Topical imiquimod versus surgery for vulvar intraepithelial neoplasia: a multicentre, randomised, phase 3, non-inferiority trial

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Summary

Background The optimal management of vulvar high-grade squamous intraepithelial lesions (vHSILs) is challenging. Surgery is the standard treatment, but recurrences are observed in half of patients. Medical treatment with imiquimod is an effective alternative, but the two modalities have not been compared in a randomised trial. The aim of this study was to compare the clinical effectiveness, histological response, human papillomavirus (HPV) clearance, acceptance, and psychosexual morbidity of primary imiquimod treatment versus surgical treatment in women with vHSIL.

Methods This study was a multicentre, randomised, phase 3, non-inferiority clinical trial done by the Austrian Gynaecological Oncology group at six hospitals in Austria. We recruited female patients aged 18–90 years with histologically confirmed vHSIL with visible unifocal or multifocal lesions. Main exclusion criteria were clinical suspicion of invasion, a history of vulvar cancer or severe inflammatory dermatosis of the vulva, and any active treatment for vHSIL within the previous 3 months. Women with known immunodeficiency, who were pregnant, or who were lactating were excluded. Patients were randomly assigned (1:1) by block randomisation to imiquimod or surgery, and stratified by unifocal or multifocal disease. Treatment with imiquimod was self-administered in a slowly escalating dosage scheme up to three times per week for a period of 4–6 months. Surgery consisted of excision or ablation. Patients were assessed with vulvoscopy, vulvar biopsy, HPV tests, and patient-reported outcomes at baseline and after 6 months and 12 months. The primary endpoint was complete clinical response (CCR) at 6 months after local imiquimod treatment or one surgical intervention. Primary analysis was per protocol with a non-inferiority margin of 20%. This trial is registered at ClinicalTrials.gov, NCT01861535.

Findings 110 patients with vHSIL (78% with unifocal vHSIL and 22% with multifocal vHSIL) were randomly assigned between June 7, 2013, and Jan 8, 2020. Clinical response to treatment could be assessed in 107 patients (54 in the imiquimod group and 53 in the surgery group), and 98 patients (46 in the imiquimod group and 52 in the surgery group) completed the study per protocol. 37 (80%) of 46 patients using imiquimod had CCR, compared with 41 (79%) of 52 patients after one surgical intervention, showing non-inferiority of the new treatment (difference in proportion –0·016, 95% CI –0·15 to –0·18; p=0·0056). Invasive disease was found in five patients at primary or secondary surgery, but not in patients with per-protocol imiquimod treatment. There was no significant difference in HPV clearance, adverse events, and treatment satisfaction between study groups.

Interpretation Imiquimod is a safe, effective, and well accepted alternative to surgery for women with vHSIL and can be considered as first-line treatment.

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Introduction

The incidence of high-grade vulvar intraepithelial neoplasia (VIN) has been rising, predominately among younger women.[1,2] More than 80% of high-grade VIN are caused by persistent infection with high-risk human papillomavirus (HPV), predominately HPV 16.[3] The disease is classified as vulvar high-grade squamous intraepithelial lesions (vHSILs) or usual-type VIN.[4,5] The optimal management of vHSIL has not been established.[6] Surgical excision and laser vaporisation are equally effective, but recurrences occur in up to 50% of women.[7] Surgery might also be associated with morbidity and psychosexual distress.[8] Topical, non-surgical alternatives, which preserve vulvar anatomy, have been studied and are included in European and US guidelines.[6] Imiquimod, a topical immune modulator, is the most investigated off-label treatment for vHSIL. The drug induces secretion of proinflammatory cytokines and alters the local immune response in favour of clearance of persistent HPV infection.[9] Imiquimod appears to be safe and well tolerated, and reported clinical response rates vary between 35% and 81%.[10–12] Imiquimod has been studied against placebo[10,11] and against cidofovir,[12] but not in a randomised trial versus surgery.

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Research in context

Evidence before this study

At the time of the trial design we searched PubMed for reports published in English or German between Jan 1, 1980, and May 30, 2012. We used the search terms "vulvar intraepithelial neoplasia", "imiquimod", and "surgery". Only two randomised controlled trials (RCT) of imiquimod for the treatment of vulvar intraepithelial neoplasia (VIN) had been reported. Both RCTs compared imiquimod against placebo and reported response rates between 35% and 81%. In addition, several observational studies on topical treatment with imiquimod for VIN had been published, reporting varying response rates. After initiation of our trial, a multicentre, open-label, randomised, phase 2 trial of imiquimod versus cidofovir for the treatment of VIN was published. It was shown that both drugs are active, safe, and feasible for treatment of VIN and warrant further investigation. The authors stated that medical treatment should be compared with excisional surgery and laser ablation, with a special emphasis on quality of life and recurrence rates. For this Article, we repeated the Pubmed search up to Aug 30, 2021. A Cochrane systematic review of medical and surgical interventions for the treatment VIN was published in 2016, reporting on five RCTs on medical treatments (four on imiquimod, including the three aforementioned studies, plus one small study [abstract only] on imiquimod *vs* placebo) and one on surgical treatments. Further comprehensive review articles were found, but no new prospective studies on imiquimod for the treatment of VIN were identified.

Added value of this study

We report results from the first randomised trial comparing imiquimod with surgery for human papillomavirus (HPV)-related VIN or vulvar high-grade squamous intraepithelial lesions (vHSIL). We found that per-protocol treatment with imiquimod resulted in a complete clinical response rate of 80% at 6 months, compared with 79% after one surgical intervention. During an observation period of 12 months, clinical and histological response, HPV clearance, adverse events, and health-related quality of life were studied and non-inferiority of imiquimod was shown.

Implications of all the available evidence

Our findings are in line with previous studies and further support the use of imiquimod for vHSIL. The effectiveness and oncological safety of imiquimod treatment has been shown. In addition, our study provides broad clinical and psychological data on advantages and disadvantages of conservative versus surgical treatment. Clinical characteristics of vulvar lesions, patients' preferences, sexual activity, and compliance should guide treatment decisions. Immunohistochemical analysis and long-term follow-up of our study cohort is ongoing and might help to identify patients who are most likely to benefit from topical treatment with imiquimod.

The aim of this randomised clinical trial was to compare the clinical effectiveness, histological response, HPV clearance, acceptance, and psychosexual morbidity of primary imiquimod treatment versus surgical treatment in women with vHSIL.

Methods

Study design and participants

For more on the **randomisation system** see http://www.randomizer.at

This study was a multicentre, randomised, phase 3, non-inferiority clinical trial done by the Austrian Gynaecological Oncology group at six hospitals in Austria. Patients with new or recurrent vHSIL were invited to participate. Inclusion criteria were histologically confirmed vHSIL with visible unifocal or multifocal lesions in women aged 18–90 years. Exclusion criteria were clinical suspicion of invasion, a history of vulvar cancer or severe inflammatory dermatosis of the vulva, known hypersensitivity to imiquimod, and any active treatment for vHSIL within the previous 3 months. Women with known immunodeficiency, who were pregnant, or who were lactating were excluded. Eligible women who were premenopausal were informed that use of contraceptives was required during the study period.

All patients provided written informed consent before randomisation. The study was approved by the Austrian Agency for Health and Food Safety and the Ethics Committee of the Medical University of Graz and institutional review boards of all participating sites.

Randomisation and masking

Eligible and consenting patients were randomly assigned to primary topical treatment with imiquimod or surgery via a central computerised system with block randomisation with block size of six, at a ratio of 1:1, and stratified by unifocal or multifocal disease. Study allocation was concealed from study investigators, but masking was not possible because of the distinct different treatment modalities. Masking of clinical assessors was not feasible, because follow-up assessments were usually done by the same investigator, who recruited patients and informed them about study allocation.

Procedures

Treatment with imiquimod was self-administered for a minimum of 16 weeks. All patients who had not achieved complete clinical response at 4 months were asked to continue application until complete clinical response, with a maximum duration of 6 months. Patients were handed a package of 5% imiquimod cream (Aldara; Meda Pharma, Bad Homburg, Germany) and received comprehensive oral and written instructions for usage. They were instructed to apply a thin layer of cream on the affected area remaining overnight without covering it in a

slowly escalating dosage scheme; once per week for 2 weeks, twice per week the following 2 weeks and, if tolerated, three times per week for the last weeks. A study diary was used to document times of imiquimod application. In case of unpleasant local side-effects, patients were advised to reduce or discontinue imiquimod application until the symptoms subsided. For prevention and treatment of systemic side-effects, such as headache and influenza-like symptoms, non-steroidal anti-inflammatory drugs were recommended.

Patients allocated to surgical treatment were informed about the surgical procedure at the study centre, and written informed consent was obtained. The type of surgery, excision or ablation, was based on clinical findings and the surgeon's judgement and was done according to the standard procedures of the clinical trial site. After excision, the specimens were histologically analysed to assess resection margins.

Comprehensive clinical assessments were done at baseline and at 6 months and 12 months. Primary assessment involved a medical history, with special attention to previous anogenital dysplasia and potential risk factors. Clinical assessments were done by experienced clinicians and included a thorough inspection of the entire anogenital region, aceto-white staining, and colposcopy. All visible vulvar lesions were marked and described on a case-report form and digital photos of the vulva were taken (appendix p 3). Computer software (Image Management System; Imagic, Glattbrugg, Switzerland) was used to outline vulvar dysplasia and calculate the total lesion size in mm². An HPV swab test was obtained from the vulva for assessment with a qualitative HPV test (cobas; Roche Diagnostics, Mannheim, Germany), which detects 14 high-risk HPV types and provides specific genotyping information for HPV type 16 and 18. At baseline at least one representative vulvar punch biopsy was required. For diagnosis of vHSIL, positive p16 immunohistochemical staining was required. Histological reports from referring medical centres were accepted, when biopsy specimens had been obtained within 3 months of study inclusion. Additional punch biopsies were done before randomisation, in case of missing or inconclusive histological reports or when clinically indicated. Location of all punch biopsies was recorded (appendix p 3). At 6 months, control biopsies were taken from the same vulvar sites. Formalin-fixed biopsy specimens were retrieved for subsequent HPV testing (Optiplex HPV kit; DiaMex, Heidelberg, Germany) and immunohistochemical analysis.

Additional clinical assessments for monitoring of adverse effects, treatment efficiency, and compliance were scheduled once per month during the first 4 months (appendix p 2). Patients with prolonged imiquimod treatment were scheduled for an extra clinical visit. If there was any clinical suspicion of progression or recurrence, additional biopsies were taken. All patients received a study diary to document severity of vulvar pain and pruritus on a numeric rating scale. At each control visit, the study diaries were collected, and patients were interviewed about local and general symptoms and received a clinical examination.

Patient-reported outcomes were assessed at baseline and at 6 months and 12 months; the Cervical Dysplasia Distress Questionnaire measures women's perception of diagnostic procedures and distress about a positive test result. The Fear of Progression Questionnaire assesses affective reactions, and the Sexual Activity Questionnaire measures the effect of treatment on sexual functioning.[13] Treatment satisfaction was assessed at 6 months, using an adopted German version of the Client Satisfaction Questionnaire CSQ8.[14]

Outcomes

The primary endpoint was clinical response at 6 months, and was assessed at the clinical centres where the patients were recruited. Complete clinical response (CCR) was defined as no clinical evidence of vulvar lesion, meaning 100% reduction of primary lesion size after primary allocated study treatment (one surgical intervention or local imiquimod treatment up to 6 months). Clinical response was determined by clinical assessment and confirmed by control biopsy. In case of discrepancy between clinical evaluation and histology, the endpoint was adjusted according to the histological result. In case of missing follow-up visits, the referring gynaecologists of the patients were contacted for clinical information (appendix p 1)

See Online for appendix

In patients with persistent or recurrent disease at 6 months, further treatment decision was based on treatment group and patient preference. After primary imiquimod treatment, repeat treatment with imiquimod or surgical therapy was offered. After primary surgery, repeat surgery was recommended (appendix p 2). During each control visit the occurrence of suspected unexpected serious adverse reactions was assessed and documented.

Statistical analysis

On the basis of previous studies, we assumed that 80% of patients with surgical treatment would have no signs of residual or recurrent disease (CCR) at 6 months.[15] Assuming the same CCR proportion in the imiquimod group[11] and a non-inferiority margin of 20%, a sample size of 50 patients per group was necessary to achieve a power of 80%. The estimation was based on a two-group large-sample normal approximation test of proportions with a one-sided 0·050 significance level. To compensate for an anticipated dropout rate of five patients per group, 110 patients were included.

Data were analysed in the per-protocol and intention-to-treat (ITT) population. The per-protocol analysis included patients who underwent surgery or applied imiquimod for at least 4 months. This was considered the primary analysis. To evaluate the non-inferiority of imiquimod to surgical treatment, the difference in

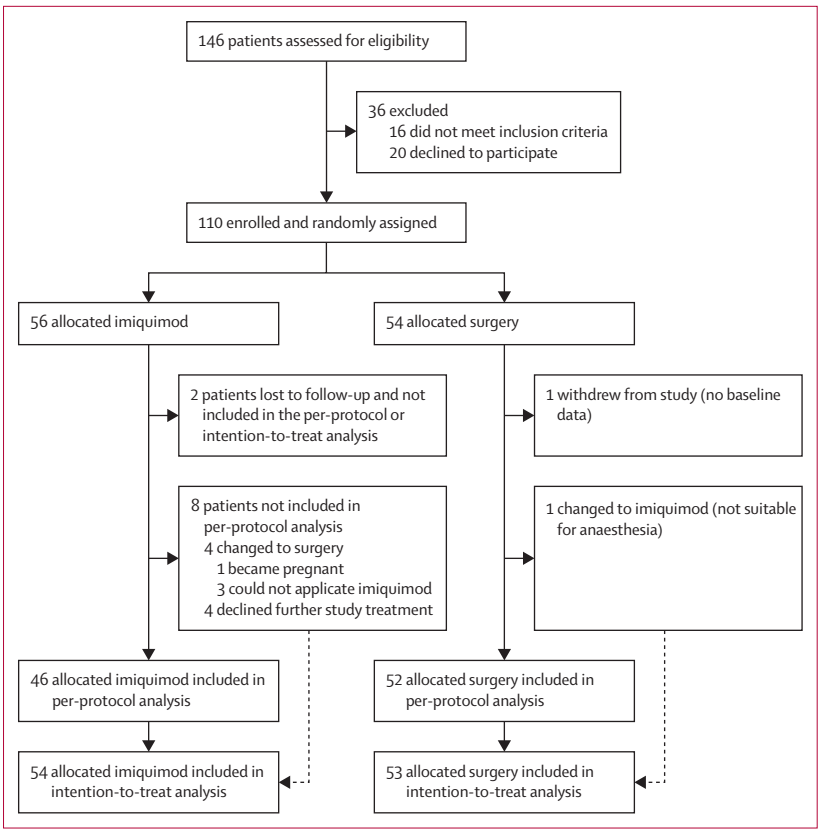

Figure: **Trial profile**

	Imiquimod (n=56)	Surgery (n=53)
Age, years	53·0 (15·7)	50·2 (14·4)
Parity	1 (0·5–2·0)	1 (0–2·0)
BMI, kg/m²	26·8 (4·6)	25·1 (4·6)
Menopausal status		
Premenopausal	22 (39%)	30 (57%)
Postmenopausal	34 (61%)	23 (43%)
Smoking status		
Never	8 (14%)	15 (28%)
Previous	13 (23%)	4 (8%)
Current	35 (63%)	34 (64%)
Lifetime sexual partners		
≤3	18 (36%)	21 (41%)
>3	32 (64%)	30 (59%)
HPV vaccination		
None	54 (96%)	48 (91%)
Started or completed	2 (4%)	5 (9%)
Previous HSIL		
CIN	10 (18%)	12 (23%)
VAIN	1 (2%)	1 (2%)
AIN	2 (4%)	3 (6%)
Previous genital warts	8 (14%)	8 (15%)
Previous malignancy	7 (13%)	7 (13%)
Previous treatment for VIN	13 (23%)	8 (15%)
1 surgery	9 (75%)	6 (86%)
2–5 surgeries	3 (25%)	1 (14%)
Imiquimod	1 (8%)	2 (25%)

Data are n (%), mean (SD), or median (IQR). BMI=body-mass index. HPV=human papillomavirus. HSIL=high-grade squamous intraepithelial lesion. CIN=cervical intraepithelial lesion. VAIN=vaginal intraepithelial lesion. AIN=anal intraepithelial lesion. VIN=vulvar intraepithelial lesion.

Table 1: **Baseline characteristics of the intention-to-treat population**

CCR proportions (surgical *vs* imiquimod) at 6 months and the corresponding 95% CIs were estimated with the Farrington-Manning method. Imiquimod was regarded as non-inferior if the upper bound of the CI did not exceed 20%. As a sensitivity analysis of the primary endpoint, we used the Cochran-Mantel-Haenszel method to adjust the proportion difference regarding the stratum used for randomisation (unifocal *vs* multifocal). Further, a sensitivity analysis evaluated the CCR proportions in two groups with different disease characteristics, unifocal and multifocal vHSIL. CCR proportions in women who were premenopausal and postmenopausal and smokers and non-smokers were investigated as subgroup analyses. Secondary endpoints included clinical response, HPV status, and patient-reported outcomes, such as treatment satisfaction. These endpoints were analysed with χ^2 tests or Fisher's exact tests, if one or more expected values were less than 5, and Mann-Whitney U tests. A two-sided significance value was set to $p<0·05$. No imputation of missing data was done. Descriptive statistics are presented by absolute and relative frequencies for categorical data and as means and SDs or medians and IQRs for continuous data, as appropriate. The statistical analyses were done with SAS software (version 9.4). This trial is registered at ClinicalTrials.gov, NCT01861535.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between June 7, 2013, and Jan 8, 2020, of 146 patients who were assessed for eligibility, 110 were enrolled. 56 women were allocated to imiquimod and 54 women to surgery (figure). The primary endpoint, clinical response to treatment at 6 months, could be assessed in 107 patients (54 in the imiquimod group and 53 in the surgery group; appendix p 1). Per-protocol analysis was done in 98 patients (46 for imiquimod and 52 for surgery). 12 months' follow-up was available for 101 patients, of whom 92 had received per-protocol treatment. 34 (61%) patients receiving imiquimod were postmenopausal, compared with 23 (43%) patients in the surgery group. Most other baseline characteristics were well balanced between study groups (table 1). 85 (78%) women presented with unifocal vHSIL and 24 (22%) with multifocal vHSIL (table 2).

	Imiquimod (n=56)	Surgery (n=53)
Current symptoms		
Yes	28 (50%)	30 (57%)
Pruritus, NRS*	4 (2–7)	4 (2–7)
Pain, NRS*	0 (0–4)	0 (0–3)
Lesion characteristics		
Unifocal	46 (82%)	39 (74%)
Multifocal	10 (18%)	14 (26%)
Total size, mm^2	129 (78–203)	166 (81–206)
Leukoplakia	33 (59%)	31 (59%)
Pigmented lesion	3 (5%)	4 (8%)
Mixed	6 (11%)	3 (6%)
Histology†		
vHSIL or VIN 2	10 (18%)	12 (23%)
vHSIL or VIN 3	46 (82%)	40 (76%)
Missing	1	0
HPV DNA on biopsy		
HPV DNA positive	41 (84%)	46 (96%)
HPV 16	38 (78%)	35 (73%)
HPV 18	0	3 (6%)
Other HPV types	3 (6%)	8 (17%)
HPV DNA negative	8 (16%)	2 (4%)
Missing	7	5
HPV Cobas test		
HPV positive	42 (78%)	35 (69%)
HPV 16	35 (65%)	29 (57%)
HPV 18	3 (6%)	3 (6%)
Other hr-HPV types‡	22 (41%)	15 (29%)
HPV negative	12 (22%)	16 (31%)
Missing	2	2
Concomitant dysplasia		
Cervix	2 (4%)	4 (8%)
Anus	2 (4%)	6 (11%)

Data are n (%) or median (IQR). NRS=numeric rating scale. vHSIL=vulvar high-grade squamous intraepithelial lesion. VIN=vulvar intraepithelial lesion. HPV=human papillomavirus. *NRS for assessment of symptom severity during the last 4 weeks before study inclusion from 0 (no complaint) to 10 (worst complaint). †Four patients with p16-positive vHSIL on biopsy had coexisting mild stages of lichen planus or lichen sclerosus. ‡Other hr-HPV types included HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

Table 2: **Characteristics of vulvar lesions at baseline**

	Imiquimod (n=46)	Surgery (n=52)	p value
Invasive disease	0	4 (8%)	..
Study outcome at 6 months			
Main study outcome			
CCR	37/46 (80%)	41/52 (79%)	p=0·0056*
Sensitivity analysis CCR			
Unifocal vHSIL	32/40 (80%)	32/38 (84%)	..
Multifocal vHSIL	5/6 (83%)	9/14 (64%)	..
Subgroup analysis CCR			
Premenopausal	12/16 (75%)	24/30 (80%)	..
Postmenopausal	25/30 (83%)	17/22 (77%)	..
Non-smoker	5/6 (83%)	12/15 (80%)	..
Previous smoker	7/12 (58%)	4/4 (100%)	..
Current smoker	25/28 (89%)	25/33 (76%)	..
Surgical treatment with excision, with or without ablation	NA	16/24 (67%)	..
Surgical treatment with ablation only	NA	25/28 (89%)	..
Further study outcomes			
Recurrent disease or partial response†	9/46 (20%)	11/52 (21%)	..
HPV positive	15/45 (33%)	11/43 (26%)	p=0·43‡
Follow-up at 12 months			
CCR	37/46 (80%)	47/52 (90%)	..
Persistent or recurrent disease	6/46 (13%)	4/52 (8%)	..
No follow-up	3/46 (7%)	1/52 (2%)	..
HPV positive	12/37 (32%)	14/42 (33%)	p=0·93‡

Data are n (%) or n/N (%). CCR=complete clinical response. HPV=human papillomavirus. NA=not applicable. vHSIL=vulvar high-grade squamous intraepithelial lesion. *p value estimated by non-inferiority analysis. †Seven of nine women with partial response after imiquimod showed a strong partial response (76–99% reduction of initial lesion size). ‡p value estimated by χ^2 test.

Table 3: **Clinical response of patients with per-protocol treatment**

By per-protocol analysis, primary treatment with imiquimod was not inferior to surgery at 6 months (difference in proportion –0·016, 95% CI –0·15 to –0·18; p=0·0056; table 3). Overall, CCR was observed in 14 (70%) of 20 patients with multifocal vHSIL and 64 (82%) of 78 patients (table 3) with unifocal vHSIL. Descriptive statistics showed no association for menopausal status and smoking habit with clinical response (table 3). By ITT analysis there was a strong tendency towards non-inferiority of imiquimod (CCR proportions, imiquimod 39 [72%] of 54 *vs* surgery 42 [79%] of 53; difference in proportion –0·07, 95% CI –0·23 to –0·09; p=0·065). A sensitivity analysis of the per-protocol population controlling for focality showed an adjusted treatment difference of –0·001 (95% CI –0·156 to 0·155) that confirmed non-inferiority. In the ITT population, the adjusted treatment difference of –0·084 (–0·243 to 0·075) did not show non-inferiority.

46 (82%) of 56 patients randomly assigned to imiquimod completed study treatment per protocol (figure). In 15 (33%) of these patients, treatment was extended up to 6 months. At 6 months, 37 (80%) of 46 patients using imiquimod per protocol had CCR, and nine (20%) had partial response (table 3). Of these nine patients, two subsequently received surgical treatment, and seven chose to continue local treatment. Prolonged imiquimod treatment was successful in two patients, secondary surgery was done in four patients, and one patient did not return for follow-up. None of the patients with imiquimod treatment per protocol had evidence of invasive disease.

Ten (18%) of 56 patients allocated to imiquimod did not complete study treatment per protocol. Two (4%) were lost to follow-up and four (7%) switched to surgical treatment; one patient became pregnant 1 month after imiquimod was started. For this patient topical treatment was halted and surgery was done at the 14th gestational

	Imiquimod (n=46)	Surgery (n=52)
Number of clinical assessments	3·7 (0·9)	2·8 (1·3)
Symptoms assessed by study diary*		
Vulvar pruritus, NRS 0–10†	3·3 (1·3–4·7)	1·5 (0·4–2·8)
Days with pruritus	18·5 (8·0–30·5)	16·0 (11·0–27·0)
Vulvar pain, NRS 0–10†	3·2 (0–4·6)	3·0 (1·5–5·0)
Days with pain	13·5 (4·0–29·0)	21·0 (16·0–28·5)
Days with analgesic drug intake	1·5 (0–10·5)	3·5 (0–12·5)
Symptoms reported by the patient		
Fatigue		
None	16 (35%)	37 (71%)
Mild	7 (15%)	2 (4%)
Moderate	18 (39%)	12 (23%)
Severe	5 (11%)	1 (2%)
Headache		
None	18 (39%)	40 (77%)
Mild	10 (22%)	1 (2%)
Moderate	9 (20%)	7 (13%)
Severe	9 (20%)	4 (8%)
Muscle or joint pain		
None	30 (65%)	44 (85%)
Mild	5 (11%)	1 (2%)
Moderate	8 (17%)	7 (14%)
Severe	3 (7%)	NA
Symptoms reported by the investigator		
Erosion		
None	29 (63%)	38 (73%)
Grade 1 (mild)	12 (26%)	10 (19%)
Grade 2 (moderate)	5 (11%)	3 (6%)
Grade 3 (severe)	NA	1 (2%)
Erythema		
None	16 (35%)	28 (54%)
Grade 1 (mild)	17 (37%)	17 (33%)
Grade 2 (moderate)	9 (20%)	7 (14%)
Grade 3 (severe)	4 (9%)	NA

Data are mean (SD), median (IQR), or n (%). NA=not applicable. NRS=numeric rating scale. *In case of incomplete or missing study diaries the mean level of vulvar pain and pruritus was estimated by the patient. †Only maximum values of the first 4 months are displayed; average symptom severity on the NRS on days with recorded symptoms divided by the number of days with recorded symptoms per month.

Table 4: **Adverse events of patients with per-protocol treatment**

week. During pregnancy, the patient developed another recurrence and topical treatment with imiquimod was restarted after the delivery of a healthy baby, resulting in CCR and histological response. Three patients switched to surgical treatment because they were not able or did not wish to apply imiquimod. In one of these women, who had not used imiquimod because of an unrelated hospital admission, invasive disease was suspected; early invasion (early tumour stage pT1a) was confirmed by biopsy and a wide excision and sentinel-node biopsy was done (negative with no tumour cells), with no evidence of recurrence during follow-up. Four patients discontinued study visits after initial imiquimod treatment; three women received surgical treatment, imiquimod treatment, or both after study completion, and one patient with unrelated severe health problems and suspected invasion refused any further treatment.

Of 52 patients, primary surgery consisted of local excision in 22 (42%) patients, laser ablation in 28 (54%) patients, and both modalities in two (4%) patients. Histology showed free margins in 17 (71%) of 24 patients and involved margins in seven (29%) of 24 patients. At 6 months, 41 (79%) of 52 patients were disease free (CCR) and 11 (21%) had recurrent or persistent vHSIL (table 3). Of these 11 patients, 5 (45%) had been diagnosed with multifocal vHSIL and six (55%) with unifocal vHSIL, treated with laser ablation in three (27%) patients and excision in eight (73%) patients. Of these 11 patients, ten underwent one (n=9) or two (n=1) repeat surgeries. At 12 months' follow-up, persistent or recurrent disease was diagnosed in four of 51 patients (table 3), and excision (n=1) or medical treatment with imiquimod (n=3) was done.

Primary surgery revealed invasive disease in four patients. Two patients were diagnosed with early invasion (<1 mm, stage pT1a) and conservative treatment with regular clinical follow-up was recommended. In two patients, surgery revealed vulvar carcinoma stage pT1b and secondary vulvar excision and bilateral sentinel lymph-node excision (negative for tumour cells) were done. Both patients had no further evidence of disease on 12 months' follow-up.

Histological assessments at 6 months' follow-up were available for 92 (86%) of 107 patients. In seven women (five in the imiquimod group and two in the surgery group), clinical diagnoses were adjusted according to the histological result of the vulvar control biopsy. In 15 women (seven in the imiquimod group and eight in the surgery group), no control biopsy specimens were obtained because the women declined (n=4) or examination was done at referring gynaecological centres (n=6), or follow-up was delayed and occurred outside the study setting (n=5).

At 6 months, vulvar HPV swabs were obtained in 88 (90%) of 98 patients (45 in the imiquimod group and 43 in the surgery group), and at 12 months in 79 (87%) patients (37 in the imiquimod group and 42 in the surgery group) with per-protocol treatment (table 3). By 6 months, overall HPV clearance was 44%. Of the nine patients with partial response after imiquimod, 56% tested positive for HPV. Overall, at 6 months, persistent HPV infection with HPV 16 was found in 14 (16%) women, and with HPV 18 in one (3%) woman. Other high-risk HPV types were identified in 18 (21%) women, with seven (8%) testing positive for more than one HPV category.

Patients attended a mean of 3·2 (SD 1·2) clinical assessments between baseline and 6 months (3·7 in the imiquimod group [SD 0·9] and 2·8 [SD 1·3] in the surgery group; table 4). Study diaries were completed by 96 (88%) of 109 (52 in the imiquimod group and 44 in the

surgery group) patients for a mean of 3 months (SD 1). The study diaries recorded the highest vulvar pain in the first month after surgery, and vulvar pruritus was highest during the first 2 months of imiquimod treatment (table 4). Vulvar erosion or erythema and general complaints such as fatigue were documented more often in patients receiving imiquimod treatment. Median analgesic drug intake was higher in patients receiving surgery (table 4).

Questionnaires assessing psychosexual distress, sexual activity, and fear of progression showed no significant differences between study groups at 6 months and 12 months. There was no significant difference in treatment satisfaction between patients with primary imiquimod and patients with surgical treatment. Further analyses on associations between health-related quality of life, clinical data, and cosmetic outcomes are ongoing.

Discussion

To our knowledge, this trial is the first prospective trial of imiquimod versus surgery for the treatment of vHSIL. The findings demonstrate that per-protocol treatment with imiquimod is non-inferior to surgical treatment during an observation period of 12 months. Complete clinical response at 6 months was observed in 80% of women, compared with 79% in women who had one surgical intervention. In the ITT analysis, there was a strong trend towards non-inferiority, with a complete clinical response rate of 72%, compared with 79% after surgery. Adverse events differed, with local pain being more intense after surgical treatment, and local pruritus and erythema being more common during imiquimod application. There was no difference in health-related quality of life and treatment satisfaction between study groups. Invasive disease was diagnosed in four patients at primary surgery and in no patients after complete imiquimod treatment

The efficacy of imiquimod for the treatment of vHSIL has been demonstrated in previous randomised clinical trials.[10–12] However, earlier reported complete-response rates were lower than those in our trial. A meta-analysis of three double-blind placebo-controlled trials yielded a complete response in 36 (58%) of 62 patients at 5–6 months, with varying results.[7] van Seters and colleagues[10] reported complete response in 35% and partial response in 46% of the 26 patients receiving imiquimod. Mathiesen and colleagues[11] reported a complete response of 17 (81%) of 21 patients. In the open-label trial by Tristram and colleagues,[12] which compared imiquimod to cidofovir, 61% of 69 patients receiving imiquimod per protocol showed a complete response, 14% a partial response, 9% stable disease, and 14% had progression.

These differences in response rates can only be partly explained by differing treatment regimens.

In our trial, imiquimod was applied up to three times per week for up to 24 weeks, which is identical to the open-label trial,[12] but longer than in the placebo-controlled trials, which allowed for a maximum treatment length of 16 weeks.[7] There were also some differences in the study populations, which might account for the differing response rates. In our study, only 19% of participants had received previous treatment for VIN, compared with 44%[12] and 69%[10] in other studies. The age, smoking habits, and lesion size of our participants were similar to previous studies.

In our trial, imiquimod-related side-effects were mostly mild to moderate. In the previous open-label study, adverse events of grade 2 or higher were observed in 87% of patients receiving imiquimod treatment, with 17% stopping treatment early because of side-effects.[12] The high number of adverse effects could be explained by the fact that patients were asked to use the contents of a whole imiquimod sachet (250 mg) three times per week. In our study, imiquimod was used in a slowly escalating regimens and patients were advised to limit the cream to a thin coverage of the affected area.

Surgery was done according to the standard procedures of the clinical trial sites and involved excision, laser ablation, or both. Although previous reports suggested higher rates of treatment failure with laser vaporisation,[16] this finding was not confirmed by a Cochrane meta-analysis[7] or our study results. At our 6-month evaluation, recurrent disease was observed in 33% of patients after local excision and in 11% after laser ablation. The overall recurrence rate was 27% within the first 12 months, which is similar to previous reported rates.[7]

Invasive vulvar cancer was diagnosed in four patients in the surgical group, indicating that early invasion, being a study exclusion criteria, was missed at preoperative clinical assessment. At 6 months' follow-up, discrepancies between clinical and histological assessment were observed in seven patients. Overall, the level of discordant clinical and histological findings appeared moderate, compared with previous published data.[17] A retrospective study of 482 vulvoscopy-directed punch biopsies found clinical detection rates of 62·3% for vHSIL and 65% for vulvar cancer.[18]

Progression to invasion during imiquimod treatment is a potential concern. In our study, none of the women who completed imiquimod per protocol developed invasive disease. In the trial by van Seters and colleagues,[10] three VIN lesions progressed to invasion, one after treatment with imiquimod and two after placebo treatment. No cases of invasive disease were reported by Tristram and colleagues.[12] In comparison, progression to invasive disease has been observed in 3% of patients who were surgically treated.[15,19]

It is still unclear why some patients do not respond to imiquimod treatment. Imiquimod binds to the toll-like receptors 7 and 8 on dendritic cells and stimulates the secretion of proinflammatory cytokines, resulting in a profound tumour-directed cellular immune response.[9] A

clinical study showed that HPV 16-specific interferon-γ-associated CD4 T-cell immunity is associated with strong clinical response to imiquimod.[20] A pre-existing coordinated inflammatory microenvironment with CD4 and CD8 T cells and CD14 inflammatory myeloid cells was associated with resolution of vHSIL.[21] Immunohistochemical analysis of the vulvar samples of our study cohort is still ongoing and is expected to provide more insights in immunological factors influencing clinical response.

Smoking has been discussed as a risk factor for persistent HPV infection and vHSIL[16] by impairing the activation of toll-like receptors.[22] In our study, no association between smoking and clinical response could be found. The effect of smoking on vHSIL recurrence after surgical treatment is unclear and differing results have been reported.[23,24]

Immunosuppression, multifocal lesions, and concomitant genital dysplasia are known risk factors for the development of new vulvar, vaginal, cervical, or anal HSIL.[24,25] In our study cohort, 27% of patients had a history of genital dysplasia and 22% had concomitant cervical or anal dysplasia at the time of study inclusion. The importance of coexisting vulvar dermatosis is unclear.[26] Previous data suggest that the presence of concomitant vulvar dermatosis does not alter the response to imiquimod.[22] In our study, four patients were diagnosed with concomitant lichen sclerosus or lichen planus, and all received surgical treatment. Prophylactic HPV vaccination was rare in our study cohort with only 9% of patients reporting incomplete or full vaccination at the time of study inclusion. Despite availability and promotion of HPV vaccination for more than a decade, HPV vaccination rates are still moderate in central Europe.[27] The efficacy of quadrivalent HPV vaccination against HPV-associated vHSIL has been shown. A combined analysis of three randomised clinical trials showed an efficacy of 100% in women who were HPV 16 or HPV 18 naive, and of 71% in women with potential previous HPV infection.[28] Countries with high vaccine uptake have already reported reduced rates of high-grade genital lesions, and over time this can be expected in the remaining countries.[29] Early trials on therapeutic vaccinations have been providing interesting results warranting further research. A genetically enhanced DNA vaccine targeting E6 and E7 oncoproteins was shown to improve HPV-specific T-cell response leading to clinical response in 43% of patients with HPV 16-associated vHSIL.[30]

Management of vHSIL could be optimised on the basis of the results of our study. This study is the first randomised controlled trial to compare surgery, the current treatment standard, to imiquimod, the most commonly used local treatment alternative. A strength of our study is the clinical, psychological, and laboratory data, which provide valuable information on efficacy, health-related quality of life, and HPV clearance. Study adherence was higher than in previous randomised trials.

A limitation of our study is the relatively long study period that was partly caused by the low incidence of vHSIL. The majority of study participants were assessed and included in the primary study centre. Hindrances to recruitment in the other study centres were lower screening rates, but also the strong preferences of patients for either treatment modality, which might limit the generalisability. Another important study limitation is selection bias; only patients compliant with the study medication were included in the per-protocol analysis. Because of the nature of the different treatments, more protocol violations occurred in the imiquimod group. Moreover, in the ITT analysis, uncertainty was introduced by patients who were not able to receive the randomised treatment. Measurement bias must be considered when interpreting treatment adverse events, given that patients receiving imiquimod had on average more assessments. Finally, assessors of clinical outcomes could not be masked to patient study treatment.

Future research needs to identify predictive markers for treatment response. Confirmation of recent immunohistochemical findings[20,21] will help to identify patients who are most likely to benefit from medical treatment and might help to estimate individual risk of vHSIL recurrence. Long-term follow-up of our study participants is ongoing and will assess the effect of treatment modality on recurrence rates. Counselling patients with vHSIL requires a discussion of potential benefits and risks of treatment options. On the basis of our results, the oncological safety of imiquimod treatment can be assumed as long as regular clinical check-ups are carried out. Treatment decision should be guided by the comorbidities and preferences of patients. A major drawback of imiquimod is the relatively long treatment period, which requires good patient compliance. In women asking for immediate treatment results and in suspected low treatment adherence, surgery might remain the treatment of choice. In all other women with vHSIL, imiquimod can be considered a first-line treatment option.

Contributors

GT, OR, and KT designed the study and wrote and reviewed the protocol. GT, EAJ, AW, AC-K, CS, GB, and ZJ were principal investigators, OR and SR did the histological assessments and MSK did the human papillomavirus testing. AB was responsible for study administration and MH did the statistical analysis. GT wrote the manuscript. GT and MH have accessed and verified the data. All authors have seen and approved the final report.

Declaration of interests

We declare no competing interests.

Data sharing

Deidentified participant data, the study protocol, and statistical analysis plan will be available on publication. Requests for specific analyses or data can be submitted by email to gerda.trutnovsky@medunigraz.at.

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