Anal intraepithelial neoplasia in HIV+ men
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Comparison of imiquimod, topical fluorouracil, and electrocautery for the treatment of anal intraepithelial neoplasia in HIV-positive men who have sex with men: an open-label, randomised controlled trial.

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Abstract

Background Anal cancer is an increasing issue in HIV-positive men who have sex with men (MSM). Screening for its precursor, anal intraepithelial neoplasia (AIN), is subject of discussion. Current treatment options are suboptimum and have not been compared in a prospective trial. We compared efficacy and side-effects of imiquimod, topical fluorouracil, and electrocautery for the treatment of AIN.

Methods In this open-label randomised trial, we included HIV-positive MSM older than 18 years visiting the HIV outpatient clinic of the Academic Medical Center, Amsterdam, Netherlands. Patients with histologically confirmed AIN were randomly assigned to receive either 16 weeks of imiquimod (three times a week), 16 weeks of topical fluorouracil (twice a week), or monthly electrocautery for 4 months. Randomisation was done with random block sizes of three and six, stratified for AIN grade (AIN grades 1, 2, or 3) and AIN location (peri-anal or intra-anal). Participants were assessed by high-resolution anoscopy 4 weeks after treatment. Responding patients returned for follow-up 24 weeks, 48 weeks, and 72 weeks after treatment. The primary endpoint was histological resolution of AIN measured 4 weeks after treatment and AIN recurrence at week 24, week 48, and week 72 after treatment. The primary analysis was done in a modified intention-to-treat population, including all patients who had received their assigned treatment at least once. The trial is registered at the Netherlands Trial Register, number NTR1236.

Findings Between Aug 12, 2008, and Dec 1, 2010, we screened 388 HIV-positive MSM for AIN by high resolution anoscopy. Of the 246 (63%) patients who had AIN, 156 (63%) were randomly assigned to either receive imiquimod (54 patients), topical fluorouracil (48 patients), or electrocautery (46 patients) following withdrawing of consent by eight patients. Modified intention-to-treat analysis showed a complete response in 13 (24%, 95% CI 15–37) patients in the imiquimod group, eight (17%, 8–30) of patients in the fluorouracil group, and 18 (39%, 26–54) of patients in the electrocautery group (p=0.027). At week 24, 11 (22%) of 50 responders had recurrence; at week 48, 22 (46%) of 48 had recurred; and at week 72, 30 (67%) of 45 had recurred. Recurrence was observed at 72 weeks in 10 (71%) of 14 patients treated with imiquimod, seven (58%
of 12 patients treated with fluorouracil, and 13 (68%) of 19 patients treated with electrocautery. Grade 3–4 side-effects were noted in 23 (43%) of 53 patients in the imiquimod group, 13 (27%) of 48 patients in the fluorouracil group, and eight (18%) patients in the electrocautery group (p=0.019). The most common side-effects were pain, bleeding, and itching. Seven serious adverse events occurred, all not related to the study.

Interpretation Electrocautery is better than imiquimod and fluorouracil in the treatment of AIN, but recurrence rates are substantial.

Introduction

The incidence of anal cancer is increasing in patients with HIV. In particular, men who have sex with men (MSM) who are HIV positive are at risk, with incidence rates of 65–109 per 100 000 person-years. As in cervical cancer, oncogenic human papillomavirus (HPV) has an important role, and anal cancer is likewise preceded by a precursor lesion: anal intraepithelial neoplasia (AIN), graded from 1 to 3. AIN of grade 1 is referred to as low-grade AIN and AIN of grades 2 and 3 as high-grade AIN. A study in HIV-positive men before the introduction of combination antiretroviral therapy (cART) showed a progression rate of 52% from low to high grade dysplasia during a follow-up period of 4 years. Progression rates from high-grade AIN to anal cancer have been reported to be around 15% for HIV-positive MSM, during median follow-up periods of 2 and 5 years. By way of comparison, malignant progression of inadequately treated cervical intraepithelial neoplasia 3 is 30% in 30 years.

Since anal cancer incidence in HIV-positive MSM is substantially higher than the incidence of cervical cancer before the introduction of standard cytological screening, and screening for cervical intraepithelial neoplasia is effective in preventing cervical cancer, AIN screening is subject of discussion. However, the efficacy of known treatment options for AIN is poor and the recurrence rate is high. Moreover, most studies of AIN treatment are retrospective single-arm case series. At present, infrared coagulation and electrocautery seem to be the best options for treatment of AIN, with moderate response rates. However, electrocautery and infrared coagulation require an outpatient setting and,
given the high recurrence rates, often need to be repeated. Therefore, topical therapies, which can be applied by the patient at home, could be an attractive alternative. A prospective study\textsuperscript{12} on imiquimod showed a complete response rate of 61\% for (mainly peri-anal) AIN. In a more recent, placebo-controlled study\textsuperscript{13} the complete response rate of imiquimod treatment for intra-anal AIN was 14\% after 4 months of treatment. Another topical option is fluorouracil. Results from a study\textsuperscript{14} from our group showed a complete response rate of 39\% for intra-anal AIN.

Since, to our knowledge, electrocautery and topical therapies have not yet been compared, we did a randomised trial comparing efficacy and tolerability of imiquimod, topical fluorouracil, and electrocautery for the treatment of AIN in HIV-positive MSM.

**Methods**

**Patients**

HIV-positive MSM older than 18 years visiting the HIV outpatient clinic of the Academic Medical Center, Amsterdam, Netherlands, were offered screening for AIN. Exclusion criteria were a history of anal cancer, treatment of AIN or anal condylomas, or both, in the past 30 days, active inflammatory bowel disease, a life expectancy of less than 12 months, and active intravenous drug use because of decreased reliability in follow-up studies. All consenting patients were screened by high resolution anoscopy using a video colposcope as described previously.\textsuperscript{15} Suspect lesions were biopsied for histopathological analysis. One pathologist (CJMvN) assessed all biopsies.

At baseline, we screened patients for anal chlamydia and gonorrhoea and demographic variables, and collected characteristics related to HIV infection. We retrieved from the electronic patient record the most recent CD4 count and plasma concentrations of HIV-1 RNA, obtained within a maximum of 6 months before inclusion.

The research protocol was approved by the local ethics committee and all participants gave written informed consent.
Randomisation and masking

Patients with histologically confirmed AIN underwent open-label block randomisation with random block sizes of three and six, stratified for AIN grade (three categories: AIN grades 1, 2, and 3) and AIN location (two categories: peri-anal and intra-anal). The allocation sequence was generated by the Clinical Research Unit of the Academic Medical Center. Participants were enrolled by OR. The treatment groups were imiquimod, topical fluorouracil, and electrocautery. For all participants, the treatment period was 16 weeks. Participants and investigators were not masked for treatment assignment except for the pathologist assessing biopsy samples.

Procedures

Electrocautery, guided by high resolution anoscopy, was done every 4 weeks up to a maximum of five times. Lesions were ablated with the ERBE ICC 50 electrosurgical unit on 20–22 W with 2–5 mm spheres. All visible lesions were treated at every visit. Patients underwent local anaesthesia (artiacaine and epinephrine) if necessary. Adverse events were recorded at every visit. Patients were recommended to refrain from receptive anal intercourse for 1 week after an electrocautery session.

The patient randomly allocated to receive imiquimod applied the drug three times weekly. Peri-anal AIN was treated with imiquimod (half a sachet, 6·25 mg, of Aldara, Meda Pharma, Amstelveen, Netherlands) in the evening. For intra-anal lesions, the volume of imiquimod was increased by an indifferent cream, lanette II, for a final concentration of 6·25 mg of imiquimod per g. This cream was manufactured and sent to the patients every 4 weeks. A standard dose consisted of 1 g of cream (6·25 mg of imiquimod; a similar dose to half a sachet of Aldara), which was applied with a standard applicator for cream in the evening. The applicator had to be inserted 3–5 cm beyond the anal sphincter before emptying it.

Patients randomly assigned to receive topical fluorouracil had to apply Efudix 2% cream twice a week (Efudix, Meda Pharma, Amstelveen, Netherlands). On those days, peri-anal lesions had to be treated twice (morning and evening). For intra-anal lesions, one dose of 1 g Efudix cream was inserted in the evening with a standard applicator for cream, as described above.
Patients on imiquimod and fluorouracil were recommended to use condoms in case of receptive anal intercourse during treatment. We contacted patients assigned to imiquimod or fluorouracil after 8 and 16 weeks for assessment of side-effects, and if deemed necessary by the study coordinator or the patient, patients were seen at the outpatient clinic. Patients with both peri-anal and intra-anal disease were treated at both locations according to the instructions given for treatment of peri-anal or intra-anal AIN.

Side-effects were graded 1–4 as reported by the patients, with grade 1 indicating mild events and grade 4 indicating potentially life-threatening events, according to the severity scale adopted in December, 2004, by the Division of AIDS at the National Institutes of Health. A serious adverse event was defined as any medical condition being fatal, life threatening, disabling, incapacitating or requiring hospital admission, or both.

In case of severe side-effects, one electrocautery session could be skipped, or local treatment could be interrupted for a week, with a maximum cumulative duration of 4 weeks. Alternatively, for patients treated with imiquimod or fluorouracil, treatment frequency could be reduced to twice or once a week respectively, guided by severity of side-effects, at the discretion of the treating physician.

We took evaluation biopsy samples 4 weeks after the last electrocautery or 4 weeks after ending topical therapy (20 weeks after inclusion). These samples were taken from the areas where AIN had been reported previously and if necessary from other suspect lesions. Patients with progression (low-grade to high-grade AIN) or persisting high-grade AIN were referred for further treatment and excluded from further study assessments. Patients with a complete response or a partial response (from high-grade to low-grade AIN) returned for high resolution anoscopy 24, 48, and 72 weeks after treatment.
Statistical analysis

At initiation of the study, in cross-section studies on average 15% of patients were expected to have AIN of grade 1, 30% of patients AIN of grade 2, and 10% AIN of grade 3.\textsuperscript{17,18} and 19 Progression rates of AIN of grades 1 or 2 are high, at least 50%. Spontaneous regression of AIN lesions of grades 2 or 3 can occur in 10% of cases.\textsuperscript{20,21} and 22 We assumed that, at week 20, efficacy of electrocautery was 50%, efficacy of imiquimod 77%,\textsuperscript{12} and, on the basis of efficacy of fluorouracil in vaginal intraepithelial neoplasia,\textsuperscript{23} we assumed efficacy of topical fluorouracil also to be 77%. We required an efficacy of alternative treatment of at least 50%: a decrease in persistence of (or progression to) high-grade AIN or anal carcinoma from 50% (electrocautery: standard treatment) to 25% (imiquimod or fluorouracil). A three-group $\chi^2$ test with a 0.05 two-sided significance level and 80% power to detect such a difference between electrocautery and each of the topical treatment groups (odds ratio [OR] of 0.333) required a sample size in each group of 51.

The primary outcome was histological resolution of AIN at 4 weeks after end of treatment, and recurrence rate at 24, 48, and 72 weeks after treatment. We defined complete response as resolution of AIN and partial response as regression from high-grade to low-grade AIN. We defined recurrence as recurrent low-grade or high-grade AIN. Secondary endpoint was tolerability of the treatment.

We included in the modified intention-to-treat analyses all participants randomly assigned to treatment that had received at least one electrocautery, or one dose of imiquimod or fluorouracil. We excluded from the per-protocol assessment patients who interrupted imiquimod or fluorouracil for more than 4 weeks, or missed more than one electrocautery session. We calculated CIs (adjusted Wald) for response rates and used $\chi^2$ analysis to test differences in response rates between treatment groups.

We analysed the association with treatment response for the following variables: treatment group, duration since HIV diagnosis, duration of cART use, current CD4 count (lower or higher than 500 cells per $\mu$L), and AIN grade. We analysed each parameter univariably in a log-binomial regression (a generalised linear model with a logarithmic link function and binomial distribution for the residual) in relation to (complete or partial) response versus no response to treatment as
dependent variable. Subsequently, we performed a multivariable log-binomial regression to assess the effect of each variable adjusted for the presence of the other potential predictors. We report results from the regression models as relative risks (RR) along with their 95% CIs. We did the statistical analysis using PASW Statistics software (Release 18.0.2). This trial is registered with Nederlands Trial Register, number NTR1236.

Role of funding source
The funding source had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding (OR) and senior (JMP) author had full access to the raw data. The senior author (JMP) had the final responsibility to submit for publication.

Results
Between Aug 12, 2008, and Dec 1, 2010, we screened 388 HIV-positive MSM for AIN by high resolution anoscopy (figure). The study period, including all follow-ups, ended in May 15, 2012. 246 (63%) men had AIN, of whom 128 (52%) had high-grade AIN. 156 HIV-positive MSM were randomly assigned to a treatment group, but eight patients withdrew informed consent before start of treatment (the main reason given was too much hassle of the study procedures or interference with study or work). The remaining 148 patients were included in the mITT analysis and randomly assigned to receive either imiquimod (54 participants), topical fluorouracil (48 participants), or electrocautery (46 participants; figure).
AIN=an intraepithelial neoplasia. *Patients were excluded for the per-protocol analysis if they interrupted imiquimod or fluorouracil for more than 4 weeks or if they skipped more than one cautery session. † One refused assessment, one patient left the study because of interference with work. ‡ Protocol violation: patient was treated with a barron ligature for haemorrhoids before assessment. § One patient died (not study related), one patient started anticoagulant therapy, three patients left the study because of interference with work.

Figure. Trial profile
63 (43%) of the 148 randomised participants had low-grade AIN and 85 (57%) had high-grade AIN (table 1). Their median age was 47 years, the median time since HIV diagnosis for participants was 7 years, and 126 (86%) of 147 participants were using cART at inclusion (one patient record was incomplete). 12 (8%) patients had an anal infection at inclusion, which was treated before the start of study treatment: eight patients had chlamydia, three had gonorrhoea, and one had both. Table 1 shows other characteristics per treatment group. 24 patients were excluded for the per-protocol analysis because of drop-out during the study, in ten (4%) of 24 cases the reason for drop-out was side-effects (figure).

Table 1. Characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>Imiquimod</th>
<th>5-Fluoruracil</th>
<th>Electrocautery</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>54</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>Age, years</td>
<td>45 (41-51)</td>
<td>47 (40-54)</td>
<td>47 (42-55)</td>
</tr>
<tr>
<td>Years since HIV diagnosis</td>
<td>7 (3-10)</td>
<td>9 (4-15)</td>
<td>10 (5-16)</td>
</tr>
<tr>
<td>Using cART</td>
<td>82% (44/54)</td>
<td>85% (41/48)</td>
<td>91% (41/45)</td>
</tr>
<tr>
<td>Undetectable plasma HIV-RNA</td>
<td>76% (41/54)</td>
<td>81% (39/48)</td>
<td>84% (38/45)</td>
</tr>
<tr>
<td>Current CD4 cell count (cells/µl)</td>
<td>535 (395-695)</td>
<td>560 (410-755)</td>
<td>590 (410-785)</td>
</tr>
<tr>
<td>Nadir CD4 cell count (cells/µl)</td>
<td>222 (110-300)</td>
<td>175 (100-280)</td>
<td>190 (110-270)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>41% (20/49)</td>
<td>35% (16/46)</td>
<td>36% (16/44)</td>
</tr>
<tr>
<td>HGAIN</td>
<td>57% (31/54)</td>
<td>60% (29/48)</td>
<td>54% (25/46)</td>
</tr>
<tr>
<td>Peri-anal AIN</td>
<td>20% (11/54)</td>
<td>15% (7/48)</td>
<td>15% (7/46)</td>
</tr>
<tr>
<td>Intra-anal AIN</td>
<td>93% (50/54)</td>
<td>98% (47/48)</td>
<td>93% (43/46)</td>
</tr>
<tr>
<td>Multifocal AIN</td>
<td>59% (32/54)</td>
<td>48% (23/48)</td>
<td>46% (21/46)</td>
</tr>
</tbody>
</table>

Data are median (IQR) or n/N (%). cART=combination antiretroviral therapy. AIN=anal intraepithelial neoplasia. *Some data were missing because of incomplete patient records.

In the mITT analysis, a complete response at 4 weeks following the end of treatment was recorded in 13 (24% [95% CI 15–37]) of the 54 patients in the imiquimod group, eight (17%, 8–30) of the 48 patients in the fluorouracil group, and 18 (39%, 26–54) of the 46 patients in the electrocautery group (table 2). The difference in complete response rate between the three groups was significant (p=0.027, table 2). Comparing electrocautery to imiquimod or fluorouracil separately, electrocautery resulted in significantly more complete responses than fluorouracil (χ², p=0.008), but not compared with imiquimod (p=0.10).
Table 2. Response to Treatment

<table>
<thead>
<tr>
<th></th>
<th>imiquimod</th>
<th>5-fluorouracil</th>
<th>Electrocautery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mITT (n=54)</td>
<td>PP (n=45)</td>
<td>PP HGAIN (n=24)</td>
</tr>
<tr>
<td>Complete Response</td>
<td>24%(^a) (13)</td>
<td>29%(^b) (13)</td>
<td>21% (5)</td>
</tr>
<tr>
<td></td>
<td>15-37% (^*)</td>
<td>18-43%</td>
<td>8-30%</td>
</tr>
<tr>
<td>Partial Response</td>
<td>11% (6)</td>
<td>13% (6)</td>
<td>25% (6)</td>
</tr>
<tr>
<td></td>
<td>5-23%</td>
<td>6-27%</td>
<td>6-27%</td>
</tr>
<tr>
<td>No Response</td>
<td>48% (26)</td>
<td>58% (26)</td>
<td>54% (13)</td>
</tr>
<tr>
<td></td>
<td>35-61%</td>
<td>43-71%</td>
<td>46-73%</td>
</tr>
<tr>
<td>Lost</td>
<td>17% (9)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>9-29%</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

mITT=modified intention to treat. PP=per protocol. AIN=anal intraepithelial neoplasia. NA=not applicable (in case of low-grade AIN partial response is not an option).\(^a\)Difference between the three groups in complete response rate was significant in the mITT analysis (p=0.027).\(^b\)Difference between the three groups in complete response rate was significant in the PP analysis (p=0.010).
Per-protocol analyses are shown in table 2; the difference in complete response rate was significant \((p=0.010)\). Comparing complete response rates in the per-protocol population showed that electrocautery was not significantly better than imiquimod \((\chi^2, \ p=0.052)\), but significantly better than fluorouracil \((p=0.0031)\).

Responding patients were invited for follow-up high resolution anoscopy, which was scheduled 24 weeks, 48 weeks, and 72 weeks after treatment. The cumulative recurrence was 22\% (11 of 50) at week 24, 46\% (22 of 48) at week 48, and 67\% (30 of 45) at week 72 (table 3). Median time to recurrence was 48 weeks in the imiquimod group, 24 weeks in the fluorouracil group, and 48 weeks in the electrocautery group. The difference in recurrence rate at week 72 was not significant \((p=0.76)\). Because of relatively small numbers, we did not analyse recurrence rates for peri-anal lesions separately.

<table>
<thead>
<tr>
<th>Table 3. Cumulative recurrence rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>24 weeks</td>
</tr>
<tr>
<td>48 weeks</td>
</tr>
<tr>
<td>72 weeks</td>
</tr>
</tbody>
</table>

Data are \%(n/N). Cumulative recurrence rates at weeks 24, 48, and 72 after treatment. Of the 54 patients initially responding to treatment, 50 patients returned for a follow up high resolution anoscopy 24 weeks after treatment. An additional two and three patients were lost to follow up at the 48-week and 72-week visits.

Table 4 shows response rates reported for intra-anal and peri-anal lesions separately. A post-hoc analysis (mITT) restricted to peri-anal AIN showed a complete response rate of 91\% (95\% CI 60–100; ten of 11 patients) for imiquimod, 57\% (25–84; four of seven patients) for fluorouracil, and 43\% (16–75; three of seven patients) for electrocautery.
In the multivariable log-binomial regression on complete or partial response, fluorouracil resulted in a significantly lower response, whereas imiquimod resulted in a non-significant slightly lower response than electrocautery (table 5). Additionally, years of cART and high-grade AIN were associated with complete or partial response to treatment (table 5). The positive association of a current CD4 count of more than 500 cells per μL was not significant (table 5).
Side-effects were reported by 48 (91%) of 53 patients treated with imiquimod, by 44 (92%) of 48 patients treated with fluorouracil, and by 42 (93%) of 45 patients treated with electrocautery ($\chi^2$, $p=0.88$; table 6). Two patients (one in the imiquimod group and one in the electrocautery group) were not available for evaluation of side-effects. Five (9%) of 53 patients in the imiquimod group, two (4%) of 48 patients in the fluorouracil group and three (7%) in the electrocautery group stopped because of side-effects ($p=0.59$). In most cases, these side-effects were related to pain or irritation, or bleeding. Influenza-like symptoms and fatigue were more frequent in the imiquimod group than in the other groups, whereas increased urge to defecate was more often reported (54%) by patients in the fluorouracil group (table 6). Bleeding occurred most frequently after electrocautery (table 6). Side-effects of electrocautery were mostly mild (grade 1) and lasted only a few days after treatment, whereas side-effects lasted for a mean of 5 weeks for imiquimod and 7 weeks for fluorouracil.

Table 6 Side effects

<table>
<thead>
<tr>
<th></th>
<th>Imiquimod (n=53)</th>
<th>Fluorouracil (n=48)</th>
<th>Electrocautery (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grade 1/2</td>
<td>grade 3/4</td>
<td>grade 1/2</td>
</tr>
<tr>
<td>any side effect, highest grade</td>
<td>25 (47%)</td>
<td>23 (43%)</td>
<td>31 (65%)</td>
</tr>
<tr>
<td>pain</td>
<td>20 (38%)</td>
<td>17 (32%)</td>
<td>25 (52%)</td>
</tr>
<tr>
<td>itching</td>
<td>8 (15%)</td>
<td>2 (4%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>bleeding</td>
<td>16 (30%)</td>
<td>0 (0%)</td>
<td>19 (40%)</td>
</tr>
<tr>
<td>slimy stool</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>urge</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>22 (46%)</td>
</tr>
<tr>
<td>incontinence</td>
<td>2 (4%)</td>
<td>0 (0%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>diarrhoea</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>flatulence</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>flu like symptoms</td>
<td>6 (11%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>fatigue</td>
<td>6 (11%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

Data are number (%). Severity of side-effects that occurred in at least 5% of participants in one treatment group. Difference between the three treatment groups in side-effects of grades 3–4 was $p=0.019$ ($\chi^2$ test). Two patients (one in the imiquimod group and one in the electrocautery group) were not available for evaluation of side-effects.
All patients treated for peri-anal AIN reported side-effects, predominantly pain. Grade 3 side-effects were noted in five (50%) of ten patients treated with imiquimod, and three (43%) of seven patients in both the fluorouracil and electrocautery groups (p=0.94). Influenza-like symptoms were reported by two (20%) of the ten patients treated with peri-anal imiquimod and by no one in the other groups. Additionally, 25 patients temporarily interrupted treatment because of side-effects: 14 treated with imiquimod and 11 with fluorouracil.

Overall, seven serious adverse events were recorded (one operation of herniated cervical disc, one operation of strabismus, one rectal perforation caused by a corpus alienum, two syphilis, one myocardial infarction, and one prostatitis). All serious adverse events were defined as such because of hospital admission of the patient, and all were unrelated to the study medication or study procedures. No treatment-related deaths were recorded.

Discussion

In this study, we compared imiquimod, topical fluorouracil, and electrocautery for the treatment of AIN in HIV-positive MSM. The number of patients with a complete response at 4 weeks following end of treatment was significantly lower for patients in the imiquimod and fluorouracil groups than for those treated with electrocautery. Additionally, side-effects were more serious and longer lasting in patients treated with imiquimod and fluorouracil than in patients treated with electrocautery. Although the study was not designed or powered to assess differences in response based on location of the AIN, the response for peri-anal lesions turned out to be substantially higher than those of intra-anal lesions.

To our knowledge, this is the largest prospective study on AIN treatment and the first one comparing currently available treatment options (panel). Two prospective studies have been published with imiquimod for AIN. The first study examined 28 patients, who mostly had peri-anal AIN. Complete response was noted in 61% of patients and, after a mean follow-up of 30 months, 74% of patients who had achieved a complete response remained disease-free at the previously treated site, although 58% developed cytological abnormalities at previously untreated
areas. In our study, in the pre-protocol analysis all nine patients treated for peri-anal AIN with imiquimod had a complete response. In the second study, which examined 53 patients with intra-anal high-grade AIN, participants were randomly assigned to receive either imiquimod or placebo. In a per-protocol analysis, only 14% of patients treated with imiquimod had a complete response and 29% a partial response after 4 months of treatment, which was even lower than the response seen in our study. Apparently peri-anal AIN responds better than intra-anal AIN to imiquimod treatment. This difference might be explained by the fact that the cream can be more effectively applied on the peri-anal skin than on the intra-anal mucosa. However, in our study, most patients experienced local side-effects such as itching and pain. This is caused by contact with the squamous mucosa, which suggests correct application of the cream.

In a recent prospective study from our group in 46 patients with AIN, topical fluorouracil was moderately effective for intra-anal AIN, with an overall response of 57% and a complete response rate of 39% 4 weeks after completion of treatment. However, side-effects were substantial and 50% of patients had a recurrence 6 months after treatment. In the current study, the overall response was significantly lower. CIs of the responses observed in the two studies, however, overlap, and side-effects in both studies are similar. In a study presented at the International Papillomavirus Conference 2009, 28 patients with diffuse high-grade AIN were treated with topical fluorouracil. 11% cleared the lesions and 57% showed a decrease in disease burden. Although the clearance rate is low, these data suggest that fluorouracil could have a role in making extensive AIN lesions more amenable to ablative therapy. Like imiquimod, fluorouracil showed a better response rate for peri-anal AIN, although the numbers are small. This again leads to the question whether the poor responses of intra-anal treatment are explained by suboptimal exposure of intra-anal lesions to the cream. However, since most patients had local side-effects, we think that the cream was applied in a correct way.

To our knowledge, two studies have been published on the efficacy of electrocautery for AIN. A prospective trial in 37 patients in an operating room setting showed that 79% of HIV-positive patients had persistent or recurrent disease (mean time to recurrence 12 months), but no recurrence was noted in eight HIV-negative participants. The authors concluded that several
electrocautery sessions might be necessary for HIV-positive patients. More recently, a retrospective study\textsuperscript{10} was published reporting the results in 232 MSM who were treated with electrocautery. In the 132 HIV-positive participants, the cure rate after the first electrocautery session was 75%. However, 61% had a recurrence during a mean follow-up of 9.1 months.\textsuperscript{10} The electrocautery scheme used in our study differs from that of these two studies.\textsuperscript{10} All participants were treated with five high resolution anoscopy guided electrocautery sessions, every 4 weeks, and at each visit all visible lesions were treated. It is therefore surprising that the complete response rate we recorded (mITT: 39%) was lower than that reported in the study by Marks and colleagues.\textsuperscript{10} An important difference is the prospective design of our study and the fact that all areas where previously AIN was reported were biopsied after treatment, also in the absence of visible lesions. Further, the electrocautery technique could differ: Marks and colleagues\textsuperscript{10} used a blade, we used small pinpoint spheres for treatment of lesions.

Years on cART and a high CD4 count were associated with response to treatment. The previously mentioned study on imiquimod treatment of intra-anal high-grade AIN\textsuperscript{13} did not show that a high CD4 count was related to treatment success. Data are likewise conflicting for the association of the CD4 count with the presence of AIN. Some studies showed a significant correlation between a low nadir CD4 count and the prevalence of AIN,\textsuperscript{27,28} whereas other studies did not.\textsuperscript{29,30}

We did not prespecify subgroup analyses of response by grade of AIN, and the study is not powered for such analyses. However, the response rate of low-grade AIN was lower than that of high-grade AIN, and in the multivariable analysis high-grade AIN was significantly associated with a successful response to treatment. The question is whether low-grade AIN should be treated at all. However, progression to high-grade AIN is seen in a substantial proportion of patients.\textsuperscript{5,32} Given our results, we think that in cases of low-grade AIN a wait-and-see policy might be sufficient, but future studies should focus on the progression rate of low-grade AIN lesions.

The prospective design, randomisation of treatments, the number of patients, and the long follow-up of our study are all strengths. One physician did all
high resolution anoscopy and one pathologist assessed all biopsies, which precluded interobserver variability. Additionally, our series of patients was unselected, and participants were treatment-naive. A limitation of the study is that spontaneous regression of AIN lesions is possible, although rates of regression of high-grade AIN lesions probably do not exceed 10%. Additionally, this issue is more relevant in uncontrolled single-arm trials. Furthermore, we did not assess the size of lesions or presence of HPV. Reduction in extent of disease and clearance of HPV could be an additional outcome measure in assessing response.

Finally, besides assessing clinical efficacy, analysing cost-effectiveness is important. For the current treatment options this analysis is probably less relevant, since electrocautery is significantly superior to imiquimod and fluorouracil. A cost-effectiveness analysis will be the subject of a separate paper. Likewise, the influence of host genetics on treatment response will be assessed separately.

In conclusion, our findings suggest that electrocautery is better than the topical imiquimod or fluorouracil for the treatment of AIN in HIV-positive MSM. Infrared coagulation has shown similar responses to electrocautery, and since both methods can easily be done in an outpatient setting, both could serve as first-choice treatment of AIN. Our data also suggest that imiquimod may be the best choice for peri-anal AIN; however, further study is needed since subgroup analyses by AIN location were limited by very small patient numbers and were not prespecified. Our study also confirms the previously reported low response of AIN to treatment, and the high rates of recurrence. Since the prevalence of high-grade AIN in HIV-positive MSM is very high, future studies should explore new and better treatment methods before AIN screening programmes be initiated.
References


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