasive squamous cell carcinoma. Treatment was complemented with laparoscopic hysterectomy and bilateral salpingectomy. The surgical pathology report did not show any residual invasive lesion and showed margins negative for precursor lesion.

Rates of histologic regression stratified by sociodemographic and clinical characteristics are shown in Table 3. As in both the PP and ITT populations, the histologic regression rate and the positivity of the high-risk HPV test varied significantly by treatment group (experimental or control: P<.20 for both) a multiple logistic regression model was designed (Table 4).

Table 3. Histologic Regression After Evaluation of the LEEP Surgical Specimen Stratified by Sociodemographic and Clinical Characteristics

| Variable | Regression | | | |
	PP Population	P	ITT Population	P
Primary outcome				.004
Group				
Control	9/40 (22.5)		9/40 (22.5)	
Experimental	23/38 (60.5)	.001	24/45 (53.3)	
Secondary outcomes		.36		.20
Age (y)				
Younger than 30	8/24 (33.3)		10/33 (30.3)	
30 or older	24/54 (44.4)		23/52 (44.2)	
Education*				
High	13/31 (41.9)	.98	14/36 (38.9)	.99
Medium	14/34 (41.2)		14/36 (38.9)	
Low	5/13 (38.5)		5/13 (38.5)	
Smoking				
No	24/57 (42.1)	.75	24/60 (40.0)	.73
Yes	8/21 (38.1)		9/25 (36.0)	
HR HPV test result				
Positive	16/59 (27.1)	<.001	16/65 (24.6)	.001
Negative	15/18 (83.3)		16/19 (84.2)	
Invalid	1/1 (100)		1/1 (100)	—
Initial biopsy[†]		.47		.45
CIN 2	9/19 (47.4)		9/20 (45.0)	
CIN 3	17/45 (37.8)		18/51 (35.3)	

LEEP, loop electrosurgical excision procedure; PP, per protocol; ITT, intention to treat; HR HPV, high-risk human papillomavirus; CIN, cervical intraepithelial neoplasia.

Data are n/N (%) unless otherwise specified.

Statistical analysis using Pearson's χ^2 test.

* Education level according to the International Standard Classification of Education 2011[2]: low, includes incomplete infant, primary and elementary education; medium, includes complete elementary education and technical–vocational education; high, includes incomplete higher education and above.

[†] In some high-grade squamous intraepithelial lesion samples, it was not possible histologically to classify as CIN 2 or CIN 3.

Table 4. Multiple Logistic Regression Model for Group Variables and High-Risk Human Papillomavirus Test in Relation to High-Grade Squamous Intraepithelial Lesions Histologic Regression (Secondary Outcomes)

Variable	n*	OR (CI 95%)	P
Analysis by ITT			
Group			.003
Control	39	1.00	
Experimental	45	24.0 (3.0–191.1)	
HPV test result			.001
Positive	65	1.00	
Negative	19	82.7 (8.4–818.4)	
Analysis PP			
Group			.001
Control	39	1.00	
Experimental	38	31.7 (3.9–257.5)	
HPV test result			.001
Positive	59	1.00	
Negative	18	76.6 (7.5–783.6)	

OR, odds ratio; ITT, intention to treat; HPV, human papillomavirus; PP, per protocol.

In this multivariable model, the HPV test and the group randomization variable were used, as they showed $P<.20$ when analyzed separately in the interference with the histologic regression of the lesion.

* Because one patient presented with an invalid HPV test, a total sample of 77 (PP) and 84 (ITT) women were considered for the calculus of logistic regression.

Table 5. Comparison of Surgical Margins and Depth of LEEP Parts Between Groups (Secondary Outcomes)

Variable	Control Group	Experimental Group	P
Analysis by ITT	n=40	n=45	
Surgical margins			.055*
Free	28 (70)	40 (88.9)	
Compromised	12 (30)	5 (11.1)	
Type of positive margins			—
Endocervical	7/12 (58.3)	1/5 (20)	
Ectocervical	1/12 (8.3)	2/5 (40)	
Both	4/12 (33.3)	2/5 (40)	
Depth of the specimen (mm)	9.3±3.1	9.5±3.4	0.73†
Analysis by PP	n=40	n=38	
Surgical margins			.004*
Free	28 (70)	36 (94.7)	
Compromised	12 (30)	2 (5.3)	
Type of positive margins			—
Endocervical	7/12 (58.3)	0	
Ectocervical	1/12 (8.3)	1/2 (50.0)	
Both	4/12 (33.3)	1/2 (50.0)	
Depth of the specimen (mm)	9.3±3.1	9.8±3.4	.38†

LEEP, loop electrosurgical excision procedure; ITT, intention to treat; PP, per protocol.

Data was n (%), n/N (%), or average±SD unless otherwise specified.

P-value was calculated to compare the control and experimental groups.

* Pearson's χ^2 test.

† Mann-Whitney U test.

As one patient had an invalid HPV test, 77 women were included in the PP population, and 84 were included in the ITT population. In the PP population, the OR was 31.7 (95% CI 3.9–257.5) ($P<.001$) for HSIL regression in patients in the experimental group, compared with patients in the control group. In the ITT population, the OR was 24.0 (95% CI 3.0–191.1) ($P=.003$) for HSIL regression in patients in the experimental group compared with those in the control group.

The status of the surgical margins in the LEEP specimen is summarized in Table 5. In the control group, the surgical margins were negative for intraepithelial lesion in 28 of 40 patients (70.0%). In the experimental group, the surgical margins were negative in 36 of 38 patients (94.7%) in the PP population ($P=.004$) and 40 of 45 patients (88.9%) in the ITT population ($P=.055$). The depth of the surgical specimens was equivalent in the two groups.

The mean interval between the diagnosis of HSIL and the LEEP procedure was 16.0±6.1 weeks in the control group and 21.0±2.6 weeks in the experimental group ($P<.001$). We analyzed whether this delay could interfere in lesion regression, persistence or progression. The mean interval between HSIL diagnosis and LEEP was 17.6±5.8 weeks in patients with histologic regression and 19.7±4.4 weeks in patients with persistent disease or progression ($P=.09$). The rate of histologic regression was higher in the experimental group than in the control group regardless of high-risk HPV type or histologic grade (Table 6).

The side effects are summarized in Table 7. Twenty-eight of 38 women (73.7%) in the PP population and 35 of 45 (77.8%) in the ITT population reported adverse events. Two patients (4%) in the ITT population had grade 2 symptoms. One of them reported intermittent vaginal pruritus with local edema on the day of previous application of imiquimod with spontaneous resolution within 24 hours. The other patient reported, when she came to the hospital for the fourth application of imiquimod, that she had experienced moderate pelvic pain that limited her daily activities for less than 24 hours after the two previous applications. These

Table 6. Histologic Regression After Evaluation of the LEEP Surgical Specimen Divided by Allocation Group and Stratified by the Type of Human Papillomavirus and Histologic Grade at the Time of Inclusion in the Study (Secondary Outcomes)

Variable	Control Group	P	Experimental Group	P
Analysis by ITT	n=40		n=45	
HPV type				
16	0/12	.04*	6/18 (33.3)	.04[†]
18	0/3	.99*	1/2 (50.0)	.99*
Others	1/20 (5.0)	.02*	14/30 (46.7)	.34[†]
16 and others	0/3	.99*	5/12 (41.7)	.50[†]
18 and others	0/3	.99*	1/2 (50.0)	.99*
Initial histology[‡]		.06*		
CIN 2	2/8 (25.0)		7/12 (58.3)	.80[†]
CIN 3	2/21 (9.5)		16/30 (53.3)	
Analysis PP	n=40		n=38	
HPV type				
16	0/12	.04*	6/14 (42.9)	.09[†]
18	0/3	.99*	1/2 (50.0)	.99*
Others	1/20 (5.0)	.02*	14/25 (56.0)	.43[†]
16 and others	0/3	.99*	5/9 (55.6)	.73[†]
18 and others	0/3	.99*	1/9 (50.0)	.99*
Initial histology[‡]		.06*		.76[†]
CIN 2	2/8 (25.0)		7/11 (63.6)	
CIN 3	2/21 (9.5)		15/24 (62.5)	

LEEP, loop electrosurgical excision procedure; ITT, intention to treat; HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; PP, per protocol.
Data are n (%) unless otherwise specified.
* Pearson's χ^2 test.
[†] Fisher exact test
[‡] In some high-grade squamous intraepithelial lesion samples, it was not possible histologically to classify as CIN 2 or CIN 3.

two patients chose not to complete treatment with imiquimod. On clinical examination, seven abnormalities were documented, six (85.7%) classified as grade 1 and one (14.3%) was classified as grade 2. Among the six patients with grade 1 findings, three had increased vaginal discharge, two had mild vaginal bleeding on the speculum examination, and one had focal and superficial erosion of the cervix. The patient with a grade 2 abnormality had a vaginal ulcer in the vaginal introitus, already undergoing epithelialization, that was diagnosed before the fourth application of imiquimod. After improvement of her condition, she completed the 12 weeks of treatment without recurrence of the ulcerated lesion.

DISCUSSION

Weekly topical treatment with imiquimod for 12 weeks is effective in promoting regression of cervical HSIL. One clinical application of these findings is the potential to use in larger lesions to achieve a higher rate of free surgical margins. We observed histologic regression (to CIN 1 or less) in more than half of patients, which suggests this might be an alternative treatment strategy to a cervical excision procedure.

Imiquimod is approved by the U.S. Food and Drug Administration for use in the treatment of external genital and perianal warts, small superficial basal cell carcinomas, and clinically typical actinic keratoses.[15] Its off-label use in vulvar intraepithelial neoplasia and vaginal intraepithelial neoplasia is common and is supported by a solid base of evidence in the literature[16–26]; however, few studies have focused on the effect of topical imiquimod treatment in patients with CIN.[14,27–29]

Most prior studies that evaluated the efficacy of imiquimod in cervical intraepithelial lesions included patients with low-grade lesions (CIN 1) (Jung PS, Kim JH, Kim D. Application of topical imiquimod for treatment of cervical intraepithelial neoplasia in young women: a preliminary result of a pilot study [abstract]. Gynecol Oncol 2016;141:103–4).[28,29] It is difficult to compare results of those studies with our results because our study only included patients with HSIL. Topical imiquimod for exclusive treatment of HSIL was examined in two randomized clinical trials.[14,30] Koeneman et al[30] interrupted their study

Table 7. Proportion of Adverse Events Observed in Patients in the Experimental Group

Variable	Analysis	
	PP (n=38)	ITT (n=45)
Patients having AEs		
Yes	28 (73.7)	35 (77.8)
No	10 (26.3)	10 (22.2)
Total no. of AEs*	41	51
Grade (CTCAE)		
1	41 (100)	49 (96.0)
2	0	2 (4.0)
Higher than 3	0	0
Type of AE		
Abdominal pain	18/41 (43.9)	21/51 (41.2)
Fever	5/41 (12.2)	5/51 (9.8)
Myalgia	4/41 (9.8)	6/51 (11.8)
Fatigue	4/41 (9.8)	4/51 (7.8)
Vaginal bleeding	3/41 (7.3)	3/51 (8.6)
Headache	2/41 (4.9)	2/51 (5.9)
Chills	1/41 (2.4)	1/51 (2.0)
Diarrhea	1/41 (2.4)	1/51 (2.0)
Dysuria	1/41 (2.4)	1/51 (2.0)
Menstrual irregularity	1/41 (2.4)	1/51 (2.0)
Vaginal discharge	1/41 (2.4)	1/51 (2.0)
Vaginal pruritus	0	1/51 (2.0)
Vaginal dryness	0	1/51 (2.0)
Abdominal distension	0	1/51 (2.0)
Pelvic pain	0	1/51 (2.0)
Vaginal pain	0	1/51 (2.0)
Findings on clinical examination		
Yes	7 (18.4)	7 (15.6)
No	31 (81.6)	31 (68.9)
Grade of findings based on clinical examination		
1		
2	6/7 (85.7)	6/7 (85.7)
Higher than 3	1/7 (14.3)	1/7 (14.3)
Clinical findings (n=7)†		
Grade 1 vaginal discharge	3 (42.8)	3 (42.8)
Grade 1 vaginal bleeding	2 (28.6)	2 (28.6)
Grade 2 vaginal ulceration	1 (14.3)	1 (14.3)
Grade 1 erosion of the cervix	1 (14.3)	1 (14.3)

PP, per protocol; ITT, intention to treat; AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events.

Data are n (%) or n/N (%).

* There are patients with more than one symptom, so the sum of the details of the symptoms is greater than 28(PP) or 35(ITT).

† According to Common Terminology Criteria for Adverse Events version 4.0.

because of poor accrual after 12 patients had been recruited. Grimm et al[14] demonstrated histologic regression in 73% of patients in the group treated with imiquimod, resulting in a NNT of 2.9. However, histologic regression was evaluated only with colposcopy-directed biopsy, not removal of the entire transformation zone.[14] In our clinical trial, all patients underwent LEEP, which allowed a thorough assess-

ment of histologic regression. In our study, the rate of positive surgical margins in the LEEP specimens was 5.3% in the experimental group, lower than rates reported in the literature after LEEP without imiquimod, which range from 27% to 46.5%.[31–34] There is evidence that the higher degree of lesion, depth of the conization specimen, and higher parity are risk factors for margin involvement.[31–34] The low frequency of positive margins in our study after imiquimod and LEEP might mean that even if there is no histologic regression, the topical treatment has reduced the lesion length.

Adverse events were frequent among the patients in our current study, with abdominal pain being the most common. In a recent case series, three patients had immunomodulatory treatment discontinued because of severe adverse events such as hyponatremia, severe headache, and corneal erosion, which required hospitalization of two of the patients.[35] Temporary hair loss also was reported in two patients treated with imiquimod as a vaginal suppository.[36] Grimm et al[14] observed adverse events in 97% of patients treated with imiquimod. In our population, no adverse event was higher than grade 2. This lower intensity of adverse effects might be related to the direct application of imiquimod to the cervix, minimizing absorption outside the target organ. In addition, we believe that the once-a-week frequency of application of imiquimod might have reduced local adverse effects.

The limitations of our study include the distance between the patients' cities of residence and Barretos Cancer Hospital, which resulted in missed follow-up visits and imiquimod applications. The delay between CIN 2 and CIN 3 diagnosis and the LEEP procedure in the control group could be a limitation, but we analyzed whether this delay could interfere in lesion regression, persistence or progression. As we showed, this period did not affect the lesion evolution.

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