

Irreversible Electroporation in Patients With Liver Tumours: Treated-area Patterns With Contrast-enhanced Ultrasound

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Research

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Abstract

Background: To describe the contrast-enhanced ultrasound imaging findings of liver tumours after percutaneous ablation by irreversible electroporation (IRE).

Methods: A prospective study of 21 malignant liver tumours (19 primary hepatic tumours and 2 hepatic metastases) treated by IRE ablation was performed. The ablation zones were evaluated by two examiners in a consensus reading performed immediately, 1 day, and 1 month after IRE ablation. The gold standard method with which the effectiveness of the treatment at 1 month is compared, is MRI.

Results: Immediately after IRE ablation and up to 1 month later, the ablation zones gradually changed from hypo-echogenicity to hyper-echogenicity on conventional ultrasound, and becomes non-enhancement on contrast-enhanced ultrasound (CEUS). There was substantial agreement ($\kappa = 0.77$, $p < 0.05$) between the results obtained with CEUS and those obtained with MRI 1 month after IRE ablation.

Conclusions: We conclude that CEUS could be an effective tool for assessing post-irreversible electroporation ablation changes after 1 month. CEUS enables the depiction of tumour vascularity in real time and provides an easy, repeatable way.

Introduction

Liver cancer is the fourth leading cause of cancer-related death worldwide[1]. The majority of patients are ineligible for curative surgical resection at the time of diagnosis. Hepatic tumour ablation, such as radiofrequency ablation (RFA), microwave ablation (MWA) or cryoablation, is potentially curative in select patients. However, the efficacy of ablation is limited by the size, number and location of the lesion. As a result, the effectiveness and safety of these techniques are limited for lesions adjacent to vital structures, such as bile ducts and gastrointestinal tract. For patients with such lesions, Irreversible electroporation (IRE) is considered an alternative treatment. Compared to other local ablation techniques, IRE is a promising technique. IRE is a non-thermal ablation method that induces tumour necrosis by inducing apoptosis and cell death. IRE treatment generates electric pulses that alter the cell membrane's electrical potential, leading to small nanopores and contributing to apoptosis[2]. The efficacy of IRE is not affected by the so-called heat-sink effect. Thus, IRE is now being applied experimentally and clinically in a wide range of tissues[3, 4].

Contrast-enhanced ultrasound (CEUS) allows for the continuous real-time imaging of contrast enhancement during arterial, portal and late phases. Thus, CEUS can dynamically assess blood flow and tissue perfusion. The feasibility of evaluating ablation zones with CEUS has been reported in several preliminary animal and clinical studies[5, 6].

CEUS is useful in assessing tumour response and evaluating the characteristics of the ablation zone. Only limited data exist about the CEUS imaging characteristics of the ablation zones after IRE ablation in humans. Cell death follows cellular membrane electroporation and histomorphologic changes in the

ablation zone after IRE ablation, so the assessment is different from other ablation methods. Familiarity with post-IRE imaging interpretation is of considerable importance in determining ablation success and in detecting recurrence.

The present study focuses on specific imaging characteristics of hepatic tumours obtained by CEUS with an intravenous contrast agent immediately, 1 day, and 1 month after US guidance percutaneous IRE ablation. Magnetic resonance imaging (MRI) was performed 1 month after ablation to investigate the therapeutic efficacy.

Materials And Methods

The study was conducted with the approval of the institutional review board. The research was conducted according to the Declaration of Helsinki. Patients gave written informed consent for imaging data analysis before treatment with CEUS.

Patients

A single-centre prospective study was conducted to evaluate and describe the CEUS imaging findings after the percutaneous IRE ablation of malignant liver tumours. From May 2016 to September 2018, 19 patients were treated with IRE at our institution. All patients met the following inclusion criteria: unresectable tumours; tumours unsuitable for thermal ablation because of close proximity to major veins; and biliary and venous systems of the liver that would cause heat-sink effects or collateral damage; these definitions were similar to the difficult location definitions set by Wei Yang et al[7]. History of hemihepatectomy was not seen as a contraindication for the present study. Thermal ablation or catheter chemical ablation did not have contraindication. The exclusion criteria were a history of epilepsy or ventricular arrhythmias, an implanted stimulation device, or a metal biliary stent. The details are presented in Table 1.

IRE procedures

All procedures were performed under general anaesthesia using the NanoKnife IRE system (Angiodynamics Inc., Latham, NY, USA). A doctor who had more than 5 years of experience in US-guided interventional procedures and IRE experienced performed all IRE procedures. The IRE generator was programmed following the manufacturer's instructions. Based on the pre-IRE image, the NanoKnife System calculated the related parameters, such as the number of electrodes, the intended area of the ablation zone, the distance between the probes, the number of probes, and the length of the active electrode tip. The ablation procedures were based on procedures described in previous studies. The required number of needles was chosen according to the nodule size. Energy deposition was applied at 2250–3000 V (pulse length, 70-90; pulse repetition, 90-270). Ablations were performed under US guidance.

Imaging procedure

US scanning was performed to assess the location, size and margin of the tumours before ablation. The first image acquisition was after the intervention (immediately after the ablation procedure), and follow-up imaging was conducted to assess the evolution of the ablation zone.

CEUS was used to evaluate the effect of IRE. All CEUS was performed using a Mylab 90 (Esaote, Italy) or an ultrasound scanner equipped with a 3-5 MHz convex transducer. The US contrast agent used was sulfur hexafluoride-filled microbubbles (SonoVue; Bracco SpA, Milan, Italy). An injection of 2.4 ml of the SonoVue agent was followed by a flush with 5 mL 0.9% normal saline solution. As soon as the US contrast agent was administered, dual B-mode images were acquired. Simultaneously, a timer was started. According to previous studies, we defined four phases: the arterial phase (15–45 seconds), the portal venous phase (60–90 seconds), the late venous phase (90–120 seconds), and the late phase (approx. 3–5 minutes). The ablation zone was observed continuously for 5 minutes. The entire examination was digitally recorded and stored on the hard disk of the US scanner for subsequent analysis.

All CEUS studies were performed before ablation and immediately, 1 day, and 1 month after tumour ablation to assess the characteristics of the ablation zone. MRI was performed 1 month after ablation to investigate the therapeutic efficacy.

CEUS analysis

One operator with 10 years of experience with CEUS, who was not blinded to the treatment results, performed all the CEUS imaging during the study. Data were analysed and defined by the consensus of two doctors. In the case of different opinions, the reviewers jointly re-assessed the saved images and then came to a consensus. The presence or absence of tumour enhancement on the immediate CEUS image was recorded. The imaging features included the following: echogenicity (hypo/iso/hyper), boundary (clear/unclear), homogeneity (homogeneous/ heterogeneous), enhancement level (non/hypo/iso/ hyper) and the enhancement pattern of the ablation zones.

Statistical methods

A p value of 0.05 was considered statistically significant. Descriptive statistics were used to present the results as absolute numbers (n), mean and standard deviation (SD), or percentages. The results of CEUS in assessment of therapeutic efficacy were compared with those of MRI by Cohen's κ values[8]. In addition, sensitivity, specificity, and positive and negative predictive values were calculated. All statistical analyses were performed using SPSS software (version 23.0, SPSS).

Results

The patient group included 19 patients (21 ablation lesions) aged 31–86 years (mean 59.9 ± 13.3 years). The identity of the tumour could be histologically confirmed in 12 lesions, while the tumours of 9 lesions were diagnosed based on imaging. The histologic findings showed hepatocellular carcinoma (HCC) in 10 lesions and metastases of gastrointestinal tumours in 2 lesions.

Immediate post-procedural assessment

On conventional US performed immediately after IRE treatment, the ablated zones were either hypo-echoic (15/21, 71%) or iso-echoic (6/21, 29%). The boundaries were unclear, and the echogenicity of the boundary was heterogeneous. On CEUS, complete non-enhancement was observed in 3 of the 21 ablated zones (14%), and the boundary of the non-enhanced area was clearly outlined (Fig.1A). Enhancement of the ablation zone was observed in 18 of the 21 ablation zones (86%): 1 ablation zone exhibited slight hyper-enhancement in the early arterial phases that was heterogeneous and washed out in the late arterial phase; and 7 ablation zones exhibited hypo-enhancement in the arterial phase. The boundaries between the enhanced and non-enhanced areas were clear.

One-day follow-up

B-mode imaging of the ablated zones performed 1 day after IRE treatment showed hyper-echoic foci (11/21, 90%), iso-echoic foci (4/21, 19%), hypo-echoic foci (6/21, 10%). The boundaries were clear. On CEUS, complete non-enhancement was documented in 13 of the 21 ablated zones (62%) (Fig.1B). Enhancement of the ablation zone was observed in 8 of the 21 ablation zones (38%). 1 ablation zone showed slightly hyper-enhancement in the early arterial phase and washed out in the late arterial phase, while the other showed hypo-enhancement in the arterial phase. During the portal venous and late phases, the enhanced foci showed hypo-enhancement.

One-month and later follow-up examinations

On conventional US performed 1 month after IRE treatment, 19 ablated zones showed hyper-echoic foci with clear boundaries (19/21, 90%), and 2 ablated zones were still hypo-echoic (2/21, 10%). Complete non-enhancement was documented in 16 of the 21 ablation zones (76%). 1 ablation zone showed slight hyper-enhancement in the early arterial phase that washed out in the late arterial phase (Fig.1C and D). Another 4 zones showed quick wash-in and quick wash-out patterns on CEUS. No evidence of peripheral contrast enhancement could be found during the arterial phase or during the portal venous phase.

1 month after IRE ablation no evidence of recurrence was found by MRI in 14 ablation zones (14/21 67%), while recurrence was identified in 7 zones (7/21, 33%). There was substantial agreement ($\kappa = 0.77$; $p < 0.05$) between the results obtained with CEUS and those obtained with MRI. With MRI as the gold

standard, the sensitivity, specificity, positive predictive value and negative predictive value of CEUS were 71.4%, 100%, 100%, and 87.5% respectively.

Discussion

IRE has been gaining increasing interest because the non-thermal properties of this method[9] permit the ability to ablate tumours adjacent to vital structures[10]. Previous studies have demonstrated that US findings after IRE application in the liver evolve sequentially over time, from seconds to at least hours[3, 11, 12]. Our results show the evolution of post-IRE ablation patterns over the clinically relevant time period.

As noted in an animal-based experiment, a strong association exists between conventional US and histopathology[5]. In our study, on conventional US, IRE ablation zones of most patients appear as a developing hypo-echoic area that demonstrates an increasingly hyperechogenic ablation zone starting one day after the procedure.

Our US findings after IRE ablation evolved sequentially over time. The findings showed good correlation with those in previous studies[5, 13]. In the study, specimens obtained immediately after ablation showed that the widened oedematous sinusoidal spaces were filled mainly by fluid, and very little haemorrhagic infiltrate was observed. As time passed, the haemorrhagic infiltrate became more dominant, which might be due to the widened fluid-filled sinusoids at the beginning of the procedure. The authors attribute this hyper-echogenicity to red blood cell accumulation over time. The degree of red blood cell infiltrate was qualitatively associated with echogenicity. Lee et al also reported the US findings of 55 ablation zones immediately and 1 day after ablation and showed that the immediate hypo-echogenicity transformed to total hyper-echogenicity in the treatment zone 1 day after the procedure[13]. Appelbaum et al speculated that red blood cells progressively infiltrated into deep regions of the ablated zone so that the hyper-echoic rim on US at 90–120 minutes transitioned to complete hyper-echogenicity within 24 hours[5]. There is concordance between our findings and those of previous studies that described the characteristics of images obtained after ablation.

In CEUS, most of the ablated zones showed hypo-enhancement immediately after IRE. Chung et al found the following zones with different enhancement patterns on CT perfusion images in normal porcine liver: an inner non-enhanced zone; a middle well-defined progressive internal enhancement zone, and an outer ill-defined arterial enhancement zone. On histopathology, the inner and middle zones accounted for the extent of cell death[14]. The histological examination suggested that the apoptotic process was involved with complete cell death in the pathophysiology of cell death caused by IRE.

The CEUS images of the IRE ablation zones in normal liver tissue differed from the images of liver tumours treated with IRE. Our study also found that 10 ablated zones appeared with hypo-enhancement immediately after IRE but became non-enhanced one day after IRE, which is due to diverse reasons. Lee et al attributed the focal hyper-attenuation to the release of contrast medium into an ablation defect caused by an IRE-induced microvasculature leakage within the defect zone[15]. Because the zones

became non-enhanced on the follow-up CEUS images, the hyper-enhancement was probably caused by extraluminal contrast material. Another opinion is about the differences in contrast agent concentrations. Guo et al suggested that nanometre-scale pores in the tissue cell membrane caused contrast agent to accumulate in the IRE zone and allowed for the contrast agent to be internalized into the intracellular environment rather than remaining extracellularly[16], which makes evaluating whether the viable tissue is a residual tumour difficult after ablation. Therefore, follow-up CEUS is necessary to assess the viable portion. However, unlike normal regeneration activity, the residual tumour can continue growing and results in a newly enhanced region.

Most of the non-enhancement pattern on CEUS 1 month after ablation is a sign of effective IRE treatment, besides there are still false negatives. Such non-enhancement patterns are different to those of the thermal ablation zone after complete ablation. Previous histologic examinations of the ablation zones showed cell death caused by apoptosis[13]. Cell death is seen with full preservation of the peri-ablative zone structures, such as blood vessels and bile ducts.

In the present study, only one patient continuously showed an enhanced ablation zone immediately and on follow-up CEUS images. The ablation zone became hyper-enhanced during the early arterial phase, slightly wash out in the late arterial phase and presented as hypo-enhanced in the late phase. To elucidate the reason for this pattern, the patient underwent a liver biopsy one month after IRE. The physiology results showed small patchy necrosis, inflammatory fibrosis tissue hyperplasia and foam cell aggregation. The result ruled out the possibility of recurrence. A reasonable explanation for this finding is hyperplasia of the inflammatory tissue. This result can cause confusion in clinical practice. Therefore, sequential follow-up is essential. Further investigation is needed to study the histological and cytological mechanisms underlying this process.

In our study, only 2 ablation zones still appeared as hypo-echoic areas, and both zones were in the same patient. After 1 month of follow-up, recurrence occurred near one of the zones treated with IRE ablation. In a previous multi-institutional review from 2009 through 2012, 31% of the patients had recurrence during a median follow-up of 18 months. Of the total 31% of patients with recurrence, 10.7% had local recurrences at the ablated site[4]. Previous work attributed these recurrences to electric field sinks that resulted from the heterogeneous structure and conductivity of the liver[17]. Later work from the authors indicated that the IRE-treated extracellular matrix (ECM) provides an environment for the activation and differentiation of progenitor cells[18], but the mechanism is not completely understood; in contrast, some studies have suggested that abnormal ECM affects cancer progression by directly promoting cellular transformation and metastasis and facilitating tumour-associated angiogenesis and inflammation, leading to the generation of a tumorigenic microenvironment[19]. The role of the IRE-spared tumour matrix in follow-up recurrences requires further research. Another theory is about the size of the treated liver tumours. Niessen et al found that large tumour volumes (> 5 cm³) portended early local recurrence[20].

In our study, the intra-hepatic blood vessels and bile duct remained almost completely intact after IRE. The hepatohilar bile duct of only one patient showed an unclear contrast agent pattern immediately after

IRE. After 15 minutes, the phenomenon disappeared. However, a consensus on the mechanism of IRE has not yet been realized. This effect could be due to the high proportion of collagenous connective tissue. A further hypothesis is that the gap junctions, which are present in large numbers in the muscularis propria of the blood vessels and the bile duct walls, could act as a conductive structure for the electrical currents; thus, the current can be passed from cell to cell without leading to destruction of the cell membrane[14]. Whether the effects of IRE are caused by a thermal or non-thermal mechanism remains unclear.

This study has several limitations. First, our study had a small sample size for the span of more than 2 years. Another limitation was that almost all of the patients we included in our study group previously underwent right or left liver resection and thermal ablation or catheter chemical ablation. The origin of the ablation zones was heterogeneous. However, this circumstance mirrors the status of our clinical treatment strategies. IRE as a novel technique and was not the first choice in our hospital. Only patients who experienced tumour recurrence after liver resection and thermal ablation were considered for IRE. Because of this situation, assessing the images of the ablated zones during subsequent follow-up imaging examinations is important. We conclude that CEUS may be a useful tool for assessing the characteristics of post-IRE ablation changes. And it is an effective way to evaluate the therapeutic efficacy 1 month after ablation. Further studies are needed to evaluate more patients to precisely depict the appearance of hepatic zones on CEUS.

Abbreviations

IRE, irreversible electroporation

CEUS, contrast-enhanced ultrasound

US, ultrasound

MRI, magnetic resonance imaging

RFA, radiofrequency ablation

MWA, microwave ablation

ECM, extracellular matrix

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Linyu Zhou participated in the study design and drafted the manuscript. Shanyu Yin and Weilu Chai analyzed and interpreted the data. Qiyu Zhao and Guo Tian collected the clinical data and performed follow-up. Linyu Zhou and Tianan Jiang participated in the study design, carried out the IRE ablation and gave final approval of the version to be published. All of the authors are in agreement with the manuscript's content.

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: Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

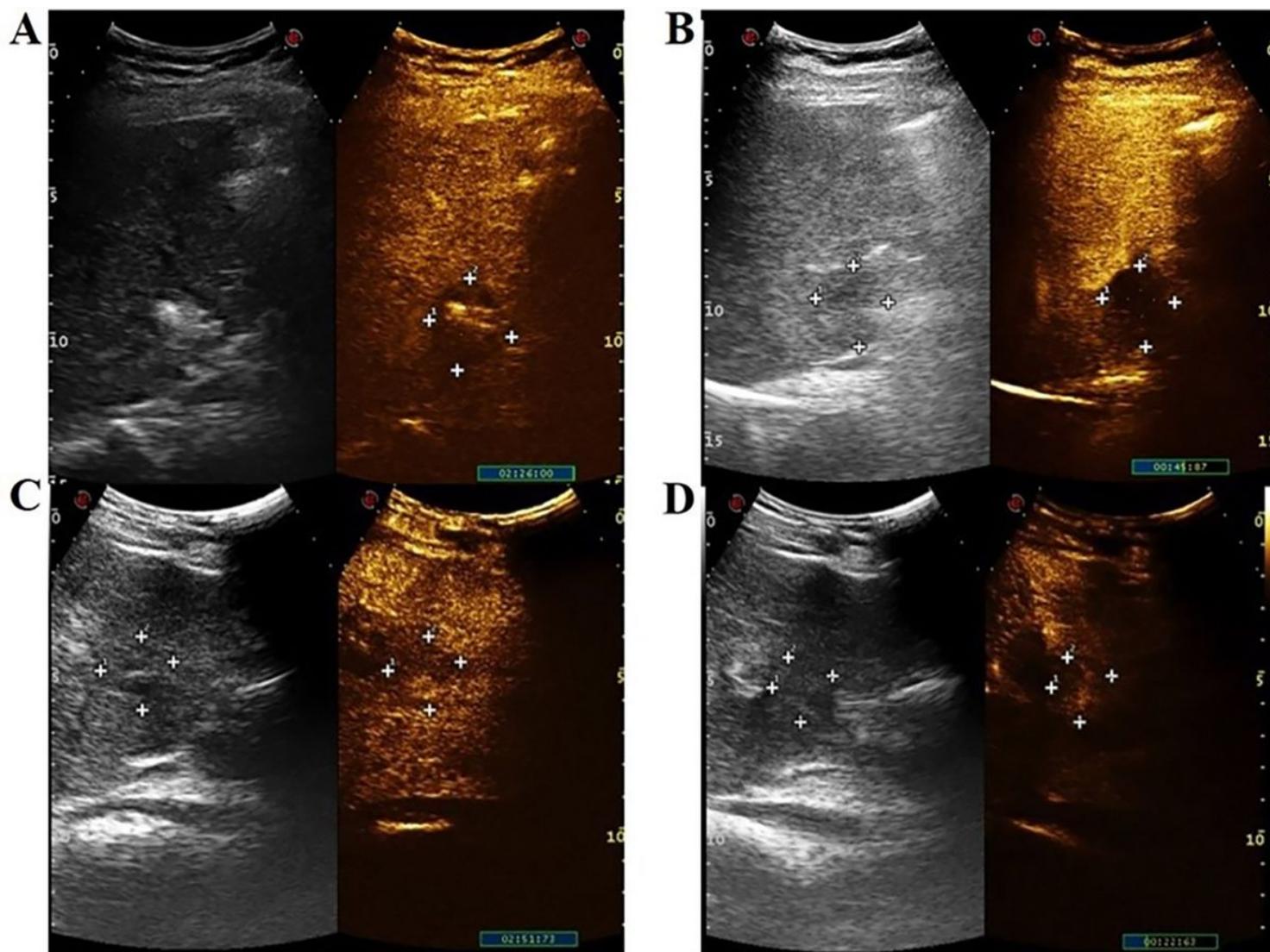


Figure 1

A Immediate post-IRE CEUS demonstrated complete non-enhancement in the ablative zone. The boundary of the non-enhanced area was clearly outlined. B A follow-up scan one day after IRE also showed complete non-enhancement. C A 46-year-old man presented with hepatic carcinoma. One month after IRE ablation, a hyper-enhanced lesion was observed in the early arterial phase. D The hyper-enhanced foci washed out during the late arterial phase.