Safety and Preliminary Efficacy of Ultrasound-guided Percutaneous Irreversible Electroporation for Treatment of Localized Pancreatic Cancer

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Abstract. *Background: Irreversible electroporation (IRE) is a local tumor treatment. Thin needles are placed percutaneously around the tumor under ultrasound guidance. Short pulses of direct current sent through the tissue irreversibly increase cell membrane permeability leading to cell death. We report a phase 1 study assessing the safety of ultrasound guided percutaneous IRE in patients with localized pancreatic cancer (LPC). Patients and Methods: Five patients (three males) with LPC, judged unsuitable for surgery, chemotherapy, or non-resectable after standard oncological treatment, were treated with IRE. The treatment was given under general anesthesia with muscle relaxation. Results: No serious treatment-related adverse events were observed. There was no 30-day mortality. One patient went on to laparotomy and had a R0 pancreaticoduodenectomy with portal vein resection. Six months after the treatment, two patients had no signs of recurrence on computed tomography or contrast-enhanced ultrasound. Conclusion: IRE for LPC can be safely performed percutaneously under ultrasound guidance, with promising initial results regarding efficacy.*

Pancreatic cancer is a severe disease. Long-term survival can be achieved by radical surgery, but only 5-12% of patients can be operated on. The reason most patients are ineligible for surgery is the presence of distant metastases (40%) or locally advanced disease (40-50%) with vascular encasement or growth on nearby organs (1). Even among patients who undergo radical resection, the five-year survival rate is only 8-15%, with a median survival of 15-25 months (1). Locally advanced pancreatic cancer has a poor prognosis, with a median survival of less than a year, although some locally advanced tumors can be down-staged with chemotherapy or radiochemotherapy and then R0-resected (2).

Irreversible electroporation (IRE) is a new tumor ablation technique that uses short direct current pulses sent through the tumor, which increases cell membrane permeability and allows easier passage through the cell membrane. Reversible electroporation can be used to allow non-permeable chemical agents, such as proteins or drugs, to cross the cell membrane by creating nanopores (3), hence the name electroporation. By changing the pulse duration and the number of pulses, the change in permeability becomes irreversible leading to cell death by necrosis (4). No thermal tissue damage occurs, thus, extracellular structures, such as vessels, remain intact (5, 6). There is no cooling (heat-sink) effect around vessels that can compromise the ablation margin, unlike with radio frequency and microwave ablation (7, 8). Due to the close proximity to blood vessels, gall ducts and the pancreatic duct, especially in locally advanced tumors, IRE appears to be a suitable treatment for localized pancreatic cancer (LPC). IRE has been used without any severe complications for local treatment of tumors in lung, kidney, and liver (9) and in the prostate (10).

In swine, ablation with IRE in the pancreas caused slight elevation of serum amylase and lipase but there were no clinical or histopatholgical signs of pancreatitis at laparotomy after treatment (11, 12), an IRE study on mouse pancreas found similar results (13). There are promising initial results regarding the safety (14, 15) and effect (16) of intraoperative and percutaneous IRE in patients with LPC, but there is lack of information on percutaneous ultrasound-guided IRE.

Therefore, a phase I/II study assessing the safety and efficacy of IRE in patients with LPC has been initiated, and the experiences from the five patients constituting the phase I part of the study, according to the protocol, is reported here.

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Table I. *Patients' characteristics before treatment with irreversible electroporation.*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age, years	72	65	89	46	65
Gender	Male	Male	Female	Female	Male
Tumor size (mm)	20×20×20	35×30×40	10×15×20	25×30×35	35×35×35
Preoperative CA 19-9 (U/ml)	360	<1.0	413	38.7	4916
ASA score	30	30	20	20	20
Previous ERCP	Yes	Yes	Yes	Yes	Yes
Previous PTC	Yes	No	Yes	No	Yes

Table II. *Patients' characteristics after treatment with irreversible electroporation.*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Elevated CRP (mg/l)	Yes (190)	No	Yes (225)	No	Yes (316)
Elevated leukocytes ($\times 10^9$)	Yes (18.6)	No	Yes (23.4)	No	Yes (23.8
Elevated amylase	Yes	No	No	No	No
Postoperative pain	No	Yes	No	Yes	Yes
Ablation zone on CEUS (mm)	20×20×20	30×35×35	25×25×25	40×45×42	38×41×40
Signs of complication on postoperative US	No	No	No	No	No
CA19-9 (U/ml)					
One month	579	Not done	160	51.3	5425
Three months	Not done	Diseased	348	147	-
Six months	Diseased	Diseased	991	23.5	Diseased
Time to metastasis	1 Month	-	-	-	3 Months
Time to local recurrence	-	-	-	-	-
Survival	108 Days	32 Days	Alive after six months	Alive after six months	138 Days

Patients and Methods

The study was approved by the Regional Ethics Committee Uppsala, Sweden (Dnr 2011/298). Five patients (three male, two female, aged 46-89 years, median 65 years) with LPC that was unrescetable after chemotherapy or radiochemotherapy, or were otherwise deemed unfit for surgery or chemotherapy, were included in the phase I part of the study. All patients had computed tomography (CT) of the thorax and abdomen and were discussed at a multidisciplinary team conference prior to study inclusion. None of the patients had liver metastases on CT and contrast-enhanced ultrasound (CEUS). All patients provided informed consent prior to any study-related procedure.

All five patients had presented with jaundice. One patient had previously undergone a laparotomy and was found to have unresectable disease and underwent a hepaticojejunostomy. Four patients had endoscopic retrograde cholangiopancreatography one of which had a successful stent placed through endoscopy, and three needed subsequent percutaneus transhepatic cholangiography. All patient diagnoses were confirmed by biopsy and the approximate median tumor volume was 13.1 cm^3 (Table I). Before treatment, three patients had an American Society of Anesthesiologists (ASA) score of 20 and two had an ASA score of 30.

Commercially available equipment (Nanoknife; Angiodynamcis System, Queensbury, NY, USA) was used for the IRE. In this method, needles are placed around the tumor and electrical pulses are sent between each needle pair: the generator produces 20-100 μs high-voltage (100-3000 V) pulses of direct current. The system is synchronized with the Electrocardiography and the pulses given are triggered by R-wave to prevent cardiac arrhythmias (17). As a safety precaution, all patients have defibrillation pads put in place.

All patients were treated under general anesthesia with deep muscle relaxation to avoid muscle contraction during IRE. In all patients, three needles, with an active length of 15 mm, were placed percutaneously under ultrasound guidance by the same interventional radiologist. Pretreatment planning was carried out using both CT and CEUS to delineate the tumor margins and define anatomical landmarks that could be used during the procedure. All tumors were well delineated on ultrasound and both the ultrasound image and the landmarks identified in the pretreatment assessment were used to define the areas to be treated. The maximum possible distance between IRE needles is 2 cm: each needle distance was therefore measured in the ultrasound image to make sure this was not exceeded, and the machine settings adjusted according to the measured distances. Three needles placed 2 cm apart and with an active length of 15 mm will give an ablation zone of 3×3 cm with a

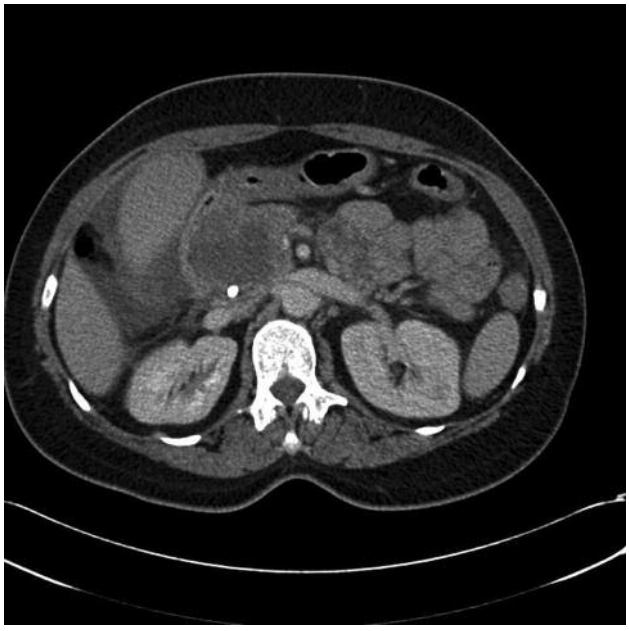

Figure 1. *The computed tomography before irreversible electroporation on a patient who then went on to undergo resection.*

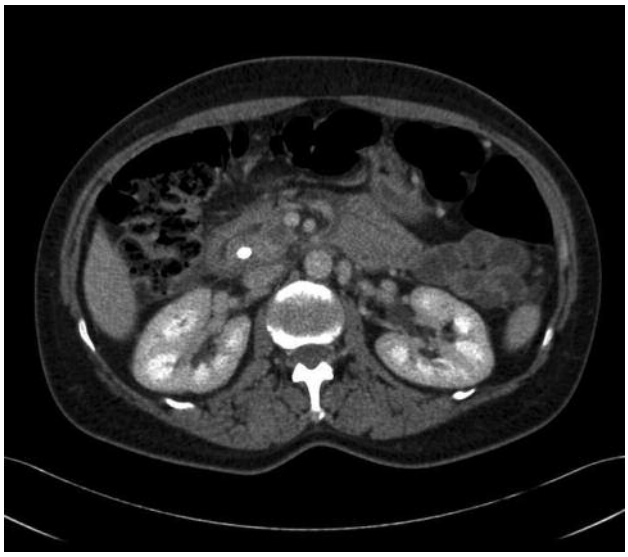

Figure 2. *The computed tomography three months after irreversible electroporation on a patient who then went on to undergo resection.*

depth of 20 mm. Therefore, the needles had to be replaced and retracted 2-4 times in order to cover the entire tumor volume as defined in the pretreatment assessment in each individual patient. It follows that needles were placed both inside the tumor and along the margins. The aim was to cover the entire tumor area with a 5 mm safety margin. The initially pulses had a pulse-length of 70 μs and a voltage of 1500 V/cm. The resulting current graph was studied and the V/cm adjusted to a level where the resulting current was expected to be 30-50 A. The upper limit is a safety cutoff level built into the machine to avoid thermoablation.

A total of 90 pulses were delivered at each needle position. For safety reasons, all patients stayed at the hospital for at least three days after the procedure: they were clinically assessed at least once a day and blood tests were taken daily. Before the patients were discharged, they underwent a CEUS to evaluate whether any tumor remained at the ablation site or whether there were any signs of postoperative complication.

The follow-up of the patients was according to a protocol, with CEUS and blood tests after one month and CT of thorax and abdomen, blood tests, and ultrasound every third month (Table II).

Results

The post-procedure CEUS revealed no residual tumor, no signs of complication, and an approximate median ablation zone of 18.4 cm^3 defined as an area with less contrast enhancement than before the ablation (Table II). No severe complications, defined as grade 3 or higher on the Dindo-Clavien scale (18), were detected post-procedure during the first 30 days.

The median postoperative hospital stay was 14 days. One patient developed sub-clinical pancreatitis with an elevation of serum amylase to 7.5 μkat/l (ref 0.15-1.1 μkat/l) and c-reactive protein (CRP) of 190 mg/l (ref <5 mg/l) but no correlating pain. This patient was discharged after 14 days, but was found to have metastases in the liver shortly afterwards, and died 108 days postoperatively.

After the treatment, three out of the five patients had elevated CRP and leukocytes (Table II).

One patient had tumor in the pancreatic head encapsulating the portal vein, however, the ultrasound examination revealed no damage to the portal vein, although it passed straight through the ablation zone. This patient had been previously treated with radiochemotherapy for a 6 cm large tumor, but the tumor was still unresctable due to encapsulation of the portal vein. Before IRE, the size of the tumor was 25×30×35 mm (Figure 1) and at the follow-up CT after three months, it was 19×9×13 mm (Figure 2). The histopathological examination after subsequent laparotomy and pyloric-preserving pancreaticoduodenectomy with portal vein resection revealed an R0 resection. One patient died of pneumonia 32 days postoperatively and another patient died of progressive disease after 138 days. Four patients had manageable pain postoperatively. Six months after the treatment two patients had no sign of recurrence on CT or CEUS.

Discussion

IRE is seemingly both a safe and active treatment for LPC. Although the median hospital stay was long (14 days), this was mostly due to pain and inflammation, and in one case, delayed gastric emptying. This patient had delayed gastric emptying prior to IRE and it did not resolve the duodenal stenosis; the patient subsequently underwent gastroenteroanastomosis.

One patient had sub-clinical pancreatitis post-procedure, but there was no severe pancreatitis. Whereas radiofrequency ablation in the pancreas can produce severe hemorrhagic pancreatitis (19), this did not occur in this study. Overall, no serious adverse event was observed in the treated patients. Furthermore, as indicated by ultrasound, all patients showed signs of complete tumor ablation shortly after IRE and after six months two patients are still alive with no evidence of pancreatic cancer relapse.

The focus of this study was on the safety of ultrasound-guided IRE in the pancreas. Therefore, the safety margin of 5 mm that we aimed for may appear insufficient. The phase II part of the study is to determine the activity of IRE in LPC, and there will be a larger tumor margin to increase the effect of the ablation. Follow-up was carried out here with both CT and CEUS, but as this was a safety study, the main aim of the follow-up was to determine that we had achieved an ablated zone in the tumor area without complications, not to assess total ablation or patient benefit on extended follow-up. Furthermore, this is a topic that will need careful consideration as IRE spares the major vessels and lack of contrast enhancement, therefore, cannot be used as a delineation of the ablation.

When this study started, there were virtually no published human cases using IRE in the pancreas. Since then, a case report has been published on percutaneous treatment, where a 6-month postoperative follow-up with magnetic resonance imaging revealed no residual tumor and decreasing carbohydrate antigen (CA) 19-9 (20). In March 2012, a case series of eight patients with CT-guided percutaneous treatment was published with similar findings: in two cases, the patients went on to have an R0 resection after IRE treatment (14). Therefore, future IRE could be a new downstaging treatment, enabling for radical resection on previously unresectable LPC.

A series of 27 cases treated with IRE intraoperatively was published in September 2012 (15): eight of these patients underwent simultaneous resection and several had additional procedures, such as gastrojejunostomy. Complications in this study were mostly related to open surgery, however, four complications were considered to be IRE-related, including portal vein thrombosis and duodenal leak after transduodenal placement of the needles. The complications related to open surgery might be eliminated if the needles are placed percutaneously. In a follow-up report, 54 patients with locally advanced pancreatic cancer treated with intraoperative IRE combined with chemotherapy or radiochemotherapy were compared with 85 patients treated with chemotherapy or radiochemotherapy: IRE was associated with an improvement in both local and distant progression-free survival, and overall survival (16).

In conclusion, ultrasound-guided percutaneous IRE appears to be safe, with limited complications and without complications related to laparotomy. Therefore, in a phase II study, LPC will continue to be treated percutaneuosly with ultrasound guidance in order to assess the antitumor activity of this new method.

Conflicts of interest

None of the Authors has any financial or personal relationships that bias their work with this study. This research received no specific grant from any funding agency in the public, commercial, or not-for profit sectors.

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