

Irreversible electroporation ablation of end-stage metastatic retroperitoneal lesions: Report on three cases and literature review

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Received October 12, 2018; Accepted April 26, 2019

DOI: 10.3892/etm.2019.7780

Abstract. Metastatic retroperitoneal tumors constitute an end-stage disease with poor prognosis that represents a heavy global health burden. The present study aimed to explore the efficacy of irreversible electroporation ablation (IRE) therapy in patients with end-stage retroperitoneal tumors. Between April 2016 and September 2017, three patients with unresectable retroperitoneal malignant tumors were enrolled. Among these cases, ultrasound (US)-guided IRE was palliatively performed for targeting 3 tumors (1 tumor per patient) located around the abdominal aorta. Post-treatment contrast-enhanced US (CEUS) and contrast-enhanced computed tomography (CECT) scans were subsequently performed to evaluate the area adjacent to the ablation zone and determine the prognosis. During the follow-up, the cases experienced a reduction of pain (mean score of 5.8 decreased to 2.2, based on the visual analogue scale), and had an overall survival rate ranging from 2 to 11 months. Case 1 remained alive at the time of submission of this study, but case 2 died within 2 months and case 3 within 11 months due to liver metastases of the primary tumor. At the 3-week follow-up, the CEUS image for case 1 exhibited

a contrast defect with a sufficient ablation margin, in accordance with the CECT at 1.5 months following IRE, exhibiting complete tumor necrosis without contrast enhancement. Overall, these results suggest that US-guided percutaneous IRE may be effective in the treatment of end-stage retroperitoneal tumors. However, further studies are required to substantiate the conclusions of the present study. The present clinical trial was registered at clinicaltrials.gov (ID: NCT02822066) on June 20th, 2016.

Introduction

Retroperitoneal sarcoma is a lethal disease with a generally poor prognosis, and retroperitoneal tumors are usually secondary metastatic tumors from other organs. Numerous patients are able to feel an abdominal mass or distension at the end-stage of the disease. Traditional treatment strategies for locally advanced retroperitoneal tumors include surgical resection (1,2), radiation therapy (3,4) and chemotherapy (5,6). The deep site of the retroperitoneum is surrounded by gastrointestinal and urologic organs and large blood vessels, which increases the difficulty of open surgery. Irreversible electroporation (IRE) is a non-thermal ablative technique, which destroys the lipid-bilayer structure of the cell membrane to generate tiny nanopores using a series of high-voltage, low-energy-current electrical pulses, resulting in apoptosis of the target cells. Previous studies have reported on the progressive use of IRE for the treatment of solid organs, including the pancreas (7), liver (8), lung (9), kidney (10) and prostate (11), and it is particularly suitable for tumors located in large vessels, the hilar region, bile duct and ureter (12). We ever reported the novel use of IRE for the percutaneous local ablation of portal vein tumor thrombus (PVTT) without heat-sink effect and thermal injury to surrounding portal vein (PV) branches (13). However, this technique is not generally suitable for patients with cardiopulmonary dysfunction, arrhythmia or cardiac pacemaker.

The present study reports on 3 cases with metastatic retroperitoneal tumors with the primary tumor originating from other organs, who received IRE palliative therapy using 3-4 electrodes according to the tumor size, and on their short-term follow-up.

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Abbreviations: IRE, irreversible electroporation; US, ultrasonography; CEUS, contrast-enhanced ultrasound; OS, overall survival; CT, computed tomography

Key words: irreversible electroporation, retroperitoneal tumor, survival, prognosis, contrast-enhanced ultrasonography, ultrasonography

Materials and methods

Patient selection. Between April 2016 and September 2017, a total of 3 patients (2 female and 1 male) who had 3 locally advanced retroperitoneal tumors received percutaneous IRE therapy. The baseline characteristics of the patients included are provided in Table I. The patients (case 1, female; case 2, female; case 3, male) were 60, 43 and 59 years old, respectively, and all had metastatic retroperitoneal tumors (case 1, ovarian cancer; case 2, gastric cancer; and case 3, pancreatic cancer), as well as a history of surgical resection or chemotherapy. On admission, the patients had complaints of waist pain for several months. The pain intensity was estimated using a visual analog scale (VAS) from 0 to 10 (where 0 represented no pain and 10 represented the worst pain imaginable) (14). Pre-operative computed tomography (CT) or contrast-enhanced ultrasound (CEUS) revealed that the tumors were between 2.0 and 6.1 cm in size. Pre-operative CEUS was generated using ultrasound machines with contrast-specific software (MyLab 70 XVG and MyLab Twice; Esaote) in case 1 revealed the course of contrast agent wash-in and wash-out as time elapsed (Fig. 1A), and pre-operative CT indicated metastatic lymph nodes of 6.1x4.3 cm in size encircling the abdominal aorta (Fig. 1B). Of note, all these metastatic tumors were situated deeply and close to important vessels, posing a risk of massive hemorrhage. The inclusion criteria for the study were as follows: i) All patients had their cancer pathologically confirmed; ii) patients were intolerant to chemotherapy and were reluctant to undergo further surgery due to the high risk associated with it; iii) measurements of the following cancer-associated blood parameters were applicable: Hemoglobin level, ≥ 8.0 g/dl (male normal range, 12.0-16.5 g/dl; female normal range, 11.0-15.0 g/dl); platelet count, $\geq 50 \times 10^9/l$ (normal range, $100-300 \times 10^9/l$) and international normalized ratio >1.5 (normal range, 0.8-1.2); and iv) the patients were void of severe coronary disease, acute or chronic infection or autoimmune diseases. This prospective study was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University (Zhejiang, China). All of the patients and their relatives were informed about the procedures and provided their written informed consent.

IRE procedure. The three cases received percutaneous IRE in the supine position under general anesthesia to avoid intense muscle contractions via an electroporation system (NanoKnife® system; AngioDynamics) using 3-4 19-gauge electrodes depending on the tumor size. All of the percutaneous procedures were performed by an interventional radiologist who had at least 10 years' experience in interventional medicine. Under the guidance of ultrasonography using MyLab Twice equipment (Esaote), percutaneous IRE ablation in case 1 was performed using 4 parallel 19-gauge electrodes, where the length of the tip was 0.5 cm and the between-electrode distance was fixed at 1.6-2.1 cm (Fig. 1C). Doppler color-flow imaging was used in real time to guide two needles, parallelly clamping the target mass surrounding the abdominal aorta (Fig. 1D). All of the mentioned intervals that are described were confirmed by US (Fig. 1E), and subsequently imported into the electroporation software in order to select the appropriate voltage and pulse-length delivery.

Preliminary 2,700-V test pulses were given to check the tissue conductivity. Subsequently, a total of 90 pulses with a length of 70-90 msec were performed in the voltage range of 2,550-3,000 V under the setting of 1,500 V/cm. The detailed procedures for treating the target lesions are summarized in Table II. Cases 1 and 2 only received one session of IRE therapy, whereas case 3 underwent a second session of therapy at 5 months after the first IRE treatment. Inter-electrode distances in the targeting lesions ranged from 1.5 to 2.0 cm. An electrocardiograph trigger was used to monitor different types of cardiac arrhythmia. After one session of electroporation, immediate CEUS images (via the injection of 2.4 ml SonoVue mixed with 10.0 ml 0.9% sodium chloride solution) were used to examine whether any potential residual lesions existed. In the procedures for case 1, by parallelly adjusting the puncture needle to a position ~ 1.0 cm away from the previous site of electroporation, the parallel puncture and needle procedures were repeated three times through a total of 12 rounds in order to maximize the curative effect (Fig. 1F). Following percutaneous IRE, follow-up by CEUS, CT scan (GE Healthcare) and measurement of cancer-associated blood parameters [carcinoembryonic antigen, alpha fetoprotein and carbohydrate antigen (CA19-9)] was performed at monthly intervals. In addition, the European Organisation for Research and Treatment of Cancer (EORTC) quality of life (QoL) questionnaire (QLQ)-C30 (Version 3.0) is a questionnaire used for health-associated assessment, which includes 5 functional dimensions (Physical, Role, Cognitive, Emotional and Social functioning), 3 symptom dimensions (Fatigue, Pain and Nausea/Vomiting), 1 global health status scale and 6 single items (Constipation, Diarrhea, Insomnia, Dyspnea, Appetite Loss and Financial Difficulties) (15). The clinical assessment of QoL in all patients was conducted at the baseline and at every three months following IRE therapy.

Results

IRE procedure characteristics and follow up. The three patients enrolled in the present study underwent palliative IRE therapy for locally advanced retroperitoneal tumors, with a mean follow-up time of 6 months post-IRE. For case 1, CEUS imaging immediately after the surgery revealed regular arterial hyper vascularization adjacent to the hypochoic area corresponding to the CEUS images at 3 weeks post-IRE (Fig. 1G and H). Furthermore, the 3-week CEUS image exhibited a clear contrast defect with a sufficient ablation margin (Fig. 1I), which was in accordance with the contrast-enhanced CT at 1.5 months after IRE, and revealed that the tumor shrank to 5.4x5.2 cm in size (vs. 6.1x4.3x4.3 cm³ in initial size), without contrast enhancement (Fig. 1J).

At several months following IRE therapy, the cases exhibited improved clinical symptoms (Table III), and their overall survival (OS) ranged from 2 to 11 months. Case 1 was alive at the time of writing. Case 2 and 3 had an OS of 2 and 11 months following IRE, respectively. Regarding the tumor markers following IRE, these were all within the normal range for case 1, although case 2 exhibited an increased carcinoembryonic antigen level, from 64.8 to 2,639.1 ng/ml, and case 3 had CA19-9 levels beyond the upper limit of normal (Table II). During the follow-up, minor procedure-associated

Table I. Characteristics of patients with metastatic retroperitoneal tumors.

Case no.	Sex	Age (years)	Primary cancer	History	Location of tumor
1	Female	60	Ovarian cancer	Oophorectomy and chemotherapy	Adjacent to abdominal aorta
2	Female	43	Gastric cancer	Gastrectomy	Adjacent to abdominal aorta
3	Male	59	Pancreatic cancer	Whipple operation	Adjacent to abdominal aorta

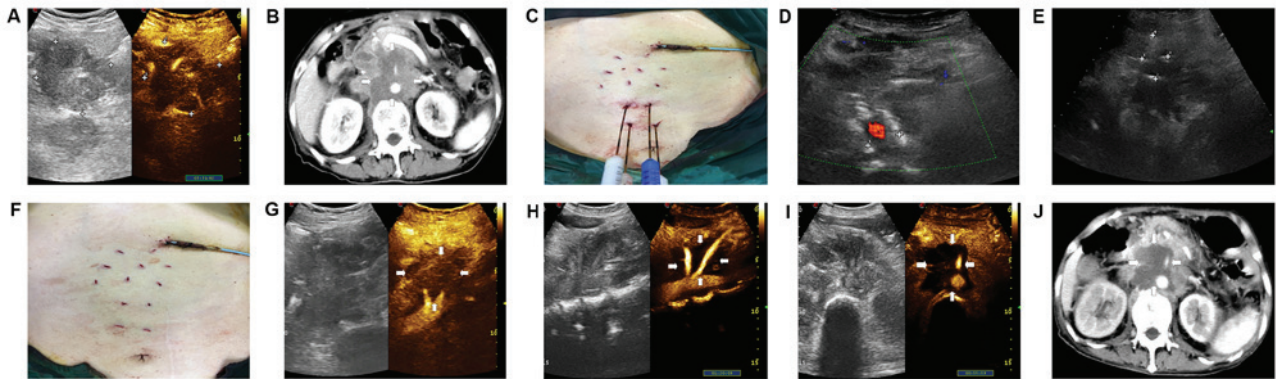


Figure 1. A 60-year-old female patient with metastatic retroperitoneal tumors was treated with IRE. (A) Pre-operative CEUS, revealing irregular arterial hyper vascularization of 6.36x6.30 cm. (B) Pre-operative CT scan revealing a tumor of 6.1x4.3 cm in size encircling the abdominal aorta. (C) The patient was placed in the supine position and US-guided electrodes were inserted parallel. (D) Grayscale sagittal US image of the retroperitoneum generated using color-flow Doppler imaging, demonstrating that the two electrodes parallelly encircled the tumor mass surrounding the abdominal aorta. (E) US-guided percutaneous IRE ablation, revealing four needles parallelly puncturing the targeted tissue. The distance between these needles was 1.85-2.13 cm. (F) Image of the post-operative abdomen revealing the needle tract. (G) Immediate post-operative image indicating the tumors with slight enhancement on CEUS. (H) CEUS 20 days after IRE revealing regular arterial hyper vascularization adjacent to the hypoechoic area, corresponding to the immediate post-operative image. (I) CEUS at 3 weeks following IRE treatment, indicating a clearly demarcated dark area without perfused tissue. (J) 6 weeks following IRE, the portal-phase axial CT image revealed tumor complete necrosis of 5.4x5.2 cm in size (vs. an initial size of 6.1x4.3x4.3 cm³), without contrast enhancement around the abdominal aorta. CE, contrast-enhanced; US, ultrasound; CT, computed tomography; IRE, irreversible electroporation.

pain was immediately detectable for the 3 cases and minor bleeding was instantly reported in case 1 and 3, which did not receive any treatment. Compared with the VAS score recorded on admission to the hospital, the patients exhibited effective pain relief (mean score of 5.8 decreased to 2.2) at the last month of follow-up. Furthermore, when comparing QoL at the baseline, QoL assessment revealed that global health status improved and pain score decreased during the last follow-up (Table III). However, case 2 died within 2 months and case 3 within 11 months due to liver metastases of the primary tumor.

Systematic review. In addition, to substantiate the results of the present study, a systematic literature search was performed in the PubMed, Embase, Web of Science, Scopus and Cochrane Library databases for studies published until September 21st, 2017, using the following predefined search terms: ‘Irreversible electroporation’, ‘retroperitoneal’ and ‘cancer’. Of 38 relevant studies selected based on the title and abstracts screened, the full-text version of 4 studies was finally reviewed. These studies comprised 1 study based on a porcine model (16) and 3 studies on human patients (17-19). Table IV provides the basic characteristics of the included studies. A total of 12 patients with 12 tumors underwent IRE treatment using 2-3 electrodes (15-17). The mean age of the patients ranged from 45.9 to 74 years. During the 1-2-month follow-up period, minor complications, including anastomotic leak, wound infection and adverse effects including pain were observed.

Discussion

At present, insufficient evidence is available to determine the optimal management of patients with retroperitoneal cancer, and this pathology continues to present a therapeutic challenge due to medical issues and anatomical challenges. Meng *et al* (20) reported a mean OS of 5.03 months using gemcitabine for the treatment of locally advanced and metastatic pancreatic adenocarcinoma. Huachansu, an injectable form of chansu, is a sterilized hot water extract of dried toad skin and when combined with gemcitabine, may not improve the prognosis of patients with locally advanced metastatic pancreatic cancer (20).

IRE was previously performed for PVTT (13). It was determined that **end-stage metastatic retroperitoneal malignant tumors** were located at risky sites (12,13), including vital structures and large blood vessels. In the present case series, the three patients were palliatively treated using IRE, with favorable results, including a mean OS of >6 months. During the follow-up, minor side effects that did not receive any treatment, including pain and bleeding from needle wounds, were recorded. The patients exhibited an improved prognosis compared with that at baseline, and the post-operative QoL of all three cases was improved in the functional and symptom dimensions, as well as on the global health status scale of the EORTC QLQ-C30. However, two cases succumbed to mortality due to liver metastases from the primary tumor. Over the course of the last few decades, numerous studies have suggested that

Table II. Procedural specifications for each patient.

Patient	Ablation area (cm)		Last follow-up	Number of needles	Type of needle (gauge)	Voltage (V)	Number of pulses	Mutual distance of needles (cm)	Time of survival the (months)	Complications	Outcome	CEA (ng/ml)		AFP (ng/ml)		CA19-9 (U/ml)	
	Initial											Pre-IRE	Post-IRE	Pre-IRE	Post-IRE	Pre-IRE	Post-IRE
1	CT:6.1x4.3; CEUS:6.3x4.9		CT:5.4x5.2	4	19	2,550-3,000	80-90	1.6; 1.8; 2.0; 2.0	5	Mild pain, minor bleeding	Alive	1.0	0.9	1.4	1.3	7.1	10.8
2	CT:2.8x2.0		CT:2.0x1.5	3	19	2,400-3,000	80	1.6; 2.0; 1.5	2	Mild pain	Deceased	64.8	2,639.1	2.6	2.7	9.8	69.0
3	CT:2.8x2.3		MR:1.8x1.4;	3	19	2,800-3,000	70-80	2.0; 2.0; 2.0	11	Mild pain, minor bleeding	Deceased	4.3	4.8	2.4	2.2	>12,000	1,374.1

G, gauge; CT, computed tomography; MR, magnetic resonance; IRE, irreversible electroporation; CEA, carcinoembryonic antigen; AFP, alpha fetoprotein; CA19-9, carbohydrate antigen 19-9.

Table III. Change in quality of life (before IRE-3 months after IRE).

EORTC Scale	Difference in quality of life	
	Before IRE-3 months after IRE	Before IRE-last month of follow-up
Global health status	-12.6 ^a	-15.2 ^a
Functional scales		
Physical functioning	-2.8	-3.2
Role functioning	-1.3	2.6
Emotional functioning	-1.2	-7.8
Cognitive functioning	3.1	5.2
Social functioning	-7.9	-6.2
Symptom scales		
Fatigue	1.3	5.2
Nausea/vomiting	2.3	3.8
Pain	16.2 ^b	15.6 ^a
Dyspnea	-0.8	-2.9
Insomnia	-4.9	-3.9
Appetite loss	2.5	4.3
Constipation	7.3	6.1
Diarrhea	4.9	6.8
Financial problems	-3.6	-3.1

^aP<0.05 vs. the baseline.

IRE is effective in prolonging the survival of patients with malignant tumors. In a multicenter prospective trial including 200 patients with locally advanced pancreatic cancer treated with IRE, the median OS was 24.9 months and only 6 patients (3%) presented with local recurrence during a median follow-up of 29 months (21). Narayanan *et al* (22) reported a similar median OS of 27.0 months. This study reported on a case with unresectable retroperitoneal malignant fibrous sarcoma treated with CT-guided IRE using 2 electrodes. The CT scan indicated that a lesion decreased in size from 7.3x7.0x7.5 to 5.1x4.0x5.2 cm, without any obvious enhancement at 2 months post-operatively (19). In a retrospective study, Underhill *et al* (17) identified that patients undergoing supplemental IRE following surgical resection for retroperitoneal neoplasms had developed few complications. Therefore, IRE has emerged as an important supplementary method to accompany surgical resection for the treatment of retroperitoneal tumors.

The clinical challenge is enhanced if the retroperitoneal tumors have a large size and then usually invade proximal organs. A previous study reported that IRE may induce cellular apoptosis rather than protein denaturation and necrosis, and the tumor cells may be removed via cellular phagocytosis; subsequently, the ablation area was rapidly replaced by normal cells, which was beneficial in terms of functional recovery (23). During these procedures, structures including blood vessels, nervous tissue or bile ducts remain intact. In terms of safety, selection of appropriate probe placement, needle exposure length and pulse length is vital. A preliminary study using a porcine model indicated that probe exposure of ~2.5 cm in the liver and ~1.5 cm in the pancreas with an inter-electrode distance of 1.5-2.3 cm were acceptable (16). The accuracy of the IRE procedure is high

Table IV. Summary of IRE therapy of end-stage retroperitoneal tumors reported in the literature.

Author, year	Study period	Design	Country	Subjects	Population characteristics	Tumor size (cm)	Treatment methods	Number of electrodes	Number of patients	Males/ females	Age (years)	Follow-up interval (months)	Complications (Refs.)
Dunki <i>et al.</i> , 2014	NA	Retrospective	United States	Porcine	6 retroperitoneal tissues	NA	US-guided IRE	4	6	NA	NA	NA	(16)
Underhill <i>et al.</i> , 2016	December 2013 to April 2015	Retrospective	United States	Human	2 sarcoma; 1 carcinosid; 1 giant cell tumor; 1 ovarian; 1 triton tumor; 1 adenocarcinoma	NA	NA	3	7	1/6	45.9±13.6	1	1 anastomotic leak; 1 wound infection
Kambakamba <i>et al.</i> , 2016	September 2012 to December 2015	Prospective	Switzerland	Human	4 retroperitoneal tumors	NA	US-guided IRE	NA	4	NA	NA	NA	(18)
Qin <i>et al.</i> , 2017	March 2016	Retrospective	China	Human	1 unresectable retroperitoneal malignant fibroussarcoma	7.3x7.0x7.5	CT and US-guided IRE	2	1	0/1	74	2	NA

NA, not available; US, ultrasonography; CT, computed tomography; IRE, irreversible electroporation; US, ultrasound.

due to real-time navigation and monitoring associated with US. Furthermore, in the present study, ablated residual lesions were easily located and rapidly identified by using CEUS. The treatment efficacy of the procedure was not affected by heat-sink effects, since IRE, as a method, is predominantly based on electrical pulse breakdown of the cell membrane (24). During the process, irreversible electroporation does not produce heat, and therefore, it is not affected by additional external temperature. Compared with thermal ablation, there are clear boundaries, which do not cause damage to adjacent normal tissue. In addition, IRE was reported to cause robust immunogenic effects, which led to increased serum interleukin-6 levels higher than those achieved through radiofrequency ablation (25). Therefore, the results of the present study highlighted that IRE may be beneficial for cases of locally advanced metastatic tumors located in proximity to major vessels.

There were certain limitations associated with the present study. First, the study had a small sample size and two of the patients were followed up until death. Furthermore, needle tract seeding is possible, but not controlled, during IRE ablation. In a previously published study, this was demonstrated to occur in 26% of the treated tumors under CT guidance in 29 patients with lesions located adjacent to major portal or hepatic veins, bile duct structures or the intestines (26). Furthermore, in the present study, confounding factors should be considered, and individual heterogeneous factors, including individual histories of therapeutic treatments and lifestyle changes, may have exaggerated the palliative treatment effects determined. Furthermore, long-term adjuvant effects may lead to selection bias (27-29). **However, taking all of these limitations into consideration, the results of the present study still suggest that patients undergoing IRE therapy were at a lower risk of complications of massive hemorrhage in at risk regions, compared with those receiving other treatment options, which may provide a novel line of investigation in the future.**

In conclusion, the present study preliminarily identified technically effective, percutaneous IRE procedures utilizing US guidance for unresectable metastatic retroperitoneal tumors. During short-term follow-up, this may assist in providing favorable palliative care in terms of improving prognosis. However, additional large-scale pairwise comparisons with control groups and long-term studies are required to substantiate these results regarding IRE therapy.

Acknowledgements

Not applicable.

Funding

The design of the present study, data collection and analysis were supported by the Natural Science Foundation of Zhejiang Province (grant no. LY16H180004), the National S&T Major Project of China (grant nos. SQ2018ZX100301 and 2018ZX10301201), the Key Research Development Program of Zhejiang province (grant no. 2018C03018), the Key Science and Technology Program of Zhejiang province (grant no. WKJ-ZJ-1923), the Science and Technology Project of Zhejiang Province (grant no. 2012C23094) and the Foundation of Zhejiang Health Committee (grant nos. 2014KYA086 and 2016DTB003).

Availability of data and materials

All data generated during the present study are included in this published article.

Authors' contributions

Study conception and design: TJ; acquisition of data: TJ, QZ, XB, GT, XC, LW; analysis and interpretation of data: QZ, GT; drafting of the manuscript: TJ, QZ; critical revision of the manuscript for important intellectual content: TJ; statistical analysis: GT; obtainment of funding: TJ, QZ, XB, XC, LW; technical or material support: TJ; study supervision: TJ. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This prospective study was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University (Zhejiang, China). Informed consent for study participation were obtained.

Consent for publication

Consent for publication of the data and images (CEUS images, CT or MRI images, laboratory findings, age, sex) was obtained from all participating patients.

Competing interests

The authors declare that they have no competing interests.

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