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# A novel application of calcium electroporation to cutaneous manifestations of gynaecological cancer

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*Objective*: Calcium electroporation (CaEP) is a new technique whereby intracellular concentrations of calcium are elevated by transient permeabilisation of the cell membrane using high-voltage electrical pulses. Tumour necrosis is induced with little damage to healthy tissue. Within gynaecological cancer, vulval cancer and vulval intraepithelial neoplasia (VIN) pose challenges for treatment, given the high recurrence rate, persistent symptoms and repeated resections required. In certain cases, CaEP may provide a suitable alternative. *Methods*: We present a case series of six patients with recurrent vulval squamous cell carcinoma (n = 2), VIN III (n = 2) and metastatic ovarian cancer (n = 2), five of whom were treated with CaEP. This is the first known application of CaEP to gynaecological cancers. *Results*: The median follow-up time was 14 months (range 2–18 months). Within the cohort of patients, CaEP was applied a total of 10 times, achieving a complete response five times and partial response four times. Symptoms improved within six weeks for eight episodes following CaEP application. Beyond six weeks, symptoms eventually recurred in all patients and four patients required more than one CaEP procedure. CaEP was useful for palliation of distressing symptoms in one case of metastatic ovarian cancer. No intra-operative or post-operative complications have been reported to date. *Conclusion*: CaEP may be a promising short-term treatment in selected patients with recurrent VIN and vulval cancer, where other treatments had failed. If validated, it could provide an acceptable alternative where surgery is unacceptable. Long term follow-up is required to evaluate effects on recurrence.

*Keywords*

Calcium; Electroporation; Ovarian cancer; Vulval cancer; VIN

## 1. Introduction

Electroporation is the transient permeabilisation of the cell membrane by short, high voltage pulses that cause the transmembrane potential to exceed threshold [1]. Once entry and passive diffusion of calcium has been permitted, tumour cell death is induced, without inducing nearby, healthy, cell death [2]. The first clinical trial of electroporation in combination with chemotherapy (electrochemotherapy 'ECT') was performed in 1991 [3]. It has since been tested in clinical trials for treatment of vulval cancer [4, 5], nonmelanoma skin cancer [6], cutaneous metastases [7, 8] and tumours in internal organs [9, 10]. When treated with ECT, tumour cells are selectively more sensitive than normal cells *in vitro* [11, 12], thus preserving healthy tissue.

Recently, the more readily available element calcium has been found to be an alternative to chemotherapy in combination with electroporation, capable of inducing cell death *in vitro* and tumour necrosis *in vivo* with striking effect [13]. Postulated mechanisms of action include depletion of ATP by increased consumption: by increasing activity of the membrane protein Calcium ATPase, or reduced production: by uncoupling of oxidative phosphorylation. Without their energy source, malignant cells demise quickly [13].

Cutaneous primary malignancies and metastases are a potential target of this therapy, particularly where healthy tissue structures should be preserved. So far, calcium electroporation (CaEP) has been used in Phase I clinical and pre-clinical studies, with promising outcomes in head and neck cancer

and sarcoma [14, 15]. Currently, phase I trials are also being conducted in 6 patients with inoperable colorectal cancer [16] and 24 patients with early rectal and sigmoid cancer as an adjuvant prior to surgery [17]. Within gynaecological oncology, electroporation could be applied to vulval cancer and vulval intraepithelial neoplasia (VIN). Recurrence after treatment of vulval cancer with standard therapy is reported to be as high as 30% [18], a similar recurrence rate to VIN [19]. The primary treatment of vulval cancer is usually radical vulvectomy.

In recurrence, the option for further resection is limited. It can be associated with adverse psychosexual outcomes in addition to recognised surgical complications, such as injury to adjacent structures [20]. Therefore, a need exists for minimally invasive techniques for the management of recurrent vulval cancer and VIN.

We present a case series of the first known application of calcium electroporation (CaEP) to gynaecological pathology: VIN III, vulval cancer on a background of recurrent VIN III and cutaneous and vaginal metastases of ovarian cancer.

## 2. Materials and methods

### *2.1 Patient cohort*

This is a retrospective case series of six patients: recurrent vulval squamous cell carcinoma (n = 2), VIN III (n = 2) and metastatic ovarian cancer (n = 2). This group consisted of highly distressed patients with severe symptoms and/or advanced disease. The disease had not been alleviated or halted with multiple standard therapies. We present this case series as an extension of already established clinical practice, with its routine use to treat cutaneous malignant lesions [14, 15, 21]. Institutional Review Board Approval was not required.

Patients were under follow-up by the gynaecological oncology team for recurrent VIN III or vulval cancer or were new referrals from the oncology team. They were seen in gynaecological oncology clinic to assess suitability for the procedure, with inclusion criteria depending on the treatment intent of CaEP:

For curative intent:

(1) Vulval carcinoma or VIN localised to skin and subcutaneous tissue, with no local or distant spread, or lymph node involvement.

(2) Failure of standard therapy including PlasmaJet® and local excision.

(3) OR unsuitable for further excision/surgery:

(a) Not fit for surgery.

(b) Surgery would result in unacceptable deformity (for example due to previous extensive surgery).

(c) Competent patient refused surgery.

For palliative intent:

(1) Skin, subcutaneous or superficially-reached metastases which are causing distress that cannot be managed conservatively.

(2) This distress exceeds the risks of CaEP.

(3) Not suitable for surgery, whether due to fitness, extensive disease or patient choice.

Exclusion criteria:

(1) Allergy to anaesthetic or calcium chloride.

(2) A tumour which is not reached by the probe/electrodes could not be seen.

(3) Pregnancy or lactation.

(4) Uncorrected coagulopathy.

(5) Chronic Kidney disease (CKD) 3b or above.

All suitable patients were treated with CaEP at the West London Gynaecological Cancer Centre, Imperial College NHS Trust (London, UK) between March 2019 to August 2020 (inclusive).

Patients were counselled about the risks of CaEP, such as hyperpigmentation, ulceration, potential (but unknown) cardiac arrhythmias [22] and unknown side effects of its application at a novel site. Beneficially, it is less scarring, minimally invasive, healthy tissue-preserving and can be repeated [2, 22]. Calcium is delivered locally into the tumour and is therefore not expected to have systemic effects.

CaEP was performed under general anaesthesia in the operating theatre. Vulvoscopy was performed in all cases prior to electroporation. Staff performing the procedure were trained by the manufacturing company Mirai Medical (Mirai Medical, Galway, Ireland).

### *2.2 Calcium solution preparation*

Calcium chloride ($CaCl_2$) solution was prepared to the recommended concentration of 9 mg/mL (225 mmol/L), having been shown to effectively induce tumour necrosis in pre-clinical studies [20] in normal and malignant cells and in phase I and II clinical trials [19], by diluting 10 mL of a stock 10 mmoL/10 mL solution of $CaCl_2$ with 35 mL of 0.9% sodium chloride (NaCl). A dose of 0.5 mL of this solution was injected into the tumour for every 1 $cm^3$ of tumour volume. Tumour volume was calculated using the formula $ab^2\ \pi/6$, where a is the longest diameter and b is the longest diameter perpendicular to a.

### *2.3 Equipment layout*

The handheld CUTIS device (Mirai Medical, Galway, Ireland) consists of needle electrodes that are inserted into the tumour tissue. The CUTIS electrode has 2 pairs of 4 needle electrodes arranged parallel apart (total of 8 needle electrodes) and can be deployed to a depth of 2 cm. The parallel rows are separated by a gap of 4 mm. The length of the needle electrodes can be adjusted according to the tumour depth into four lengths: 5, 10, 15 and 20 mm (Fig. 1). The handheld device is connected to the CE approved *ePORE* generator (Mirai Medical, Galway, Ireland) which delivers the electrical impulse (Fig. 1).

### *2.4 Electroporation technique*

The lesion is mapped by drawing round it to ensure that all the lesion is treated with minimal re-treatment of the areas already electroporated. Needle electroporation is repeated in different sections of the lesion according to its size. Following

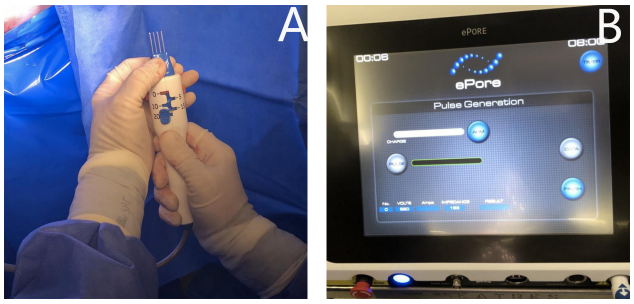


**Fig. 1. There is no associated patient.** (A) *ePORE* handheld device with needle electrodes adjusted to 20 mm length. (B) *ePORE* electrical generator during procedure.

injection of $CaCl_2$, electroporation pulses are delivered in 2 microsecond intervals, alternating from positive to negative polarity with a gap of 0.5 microseconds between the change in polarity. The pulses are delivered in 50 pairs (2 microseconds positive, 2 microseconds negative) for an energised time of 200 microseconds per train of pulses at a frequency of 166 KHz (as opposed to the standard 5000 Hz used in ECT previously). Transmission of electroporation pulses at higher frequency to the tissue means that the muscle has no time to react and hence the procedure could, potentially, be carried out with local anaesthesia. Each pulse has an amplitude of 520 V (positive 520 V and negative 520 V) for an applied electrical field strength of 1300 V/cm. A total of 30 'trains' of pulses are delivered in total at a frequency of 10 Hz and a total energised time of 6 milliseconds. Needle electroporation is repeated in different sections of the lesion according to its size.

Patients were observed for a minimal period of 4 hours, observations were recorded, pain controlled and all patients were confirmed to have passed urine. All patients were informed of the direct contact and open-door policy at the department for any complications within 6 weeks of a procedure. There was a low threshold for repeat biopsy given the novelty of the technique.

### *2.5 Follow up*

All patients were followed up within four weeks of the procedure in the specialist gynaecological oncology clinic. Electroporated lesions were assessed macroscopically in clinic, with a complete response describing full macroscopic resolution and relief of symptoms, partial response for partial macroscopic resolution and partial symptomatic relief, stable disease for no change and progressive disease for worsening and/or additional macroscopic lesions and symptoms. When new lesions appeared after an initial, complete response, this was described as recurrence. Biopsies taken after CaEP to assess microscopic response were not routinely done.

### *2.6 Case series*

We summarise below, and in Table 1, six patients that underwent the procedure consecutively. The median follow-up time for cases was 14 months (range 2–18 months).

### *2.7 Patient A*

Patient A (44 years old) presented with severe vulval pain, on a background of previous vulval cancer in November 2017, treated with clitoral sparing vulvectomy. Her treatment history, including surgery, is described in Table 1. A thickened and erythematous vulval lesion was treated with CaEP; applied to the right labium minus, clitoris, clitoral hood and perianal region, in July 2019. The procedure was uncomplicated and she was fit for discharge on the same day with good pain control and bladder function. Before CaEP, biopsies were taken perianally, which subsequently showed intraepithelial neoplasia. Four weeks post-procedure, there was a complete response, with report of significantly reduced pain and minimal erythema of the vulva on examination.

At three months after the first CaEP treatment, she reported two painful areas in the perianal and clitoral regions. She underwent a second CaEP in December 2019. Biopsies prior to the procedure showed vulval intraepithelial neoplasia stage II (VINII) and HPV change. After six weeks, there was a partial response, with improvement in the clitoral lesion had much but some residual discomfort from the perianal lesion. Due to this, a third CaEP was performed in August 2020. Biopsies prior to the procedure showed severe dysplasia but no evidence of invasion. She is due to be followed up 4 weeks post-procedure.

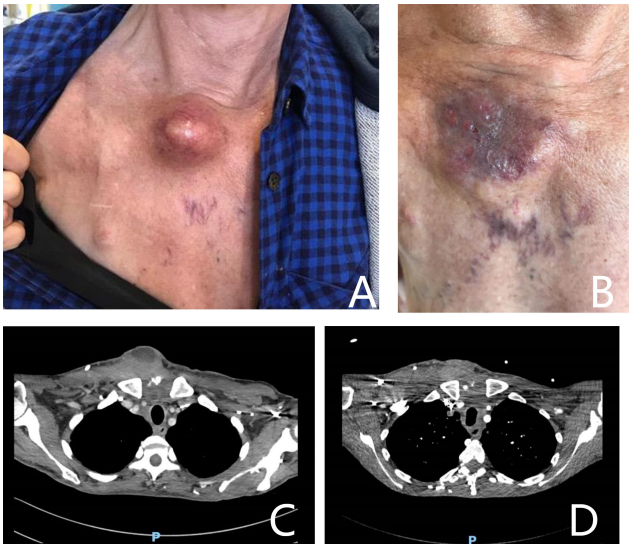


**Fig. 2. Patient B**. (A) Pre-sternal lesion before CaEP. (B) Resolution of the lesion after CaEP. (C) CT image of pre-sternal lesion before CaEP. (D) CT image of the lesion after CaEP.

**Table 1. A summary of pathology, treatment history, treatment intent, results including complications and follow up for all of the six patients.**

| Patient | Type of lesion | Past treatment | Date CaEP provided | Treatment intent | Histology (pre-CaEP) | Macroscopic response | Microscopic response | Symptomatic response | Complications |
|---|---|---|---|---|---|---|---|---|---|
| A: Age 44 | VIN III | 1. PlasmaJet® ablation x2, 2015, 2016 | 1st CaEP: July 2019 | Curative | July 2019: VIN III and PAIN (peri-anal intraepithelial neoplasia) | Complete response | N/A | At 4 weeks: vulval pain significantly reduced | None |
| | | 2. Cervical cone excisions | | | | | No biopsies taken after CaEP | At 3 months: mild discomfort in two areas (perianal and clitoral) | Discharged the same day |
| | B/G: previous vulval cancer | 3. Vulvectomy (clitoral sparing) and a bilateral V-Y advancement flap 2017 (technique used to repair cutaneous defects whereby V-shaped incision is made and broad base of V is advanced into the defect, which is then closed in a Y-shape, for better cosmesis) | 2nd CaEP: Dec 2019 | Curative | December 2019—VIN II, HPV change and AIN II | Partial response<br>Reduction in clitoral lesion, remaining erythema and thickening in perianal lesion | N/A<br>No biopsies taken after CaEP | At 6 weeks: partial-symptoms resolved<br>from clitoral lesion, residual symptoms in perianal lesion | None<br>Discharged the same day |
| | | 4. Local excision 2018 | 3rd CaEP: Aug 2020 | Curative | August 2020—severe dysplasia in all biopsies. No evidence of invasion | N/A—awaiting follow-up in late Sept 2020 | N/A | N/A | None<br>Discharged the same day |
| B: Age 48 | Metastatic chest wall deposit—FIGO Stage III mucinous ovarian cancer | Primary debulking surgery 2015 | Aug-19 | Palliative | August 2019: Mucinous ovarian carcinoma metastatic deposit | Complete response | N/A | Partial, but transient: reduction in pain before abscess formation 10 days later | None |
| | | Radiotherapy for recurrence 2018<br>Secondary debulking surgery 2018<br>Brachytherapy 2019<br>Chemotherapy 2019 | | | | | No biopsies taken after CaEP | Voice hoarseness persisted however | Discharged the same day |

**Table 1. Continued.**

| Patient | Type of lesion | Past treatment | Date CaEP provided | Treatment intent | Histology (pre-CaEP) | Macroscopic response | Microscopic response | Symptomatic response | Complications |
|---|---|---|---|---|---|---|---|---|---|
| C: Age 80 | VIN III + vulval SCC | 1. Radical vulvectomy and bilateral groin node dissection for stage III squamous cell carcinoma of the vulva 1996 | 1st CaEP: March 2019 | Curative | March 2019: vulval squamous cell carcinoma | Complete response | Complete response | Yes | None |
| | | 2. Repeat vulvectomy for recurrence 2013 | | | | | Biopsies May 2019 (2 months post-CaEP): no evidence of malignancy | | Discharged the same day |
| | B/G: FIGO stage III vulval SCC | 3. Chemoradiotherapy + lymph node excision secondary to a right inguinal lymph node recurrence 2016 | 2nd CaEP: Sept 2019 | Curative | September 2019: well-differentiated vulval squamous carcinoma + VIN III | Partial response | N/A | Discomfort still present, especially in the groin | None |
| | | 4. Partial vulvectomy + bilateral VY advancement flaps for recurrence 2017 | | | | Macroscopic carcinosis still present in some areas | No biopsies taken after CaEP | | Discharged the same day |
| | | (wound became infected and healed poorly) | 3rd CaEP: October 2019 | Curative | October 2019: Differentiated VIN + granulating tissue | Complete response | Recurrent disease at 3 months | 2 weeks: yes, reduced pain | None |
| | | | | | | | | 3 months: recurrence of symptoms | Discharged the same day |
| D: Age 50 | VIN III recurrent | Local excision 2016 | 1st CaEP: September 2019 | Curative | September 2019 (pre-CaEP): VIN III | Partial response | N/A | 4 weeks: pain free. Occasional itching (much reduced) | None |
| | | | | | | At 4 weeks: right upper vulva-single 1 mm spot on labium minus of possible macroscopic VIN | | | |
| | | | | | | At 5 months: progressive disease | No biopsies taken after CaEP | 6 months: intense pruritis in labia (area not previously electroporated) + bladder urgency | Discharged the same day |
| | | PlasmaJet® treatment x2 2016, x2 2017, 2018 | 2nd CaEP intended for: March 2020 | Curative | N/A—Procedure rescheduled due to COVID-19 | N/A | N/A | N/A | N/A |

**Table 1. Continued.**

| Patient | Type of lesion | Past treatment | Date CaEP provided | Treatment intent | Histology (pre-CaEP) | Macroscopic response | Microscopic response | Symptomatic response | Complications |
|---|---|---|---|---|---|---|---|---|---|
| E: Age 72 | FIGO stage III serous ovarian cancer | Chemotherapy 2019 | Abandoned (could not safely be carried out) | Palliative | N/A | N/A | N/A | N/A | N/A |
| F: Age 75 | FIGO stage III Vulval SCC | 1. Chemotherapy for both lung and vulval cancer 2019 | 1st CaEP: September 2019 | Palliative | September 2019 (pre-CaEP): vulval squamous cell carcinoma | Complete response | N/A | Pain free for 4 weeks | None |
| | B/G | | | | | | No biopsies taken after CaEP | Increased mobility<br>At 1 month: recurrence of pain | Discharged the same day |
| | T4N2M1a lung cancer | 2. Radiotherapy of pelvis alone | 2nd CaEP: October 2019 | | N/A—no biopsies taken pre-CaEP | Partial response<br>Mild, residual, possible macroscopic VIN present | N/A<br>No biopsies taken after CaEP | Reduction in pain and anxiety | None<br>Discharged the same day |

Key. B/G, diagnosis background.

*2.8 Patient B*

Patient B (48 years old) presented with voice hoarseness and a superficial, discharging, pre-sternal mass on a background of stage III mucinous ovarian cancer, for which she had undergone primary and secondary debulking surgery. She was referred by the medical oncologists for consideration of CaEP, currently receiving Folfirinox/Avastin chemotherapy, and previously brachytherapy, with no effect on the lesion. CT scans demonstrated a raised, fluctuant tumour with a possible necrotic core, in the upper mediastinal region of the recurrent laryngeal nerve (Fig. 2).

CaEP was applied to this 5 × 5 cm tumour in August 2019. The procedure was uncomplicated and Patient B was discharged the same day. Two weeks later, complete macroscopic response of the lesion was noted in clinic and on CT imaging (Fig. 2). On the right side, some areas which had not undergone electroporation demonstrated residual disease on CT (Fig. 2). She was discharged to a hospice and passed away in October 2019.

*2.9 Patient C*

Patient C (80 years old) was noted to have possible VIN III during a routine gynae-oncology clinic appointment, following a history of recurrent stage III vulval squamous cell carcinoma. She had already undergone a radical vulvectomy and bilateral groin node dissection (1996), a repeat vulvectomy (2013), chemoradiotherapy followed by lymph node excision (2016) and a partial vulvectomy with bilateral V-Y advancement flaps (2017). The latter was complicated by wound infection and poor healing. VIN III was subsequently confirmed on histology from biopsies taken during vulval mapping in December 2018. CaEP was deemed a reasonable alternative for management of the VIN III given this patient's poor healing history after surgery. CaEP was eventually applied to a 3 × 3 cm lesion in March 2019 (delayed due to equipment unavailability), of which pre-CaEP biopsies demonstrated vulval squamous cell carcinoma.

Patient C then required two further CaEP procedures (Table 1): in September 2019, for one 3 × 3 cm lesion and three 1 × 1 cm lesions, of which pre-CaEP biopsies later confirmed well-differentiated vulval squamous cell carcinoma and some differentiated VIN III, as well as in October 2019, partial response after her 2nd CaEP (although the vulva was healing well at the time). This further treatment was duly warranted considering pre-CaEP biopsy findings of squamous cell carcinoma in September 2019 (above) and taking into account Patient C's wishes to avoid radical surgery. Her third CaEP is illustrated in Fig. 3. Superficial lesions present on the left side of the vulva were injected with $CaCl_2$ solution and electroporated. Biopsies taken prior to the procedure showed differentiated VIN and granulating tissue. On review two weeks later, there was a complete response to CaEP; with the previously seen lesions no longer visible.

All three CaEP procedures this patient underwent were uncomplicated and the patient was able to be discharged the same day.

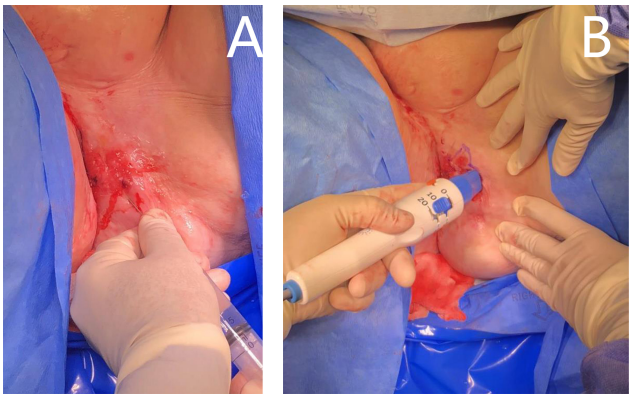


**Fig. 3. Patient C.** (A) $CaCl_2$ solution injection to the superficial lesion. (B) Electroporation of the superficial lesion.

Three months following the final CaEP, the patient was reviewed in clinic with recurrence of pain. On examination, there was macroscopic evidence of likely vulval squamous cell carcinoma recurrence after initial, complete response. She underwent vulval mapping and biopsies in January 2020. Biopsies confirmed a recurrence of well differentiated squamous cell carcinoma on the left of the vulva. A radical left vulvectomy with V-Y flap formation and PlasmaJet to the remaining areas was performed in March 2020. Excision margins were clear of tumour (1 mm on the perianal margin). On clinic review four months following the procedure, a new macroscopic lesion was noted on the right side of the vulva. Further biopsies were taken in August 2020, showing ulceration and inflammatory change from the right vulva and changes suggestive of squamous cell carcinoma in the right perineal region. She is due for follow-up in late September 2020.

*2.10 Patient D*

Patient D (50 years old) presented with significant vulval itching, following multiple PlasmaJet® ablations for recurrent VIN III (Table 1). Vulvoscopy and mapping was performed in September 2018. Biopsies taken at the time confirmed VIN III in the right and left labium minus and perianally.

In view of multiple PlasmaJet® treatment failures in the past, the patient opted for CaEP, which was eventually performed in September 2019 (the patient missed several appointments) to the right and left superior labium minus, right and left vestibules, perineum and perianal area. VIN III of the vulva and perianal area was confirmed on pre-CaEP biopsies. On review in October 2019, the itching had settled to a mild level. The lesions, however, demonstrated a partial response to CaEP. Although there was a significant macroscopic reduction of VIN peri-anally, a single 1 mm spot on the right labium minus was noted with no macroscopic evidence of VIN on the left upper vulva. In February 2020, the patient reported intense pruritis in two small areas in the right and left labia, and bladder urgency. Examination showed disease progression, with macroscopic lesions in the pruritic areas. Ar-

eas previously electroporated were normal. She was offered repeat CaEP treatment in March 2020 which was cancelled due to the COVID-19 pandemic. To date, the team have been unable to contact the patient to reschedule the procedure.

*2.11 Patient E*

Patient E (72 years old) was referred for palliative CaEP due to a painful vaginal vault lesion, with vaginal bleeding and intermittent vaginal discharge, secondary to stage III serous ovarian cancer, for which she had been receiving chemotherapy. Unfortunately, the instrument was too wide to pass through the vaginal introitus and the needle electrodes could not be visualised. CaEP was therefore abandoned as it was unsafe to proceed. The patient was subsequently advised to continue with chemotherapy as the size of the vaginal lesion, and the mix of cystic and solid components, reduced the likelihood of radiotherapy being effective.

*2.12 Patient F*

Patient F (75 years old) presented with severe vaginal pain and a visible vulvo-vaginal tumour on a background of stage III vulval squamous cell carcinoma, for which she had been receiving radiotherapy. She also had concomitant lung cancer (Table 1). Her vulvo-vaginal pain was debilitating and affecting her mobility to such a degree it rendered her housebound.

Pelvic exenterative surgery including removal of the bladder, urethra, vulva and right lymph node dissection was offered. She declined this to avoid major surgery and opted for CaEP, performed in September 2019. Vulvoscopy demonstrated a large, ulcerated vaginal tumour incorporating the right vulva and urethra and fixed, enlarged right inguinal lymph nodes. Eight treatments with CaEP at 10–15 mm depth were performed without complications.

There was a complete response to CaEP; rendering Patient F markedly pain-free for over a month and significantly increasing her mobility (no longer housebound). Her pain recurred eventually and a repeat CaEP was performed at the end of October 2019 for ongoing symptomatic relief.

During examination under anaesthesia, a large right vulvo-vaginal lesion was noted associated with erythema and oedema in the surrounding tissue (Fig. 4). Intra-operative ultrasound (IOUS) was performed to appreciate the depth of the lesion (Fig. 4) and guide the infiltration of $CaCl_2$ and length of the needle electrodes. Cystoscopy was not performed as there was stenosis of the urethra secondary to local invasion. As the patient had been experiencing dysuria, a suprapubic catheter was inserted. The patient was discharged the same day. At clinic review two weeks later, there was no macroscopic evidence of recurrence of cancer but possible mild macroscopic VIN only, demonstrating a partial response to CaEP. She passed away in January 2020.

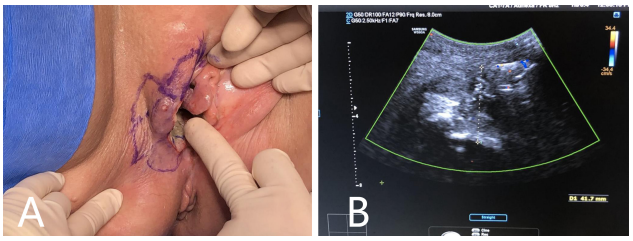


**Fig. 4. Patient F**. (A) Large tumour affecting mostly the right lower vagina and vulva. (B) Patient F: Intra-operative ultrasound on the right vulval lesion.

## 3. Discussion

*3.1 Main findings*

In summary, CaEP was performed a total of ten times amongst this group of patients (n = 5); four times for vulval squamous cell carcinoma, five times for VIN III and once for a metastatic ovarian carcinoma deposit. One of the original six patients (patient E) was not treated with CaEP because of equipment failure.

CaEP resulted in a complete macroscopic reduction of the lesion in five episodes of CaEP application and partial macroscopic reduction in four other episodes. For the remaining episode of CaEP application, there has not yet been clinic follow up. Symptoms had resolved or significantly improved within six weeks for five episodes of CaEP application, partially improved in three, persisted in one and could not be ascertained in one because of pending clinic follow-up. Beyond six weeks, symptoms recurred in all patients that had initially resolved or significant improvement of symptoms within six weeks. Of the five patients that received CaEP, four required more than one CaEP procedure (maximum three instances) for recurrence of VIN (n = 2) and vulval squamous cell carcinoma (n = 2), although one of these cases has not yet had their second CaEP procedure because of COVID-19. In one of the cases of recurrence of vulval squamous cell carcinoma (Patient C), treatment proceeded to a radical left vulvectomy five months after the third CaEP. CaEP was useful for palliation of distressing symptoms in one case of metastatic ovarian cancer (Patient B).

In all ten episodes where CaEP was carried out, no intra-operative or post-operative complications were noted, and all patients were able to be discharged on the day of the procedure. No longer-term complications as a result of CaEP were reported in the 14–18 months that the remaining living patients have been followed-up.

In this small case series, 8/10 episodes of CaEP application achieved a clinical response at 6 weeks. Recent trials of electrochemotherapy (ECT), with larger study numbers have shown comparative effects. ECT with bleomycin in 15 patients with vulval cancer had a response rate of 80% within a month, however at 1 year only 50% of patients survived [5]. ECT with Bleomycin in 61 patients was more successful, with a clinical response rate of 86.3% at 2 months.

### 3.2 Review

CaEP has been shown to be effective in other types of cutaneous malignancy. In a randomised double-blind study of seven patients with a total of 47 cutaneous metastases of melanoma and breast cancer, CaEP was found to be as effective as electrochemotherapy with bleomycin [21]. CaEP resulted in a 72% objective response and a complete response in 66% of cases [21]. A phase I trial of CaEP for recurrent head and neck cancer in six patients found a clinical response in half of the patients and no complications related to the procedure. It concluded that CaEP is effective, safe and feasible [14].

There is growing evidence that CaEP is selective in its effect on tumour cells and therefore, unlikely to affect surrounding normal cells [15]. CaEP was shown to be significantly more efficient in rhabdomyosarcoma cell lines compared to normal murine muscle cells. A significant increase in intracellular calcium and decreased expression of the calcium/sodium exchanger (NCX1) was demonstrated. This, in itself, reduces the capability of the malignant cells to extrude calcium [15].

Furthermore, calcium levels in normal skin tissue were found to return to levels similar to untreated controls, within four hours after CaEP [23]. The expression of plasma membrane calcium ATPase (PMCA), which is responsible for extruding intracellular calcium, was reduced in cancer cells. Normal surrounding tissue of breast cancer lesions demonstrated minimal necrosis, even when directly treated with CaEP [23]. It is thought that differences in expression of plasma membrane transporters, along with possible alterations in membrane repair and electric impedance, underlie the differences in sensitivity of malignant and normal cells to CaEP [22].

### 3.3 Limitations

CaEP is a new application in clinical practice. Therefore, its equipment can be challenging to obtain, and if there is failure, it can be arduous to rectify. Whilst still developing an optimal model, limitations in access were demonstrated in our case series (Patient E). We are confident that such limitations will be addressed with more frequent use of the equipment.

We do not yet fully know how effective CaEP will be in altered cancer cell types and in diverse conditions. In fact, a recent study based on cell lines of colorectal cancer, breast cancer and normal fibroblasts showed that membrane composition may influence the effectiveness of CaEP owing to the calcium cation interaction with the lipids of the outer membrane [24]. There is also some degree of temperature dependency, especially in cell membranes with a high concentration of ether lipids [24]. Moreover, a disparity in sensitivity was also observed in cell lines of various tumour cell types [23]. This highlights the limitations of concluding the effects of CaEP within our own heterogenous study population.

Our follow-up data is limited to a maximum of 14–18 months after the first CaEP for the remaining living patients. We are therefore unable to comment on longer-term symptom and cosmetic effects of this technique beyond this timeframe, especially where more than one CaEP procedure has taken place. Patients who have been treated with such a novel technique are still being followed-up.

Furthermore, the recurrence of symptoms, positive histology and need to do more than one CaEP procedure in our patient cohort critically highlights that for curative intent, CaEP may be transient, although larger studies are required to confirm this.

### 3.4 Strengths

Our cases series has demonstrated promising short-term symptomatic benefit in a small patient cohort. The fact that all procedures were performed with no complications such as post-procedure pain or wound infection makes CaEP an attractive alternative option to surgery. This is of significant importance as a large proportion of patients with vulval cancer or VIN tend to be elderly with significant co-morbidities including high BMI and poor healing. This is secondary to a hypo-oestrogenised and/or irradiated vulvo-vaginal epithelium rendering them very susceptible to post-excision wound infection.

In addition, it is worth highlighting that we are proposing this novel technique for patients with recurrent vulval cancer and VIN, who have continued symptoms, have already undergone numerous surgical resections, and hence, all current options have been exhausted.

### 3.5 Future work

Future research on CaEP should include further phase I studies to determine the optimal dosing and efficacy of CaEP in vulval squamous cell carcinoma and VIN. In larger study populations, this could be stratified into human papilloma virus (HPV) dependent and HPV-independent VIN and Vulval dysplasia. This can be followed by appropriately sized phase II trials to draw reliable conclusions on the effectiveness of this novel technique. Validation studies require patients to be followed up over a longer length of time to assess long term outcomes, particularly survival and recurrence rates.

Given that the preliminary data for CaEP in various types of cancer is promising, this technique could also be applied at an experimental level in other types of cancer or premalignant conditions when conventional approaches are not acceptable to the patients. In the field of gynaecological oncology, this could potentially relate to a young patient with new or recurrent cervical intraepithelial neoplasia (CIN) III or stage IA1 cervical cancer. This patient may not wish to embark on an excisional procedure, which may have an adverse effect on their future fertility. Similarly, CaEP could be used in cases of endometrial hyperplasia or stage IA endometrial cancer as an alternative fertility-sparing option. In that case, hysteroscopy or intra-operative ultrasound could be utilised, after modifying the handheld device, to deliver CaEP directly into the abnormal endometrium. As demonstrated by Frand-

sen *et al*, the normal surrounding tissue does not seem to be affected by CaEP. Hence, healthy endometrial tissue would be preserved [23].

## 4. Conclusions

In a small, selected cohort of patients with cutaneous manifestations of gynaecological cancer, CaEP has shown promise in macroscopic resolution of the lesions and symptomatic improvement in the short term, with lack of any complications. In the longer term, however, most cases of VIN and vulval cancer recur. Despite this, if validated, CaEP may prove an effective and minimally invasive option in women for whom either the risks of extensive surgery are unacceptable or all current management options have been exhausted. Hence, CaEP may also be acceptable to women who have already undergone extensive surgery (ies) and thus further surgery would result in severe disfigurement. Long-term follow-up is required to conclusively evaluate its effects, particularly recurrence.

## Author contributions

YAS was the main contributor in preparation, writing and revision of the manuscript and gathering of data. HEM, NG, BPJ, MC, DLP and MehC were involved in preparation of the manuscript, collecting the data and preparing the tables. KL, CF, SGM and HG were involved in assessing the manuscript from a clinical perspective and the care of the patients described. SS and JRS were also responsible for the original manuscript design, drafting and revision for important intellectual content. JRS is also the guarantor for this paper and accepts full responsibility for the work and/or the conduct of the study. All authors approve.

## Ethics approval and consent to participate

Institutional Review Board approval was not required for this case series as it is an extension of already established clinical practice. Informed, written and signed consent was obtained and the patients agreed for their case histories and photographic material related to the lesions to be published for research purposes.


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## Conflict of interest

The authors declare no conflict of interest.

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