Evaluation of thermal injury to liver, pancreas and kidney during irreversible electroporation in an in vivo experimental model

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Background: Irreversible electroporation (IRE) is a new technique for tumour cell ablation that is reported to involve non-thermal-based energy using high voltage at short microsecond pulse lengths. In vivo assessment of the thermal energy generated during IRE has not been performed. Thermal injury can be predicted using a critical temperature model. The aim of this study was to assess the potential for thermal injury during IRE in an in vivo porcine model.

Methods: In vivo continuous temperature assessments of 86 different IRE procedures were performed on porcine liver, pancreas, kidney and retroperitoneal tissue. Tissue temperature was measured continuously throughout IRE by means of two thermocouples placed at set distances (0.5 cm or less, and 1 cm) from the IRE probes within the treatment field. Thermal injury was defined as a tissue temperature of 54°C lasting at least 10 s. Tissue type, pulse length, probe exposure length, number of probes and retreatment were evaluated for associations with thermal injury. In addition, IRE ablation was performed with metal clips or metal stents within the ablation field to determine their effect on thermal injury.

Results: An increase in tissue temperature above the animals' baseline temperature (median 36.0°C) was generated during IRE in all tissues studied, with the greatest increase found at the thermocouple placed within 0.5 cm in all instances. On univariable and multivariable analysis, ablation in kidney tissue (maximum temperature 62.8°C), ablation with a pulse length setting of 100 μs (maximum 54.7°C), probe exposure of at least 3 cm (maximum 52.0°C) and ablation with metal within the ablation field (maximum 65.3°C) were all associated with a significant risk of thermal injury.

Conclusion: IRE can generate thermal energy, and even thermal injury, based on tissue type, probe exposure lengths, pulse lengths and proximity to metal. Awareness of probe placement regarding proximity to critical structures as well as probe exposure length and pulse length are necessary to ensure safety and prevent thermal injury. A probe exposure of 2.5 cm or less for liver IRE, and 1.5 cm or less for pancreas, with maximum pulse length of 90 μs will result in safe and non-thermal energy delivery with spacing of 1.5–2.3 cm between probe pairs.

Surgical relevance

In this experimental investigation, irreversible electroporation (IRE) is studied in a porcine model to establish set device and probe guidelines, so that any type of thermal damage can be avoided. This IRE device is currently in use in the treatment of locally advanced soft tissue tumours, and the issue of potential thermal damage is therefore of clinical interest. This is further underlined by the fact that there is an underestimation among some IRE end-users that IRE energy delivery is non-thermal regardless of the circumstances. It is shown here that IRE can generate thermal energy, and even thermal injury, based on tissue type, probe exposure lengths, pulse lengths and proximity to metal (clips and stents).

Introduction

Tumour ablation using a thermal-based coagulative necrosis method has become an established technique for treatment of unresectable malignancies when indicated1,2. The most common methods of tumour ablation involve thermal-based destruction of the lesion by radiofrequency...
or microwave energy delivery systems, which lead to complete cellular, protein and collagen destruction\(^1\). Although these systems have demonstrated safety and efficacy, there are contraindications that limit their use. The primary contraindication to thermal ablation is tumor location near vital heat-sensitive structures such as large vessels, ducts or nerves, which, if injured, would result in morbidity or death\(^1\). In addition, the heat-sink effect created by large vessels limits the efficacy of thermal destruction of adjacent malignant tissue\(^5,6\).

Irreversible electroporation (IRE) is an alternative ablative technology that uses a different method of cellular destruction, reported as a non-thermal cell membrane electroporation (causes increased porosity). IRE uses high voltage delivered at very short pulse lengths to alter permanently the permeability of cell membranes, which results in delayed cell death by apoptosis\(^7,8\). In addition to cellular destruction without the use of thermal energy, IRE spares the underlying connective tissue scaffolding, and so stricture of vital structures can be avoided\(^8\). These characteristics have made IRE an ideal technology for the ablation of tumours in locations where thermal ablation is contraindicated\(^11\).

Although there is great potential for IRE as a non-thermal method of tumour ablation, an \textit{in vivo} assessment of thermal energy generated during IRE is needed to ensure safe clinical practice. Early clinical application in hepatopancreaticobiliary surgery has raised the question of thermal injury during general use\(^12\) and, more specifically, in the setting of metal stents. Metal stents are frequently placed before surgery in patients with obstructive jaundice owing to extrahepatic cholangiocarcinoma or pancreatic adenocarcinoma. The safety of performing IRE when there is metal within the ablative field is unknown. Given the conductivity of metal, a precise evaluation of the ability to deliver IRE safety is needed.

The aim of this study was to assess the potential for thermal injury during IRE \textit{in vivo} in a porcine model, and to evaluate the potential for thermal injury in the presence of metal stents.

**Methods**

Living, anaesthetized pigs (\(n = 30\)) were used for all IRE tissue ablations. The study was approved by the University of Louisville Institutional Animal Care and Use Committee (IACUC), and the care of all study animals was in accordance with IACUC guidelines and also the Animal Research: Reporting \textit{In Vivo} Experiments (ARRIVE) guidelines. A total of 86 ablation trials were carried out: 30 in liver, 30 in pancreas, 20 in kidney and six in the retroperitoneum.

**Irreversible electroporation procedure**

All ablations were performed using the NanoKnife\textsuperscript{®} Tissue Ablation System (Angiodynamics, Latham, New York, USA) with cardiac gating timed to the R wave of each cardiac cycle. Standard 19-G monopolar probes were used for all ablations. For all procedures, probe placement was performed with ultrasound guidance (Flex Focus 800; BK Medical, Herlev, Denmark). Each temperature evaluation trial was performed with a series of three electroporations (a total of 90 pulses delivered). A primary electroporation (90 pulses) was carried out, followed by two repeat electroporations with a pause of 45–60 s between each. The probes were not repositioned before the two repeat electroporations. Tissue temperature was measured continuously throughout each ablation by means of two electrically insulated UniBlate\textsuperscript{™} thermal monitoring devices (thermocouples) (Angiodynamics) placed at set distances from the IRE probes and located within the treatment field. Thermocouple 1 was placed 0.5 cm or less from probe 1; thermocouple 2 was placed 1.0 cm from probe 1 and probe 2. Ablation probes were fixed at a distance of 2 cm from one another in arrangements of either two or four probes. Four-probe arrays were always arranged in a box formation (Fig. 1), as this is the most common probe configuration used for clinical IRE.

Thermal injury or the critical temperature was assessed by tissue temperature alone, and was defined as a tissue temperature equal to or greater than 54°C for more than 10 s\(^{13–19}\). This definition was confirmed by the pathological finding of coagulative necrosis on haematoxylin and eosin staining; tissue temperatures below this threshold demonstrated only inflammation and electroporation changes, as described previously\(^8\). The peak temperature was defined as the maximum temperature achieved during any of the electroporation pulses.

Variables that were altered between ablation trials were: tissue type (liver, pancreas, kidney, retroperitoneal tissue), and, within each tissue type, pulse length (100 or 70 ps), probe exposure length (3, 2 or 1.5 cm) and number of probes (2 or 4). These parameters were evaluated in this porcine model, based on the reported clinical practice of IRE\(^{11,20–22}\). Thermocouple orientation was constant for all treatments and tissue temperatures for the two locations were measured separately (Fig. 1). The effect of retreatment with the probes in the same location on tissue temperature was also assessed. All ablations comprised 90 pulses with the voltage set at 3000 V, based on the default setting of the IRE device of 1500 V/cm, with all evaluations performed with probes spaced 2 cm apart.
Additional electroporation trials were carried out to assess the effect of the presence of metal within the zone of ablation on the tissue temperature. This was done by placing a 0.5-cm metal clip (Ethicon, Johnson and Johnson, Cincinnati, Ohio, USA) deep in the hepatic tissue and then treating the tissue surrounding the clip. Tissue temperature was recorded during energy delivery. Likewise, metal stents (WallFlex™ (fully covered), Boston Scientific, Marlborough, Massachusetts, USA; Zilver™, Cook Medical, Bloomington, Indiana, USA) were deployed within the hepatic bile ducts near the bifurcation. IRE ablation was then carried out using a four-probe array to bracket the stent within the ablation field. Again, tissue temperature was recorded during the ablation.

**Statistical analysis**

The $t$ test was used to analyse mean maximum temperature and mean change in temperature in ablation trials evaluating the effect of tissue type, number of probes, probe exposure length and presence of metal stents. A paired $t$
Fig. 2 Changes in temperature during single trials of three repeat ablations in the same location. The dotted line denotes the critical temperature of 54°C. 

a Kidney with 3-cm probe exposure and 100-μs pulse length; thermal injury observed.

b Pancreas with 1·5-cm probe exposure and 100-μs pulse length; no thermal injury observed.

c Liver with 3-cm probe exposure and 100-μs pulse length; thermal injury observed.

d Liver, using pulse length 100 μs (thermal injury) versus 70 μs (no thermal injury for probe exposure of less than 3·0 cm).

e Liver with probe exposure 3 cm (thermal injury) versus 2 cm (no thermal injury). The probe spacing was 2 cm (1500 V/cm, total 3000 V) in all these evaluations.
test was used to compare the mean maximum temperature and the mean change in temperature at each thermocouple location. ANOVA was used to assess the effect of retreatment on tissue temperature. χ² statistics were used to assess the frequency of thermal injury for each variable tested. Multivariable analysis of thermal injury was performed using linear regression modelling. Statistical analysis was done using SPSS® version 21.0 (IBM, Armonk, New York, USA).

**Results**

**Effect of tissue type**

Thermal data were obtained for IRE in porcine liver, kidney, pancreas and retroperitoneal tissue. Thermal energy, as indicated by an increase in tissue temperature over the subject's baseline temperature (median 36°C), was noted during IRE ablation for all tissue types, regardless of probe exposure length or pulse length (Fig. 2). The mean maximum temperature and mean thermal change were significantly higher for kidney than for all other tissue types (Table 1). Thermal injury occurred significantly more often in kidney than in other tissues (P = 0.016) (Table 2). Histological analysis confirmed the presence of coagulative necrosis in the electroporations that demonstrated thermal injury, as defined above (Fig. 3).

**Effect of probe location**

Tissue temperature was compared at two locations within the ablation zone. At no more than 0.5 cm from probe 1, the mean maximum temperature was 55.8°C. At 1.0 cm away from probe 1 and probe 2, the mean maximum temperature was 49.8°C (P < 0.001). The mean change in baseline temperature at 0.5 cm or less from probe 1 was 16.0°C, whereas that at 1 cm from probe 1 and 2 was 10.2°C (P < 0.001) (Table 1).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean maximum temperature (°C) ≤ 0.5-cm probe location</th>
<th>Mean maximum temperature (°C) 1.0-cm probe location</th>
<th>s.d.*</th>
<th>P†</th>
<th>Mean change in temperature (°C) ≤ 0.5-cm probe location</th>
<th>Mean change in temperature (°C) 1.0-cm probe location</th>
<th>s.d.*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney (n = 20)</td>
<td>62.8</td>
<td>57.4</td>
<td>10.0</td>
<td>&lt; 0.001</td>
<td>21.5</td>
<td>15.6</td>
<td>6.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Retroperitoneum (n = 6)</td>
<td>51.5</td>
<td>47.4</td>
<td>5.0</td>
<td>&lt; 0.001</td>
<td>12.3</td>
<td>9.0</td>
<td>5.1</td>
<td>&lt; 0.001</td>
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<td>Pancreas (n = 30)</td>
<td>51.0</td>
<td>46.3</td>
<td>5.8</td>
<td>10.7</td>
<td>6.2</td>
<td>4.3</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Liver (n = 30)</td>
<td>51.8</td>
<td>48.0</td>
<td>4.6</td>
<td>13.6</td>
<td>10.7</td>
<td>5.5</td>
<td>6.3</td>
<td></td>
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<tr>
<td>Probe location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of probes</td>
<td>55.8</td>
<td>49.8</td>
<td>8.8</td>
<td>&lt; 0.001</td>
<td>16.0</td>
<td>10.2</td>
<td>7.2</td>
<td>&lt; 0.001</td>
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<td>70 (n = 63)</td>
<td>50.6</td>
<td>45.8</td>
<td>3.4</td>
<td>10.3</td>
<td>8.5</td>
<td>2.5</td>
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<td>100 (n = 23)</td>
<td>54.7</td>
<td>46.5</td>
<td>4.3</td>
<td>16.6</td>
<td>8.7</td>
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<td>Probe exposure (cm)</td>
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<td>2 (n = 53)</td>
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<td>45.7</td>
<td>1.5</td>
<td>12.0</td>
<td>9.7</td>
<td>1.8</td>
<td>0.011</td>
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<td>3 (n = 13)</td>
<td>52.0</td>
<td>46.3</td>
<td>1.0</td>
<td>10.0</td>
<td>8.2</td>
<td>4.4</td>
<td>0.08</td>
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<td>1.5 (n = 20)</td>
<td>49.1</td>
<td>43.4</td>
<td>1.3</td>
<td>6.1</td>
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<td>1.5</td>
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<td>No. of probes</td>
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<td>10.0</td>
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<td>4.4</td>
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<td>2 (n = 33)</td>
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<td>45.3</td>
<td>4.6</td>
<td>9.2</td>
<td>7.2</td>
<td>0.8</td>
<td>0.007</td>
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<td>4 (n = 53)</td>
<td>52.0</td>
<td>46.1</td>
<td>1.0</td>
<td>10.0</td>
<td>8.6</td>
<td>4.4</td>
<td>0.08</td>
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<td>45.5</td>
<td>2.8</td>
<td>12.4</td>
<td>9.5</td>
<td>2.8</td>
<td>0.002</td>
<td></td>
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<td>0.7</td>
<td>9.6</td>
<td>4.5</td>
<td>1.8</td>
<td>0.004</td>
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<td>Treatment 3</td>
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<td>1.3</td>
<td>9.8</td>
<td>5.0</td>
<td>3.1</td>
<td>0.007</td>
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<td></td>
</tr>
<tr>
<td>None (n = 5)</td>
<td>47.3</td>
<td>45.8</td>
<td>1.5</td>
<td>11.3</td>
<td>8.5</td>
<td>1.5</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Present (n = 20)</td>
<td>65.3</td>
<td>55.3</td>
<td>5.9</td>
<td>29.3</td>
<td>19.3</td>
<td>5.9</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

*Standard deviation of the differences between temperatures at thermocouples positioned at 0.5 cm or less and 1.0 cm from the irreversible electroporation probe. † test (probe location 0.5 cm or less versus 1.0 cm).

<table>
<thead>
<tr>
<th>Tissue type – kidney</th>
<th>Univariable P</th>
<th>Multivariable P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse length – 100 µs</td>
<td>0.016</td>
<td>0.024</td>
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<td>Probe exposure length – 3 cm</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>No. of probes</td>
<td>0.630</td>
<td>–</td>
</tr>
<tr>
<td>Metal – yes</td>
<td>0.016</td>
<td>0.001</td>
</tr>
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</table>

*χ² test; † linear regression.
Effect of pulse length

IRE was performed on hepatic tissue at two different pulse lengths: 70 and 100 μs. The mean maximum temperature at 0.5 cm or less for a pulse length of 70 μs was 50.6°C, compared with 54.7°C for a pulse length of 100 μs (P = 0.035). The mean temperature change was 10.3 and 16.6°C for pulse lengths of 70 and 100 μs respectively (P = 0.007) (Table 1). A pulse length of 100 μs was significantly associated with thermal injury (P = 0.001) (Table 2). There was no evidence of thermal temperature change at the 1.0-cm thermocouple.

Effect of probe exposure length

IRE was performed on hepatic tissue at probe exposure lengths of 2 and 3 cm. The mean maximum temperature at 0.5 cm or less for the probe exposure of 2 cm was 47.3°C compared with 52.0°C for an exposure length of 3 cm (P = 0.011). The mean temperature change was 12.0 and 10.0°C respectively (P = 0.011) (Table 1). Although thermal injury was more common with a probe exposure of 3 cm, this did not reach statistical significance (P = 0.294) (Table 2).

Effect of number of probes

IRE was performed on hepatic tissue using either a two- or a four-probe array. The mean maximum temperature at 0.5 cm or less was 52.0 and 50.2°C respectively (P = 0.541). The mean temperature change was 10.0°C for the two-probe array and 9.2°C for the four-probe array (P = 0.689) (Table 1). The number of probes was not associated with an increased risk of thermal injury (P = 0.630) (Table 2).

Clinical scenarios for liver, pancreas and kidney tissue

Each organ was treated within previously reported treatment parameters with differences in the potential for thermal damage. In liver, when probe exposure was 2 cm there was no evidence of thermal injury (Fig. 2e). If a probe exposure of 3 cm was used then thermal injury could be mitigated with a drop in pulse length to 70 μs (Fig. 2d). In pancreas, where all previous reports have demonstrated that only a 1.5-cm probe exposure can be used clinically, there was no evidence of thermal injury regardless of pulse length and the number of retreatments delivered (Fig. 2b). In kidney, the risk of thermal injury was more pronounced, most likely because of the conductivity of urine, and so thermal injury was evident with probe exposure of 3 cm and pulse lengths of 100 μs (Fig. 2a, Table 1).

Effect of the presence of metal on tissue temperature

IRE was performed on hepatic tissue using a four-probe array with either a 0.5-cm metal clip or a metal bile
duct stent placed within (defined as 1 cm or less) the field of ablation. The mean maximum temperature was 65.3°C, compared with 47.3°C for hepatic tissue without metal within the zone of ablation ($P = 0.007$). The mean change in temperature was 29.3 and 11.3°C respectively ($P = 0.007$) (Table 1). Ablation in the presence of metal was significantly associated with thermal injury on univariable analysis ($P = 0.016$) as well as significant on multivariable analysis ($P = 0.001$) (Table 2). Ultrasound imaging demonstrated significant electroporation deflection as well as evidence of thermal injury following ablation in the presence of metal (Fig. 4).

**Discussion**

IRE is reported to be a non-thermal tumour ablation technique that has been advocated to protect non-target tissue from thermal injury. Although safety and efficacy data have been reported, there are still many questions regarding the mechanism by which IRE achieves cell death. Of particular interest is the ability of IRE to remain non-thermal given the wide range of ablation parameters that can be used with the current clinical device – with probe exposures ranging from 1.0 to 4.0 cm, pulse lengths between 70 and 100 μs, number of probes ranging from two to six, and probe spacing between 1.0 and 2.5 cm. In addition, IRE energy delivery in the presence of metal clips or stents as a highly conductive low-resistance entity must be evaluated, given that this is a common clinical scenario in the treatment of unresectable hepatopancreatobiliary malignancies.

The present study evaluated the potential of IRE to cause thermal injury, using established definitive non-thermal parameters in three of the most common tissue types, namely liver, pancreas and kidney. For liver tissue, a probe exposure of 2.5 cm or less and pulse length of 70–90 μs consistently resulted in non-thermal injury. In pancreatic tissue, for which a probe exposure of 1.0 or 1.5 cm is most commonly used in clinical treatment, there was no evidence of thermal injury with the pulse lengths evaluated, and with at least 270 pulses delivered in the same location. For kidney tissue, a probe exposure of 2 cm and a pulse length of...
70–90 μs resulted in the least thermal injury for this organ. This injury is confounded and compounded when the renal collecting system is violated, leading to urine leakage into the planned electroporation zone. In this clinical scenario, a shorter probe exposure of 1-5 cm is recommended, with subsequent drawbacks in treating lesions of the size commonly found within the renal parenchyma.

The safe use of IRE around vascular and ductile structures has been shown previously in chronic large animal models. Organ-specific safety and efficacy data for use of IRE in liver and pancreas have been published recently. When IRE is delivered appropriately, it affects only the target tissue and spares the surrounding structures. Proteins, the extracellular matrix, and critical structures such as blood vessels and nerves, are all unaffected by this treatment. IRE expands the scope of palliative and/or definitive treatment of lesions near major vascular, biliary and urinary structures, which in the past could be treated only with external-beam radiation therapy. The disadvantage of IRE is the need for general anaesthesia (deep paralysis) for safe and effective delivery.

However, the present study has shown that IRE does generate thermal energy, and that this energy is greatest in tissue adjacent to the probe and decreases with distance from the probe. In every instance, the temperature measured by the thermocouple placed no more than 0.5 cm from the IRE probe was higher than that measured at the thermocouple placed at 1.0 cm. In addition to these location-specific variables, two device-specific variables were shown to be associated with thermal energy: prolonged pulse length and increased probe exposure length. Finally, the presence of metal, in the form of either a clip or a stent, was significantly associated with the generation of thermal energy.

Thermal injury, as defined above, was associated with certain tissue types (specifically kidney), pulse length and the presence of metal within the ablation field in both univariable and multivariable analysis. The association between metal in the IRE field and thermal injury is of specific interest. The closer the metal to the ablation probe, the greater the thermal energy generated. This was also demonstrated by an increase in current draw, which on occasion automatically terminated the ablation owing to violation of the generator’s default safety settings; these do not allow energy to be delivered when more than 50 A will be drawn from the machine. Based on these findings, it is recommended that metal structures (such as biliary stents) should be removed before IRE. If this is not feasible, the probe pairs should not bracket the metal and if possible be at least 1 cm away from the IRE probes. When this distance from metal structures cannot be achieved, optimal efficacy cannot be guaranteed and thermal risk related to deflection is possible.

The temperature changes observed during this study suggest that, although IRE is less thermal than radiofrequency or microwave ablation, the potential still exists for generation of significant thermal energy, which can result in thermal injury, in certain tissue types and with certain treatment parameters that can be mitigated by the end user. In particular, IRE ablation of kidney tissue should be done with caution if carried out with non-thermal intent. In addition, shorter pulse lengths and shorter probe exposure lengths should be used to minimize the risk of thermal injury. Finally, IRE ablation in the presence of metal clips or stents will significantly increase the chance of thermal injury.

This evaluation of thermal energy generated by IRE is not without weaknesses. Thermal injury can be defined by many different mechanisms. Many temperature-based models exist for prediction of thermal injury and have been accepted as alternatives to pathological confirmation. One such model suggests that thermal injury can be predicted by reaching a temperature of 50°C for at least 5 min. If this model had been used, the predicted incidence of thermal injury would perhaps have been lower. However, all of these models probably oversimplify a process that is quite variable and tissue-specific. Awareness of possible thermal injury from IRE is warranted and can be avoided with appropriate use of the device.

Disclosure

R.C.G.M. is a consultant for Angiodynamics. The authors declare no other conflict of interest.

References


Temperature evaluation in electroporation


