Myocardial Lesion Size after Epicardial Electroporation Catheter Ablation following Subxiphoid Puncture

Running title: Neven et al.; Lesion size after epicardial electroporation ablation

Kars Neven, MD$^{1,2}$, Vincent van Driel, MD$^1$, Harry van Wessel, BSc$^{1,3}$, René van Es, MSc$^1$, Pieter A. Doevendans, MD, PhD$^{1,4}$, Fred Wittkampf, PhD$^1$

$^1$Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands; $^2$Department of Rhythmology, Alfried Krupp Krankenhaus, Essen, Germany; $^3$St. Jude Medical, Veenendaal; $^4$ICIN – Netherlands Heart House, Utrecht, The Netherlands

Correspondence:
Kars Neven, MD
University Medical Center Utrecht
Dept. of Cardiology
P.O. Box 85500
3508 GA Utrecht
The Netherlands
Tel: +31-88-7556184
Fax: +31-86-8480699
E-mail: kars_neven@hotmail.com

Journal Subject Codes: [22] Ablation/ICD/surgery
Abstract:

**Background** - Irreversible electroporation is a promising non-thermal ablation modality able to create deep myocardial lesions. We investigated lesion size after epicardial electroporation catheter ablation with various energy levels following subxiphoid pericardial puncture.

**Methods and Results** - In 6 six-month-old pigs (60-75 kg), a custom deflectable octopolar 12 mm circular catheter with 2 mm ring electrodes was introduced via a deflectable sheath after pericardial access by subxiphoid puncture. Non-arcing, non-barotraumatic, cathodal 50, 100 and 200-joule (J) electroporation applications were randomly delivered on the basal, mid and lateral left ventricle. After 3 months survival, myocardial lesion size and degree of intimal hyperplasia of the coronary arteries was histologically analyzed. Five animals survived the follow-up without complications, 1 animal died of shock after the subxiphoid puncture. At autopsy, whitish circular scars with indentation of the epicardium could be identified. Average lesion depth of the 50J, 100J and 200J lesions were 5.0 ± 2.1 mm, 7.0 ± 2.0 mm and 11.9 ± 1.5 mm, respectively. Average lesion width of the 50J, 100J and 200J lesions were 16.6 ± 1.1 mm, 16.2 ± 4.3 mm and 19.8 ± 1.8 mm, respectively. In the 100J and 200J cross-sections, transmural left ventricular lesions and significant tissue shrinkage was observed. No intimal hyperplasia of the coronary arteries was observed.

**Conclusions** - Epicardial electroporation ablation following subxiphoid pericardial puncture can create deep, wide and transmural ventricular myocardial lesions. There is a significant relationship between the amounts of electroporation energy delivered epicardially and lesion size in the absence of major adverse events.

**Key words:** ablation, epicardium, lesion, dosing, irreversible electroporation
Introduction:

Radiofrequency catheter ablation has become the standard ablation technique for the treatment of cardiac arrhythmias after direct current catheter ablation was abandoned, this was because of the high complication rate due to the generation of vapour globe, sparking, explosion, and pressure waves.\(^1\)\(^-\)\(^4\) Since its introduction, epicardial radiofrequency ablation is increasingly being performed for the treatment of ventricular arrhythmias due to ischemic and especially non-ischemic cardiomyopathies.\(^5\) Radiofrequency causes heat damage to all tissue near the ablation site. Ablation near coronary arteries can therefore have hazardous adverse effects, like coagulation of blood inside the vessel and vessel stenosis with subsequent myocardial infarction.\(^6\)\(^-\)\(^8\) In addition, the cooling effect of arterial and endocardial blood flow may limit lesion formation and success of the procedure.\(^9\),\(^10\)

In recent years, irreversible electroporation has been proposed as a new ablation modality for cardiac arrhythmias.\(^11\)\(^-\)\(^14\) Du Pré et al. showed that epicardial electroporation ablation over coronary arteries, with a follow-up of 3 weeks, has a low risk of coronary damage and that the use of this technique near or even on large coronary arteries is relatively safe. In addition, lesion formation by electroporation did not appear to be affected by the presence of arterial blood flow.\(^15\) We recently demonstrated that epicardial catheter ablation using 200 Joules (J) electroporation applications can create extensive and deep myocardial lesions without significant damage to the coronary arteries after a 3-month follow-up period.\(^16\)

The purpose of the present study is to investigate the relationship between the magnitude of an epicardial electroporation application and lesion size.

Methods:

All studies were performed after prior approval from the Animal Experiments Committee of the
University Medical Center Utrecht, Utrecht, the Netherlands and were performed in compliance with the Guide for the Care and Use of Laboratory Animals.17

Study protocol

The study was performed in 6 pigs (weight 60–75 kg). Amiodarone was started 1 week before the procedure (400 mg once daily) to prevent procedure-related arrhythmias. Carbasalate calcium (80 mg once daily) and clopidogrel (75 mg once daily) were started 3 days before the procedure and continued until euthanasia. The animals were sedated, intubated and anesthetized according to standard procedures. Using a subxiphoid pericardial puncture, a custom deflectable octopolar 12 mm circular catheter with 2 mm ring electrodes was introduced in the pericardial space via a 40 cm long 8.5 French deflectable sheath (Agilis EPI Steerable Introducer, St. Jude Medical, St. Paul, MN) (Figure 1). After LAD and RCx angiography, three single, QRS-wave triggered, cathodal 50J, 100J or 200J applications were delivered on three non-overlapping left ventricular (LV) locations: the mid anterior LV, the anterior-apical LV and the mid-lateral LV. In each animal, 50J, 100J and 200J, were delivered in random sequence. The energy was generated by a monophasic external defibrillator (Lifepak 9, Physio-Control, Inc., Redmond, WA). A large skin patch (7506, Valleylab Inc., Boulder, CO) on the lower back served as indifferent electrode.13, 14 A cathodal polarity was chosen because that has the highest threshold for arcing in a blood environment.18 The impulse waveform was similar to that applied in our earlier studies.13, 14 Coronary arteries were not specifically targeted, but coronary angiography was repeated after the last application.

After 3 months survival, coronary angiography of the LAD and RCx arteries was repeated, the thorax was opened by sternotomy and the animal was euthanized by exsanguination. After the heart was removed, the pericardium was peeled off and the areas with
Ablation lesions were excised and fixated in formalin.

**Histological evaluation**

After fixation, three 4.0 mm wide segments were dissected from each lesion perpendicular to the epicardial surface to facilitate measurement of lesion width and depth. The first segment (A) was taken through the middle of what visibly appeared to be the center of the circular lesion; two other segments (B and C) were dissected perpendicular to that first segment, again through the apparent center of the lesion. (Figure 2) After embedding in parafin, these segments were sectioned and stained with hematoxylin-eosin and with elastic–van Gieson. All histological sections were digitally scanned and analyzed using Imagescope (Aperio Technologies). Lesion size was measured in each section.

**Measurement of lesion size**

Two investigators, blinded for the energy settings used, independently measured lesion depth and width: maximal lesion depth was measured in segment A. Lesion width was calculated as the average value of: lesion width in segment A and the sum of lesion widths in segments B and C plus 4.0 mm. Values obtained by the 2 blinded investigators were averaged. Large lesions often showed tissue shrinkage, as also seen after myocardial infarction. When sufficient undamaged myocardium was present in the histological section, the estimated original epicardial contour was used to measure lesion depth. With relatively deep lesions, tissue shrinkage apparently also affected the endocardial contour and then also the estimated original endocardial contour was used to measure lesion depth.

**Evaluation of the coronary arteries**

All pre- and post-ablation angiograms were qualitatively analyzed by 2 experienced investigators. Consensus had to be reached on the degree of luminal narrowing. All histological
cross-sections were analyzed for the degree of intimal hyperplasia of the coronary arteries.

**Statistical analysis**

The relation between delivered peak current and mean lesion depth and width was calculated by randomized block regression analysis using Tukey’s multiple comparison post-hoc test. Continuous variables were expressed as mean ± standard deviation (SD). Statistical significance was defined as \( p \leq 0.05 \).

**Results**

Five animals survived the procedure and the 3-month follow-up period without complications. One animal had to be euthanized acutely before electroporation applications had been delivered, because of complications due to failed subxiphoid puncture. The novel ablation catheter had never been deployed in this animal.

**Pericardial ablation**

All recorded voltage and current waveforms were smooth suggesting the absence of arcing. Average peak currents of the 50J, 100J, and 200J applications were 11.6 ± 1.4, 19.0 ± 1.5, and 27.1 ± 0.7 amperes, respectively (Table).

**Macroscopic findings**

Careful inspection of the organs adjacent to the pericardium showed no abnormalities in any animal. No macroscopic signs of bleeding, scarring or excessive fibrotic tissue proliferation were found.

**Lesion size**

In five animals, 43 cross-sections from 15 electroporation lesions were analyzed. In total we obtained 15 cross-sections from five 50J lesions, 13 cross-sections from five 100J lesions and 15 cross-sections from five 200J lesions (Figure 3).
At visual inspection after euthanization, the lesions had a circular, whitish aspect on the epicardial surface with denting in the center of the lesion, due to tissue shrinkage. All but one of the lesions showed continuous lesions, with a sharp demarcation between the ablation lesion and the surrounding tissue. Due to vital myocardium in the center of the lesion, one 50J lesion did not show one continuous lesion, but two separate lesions in segment A (Figure 4). For this lesion we averaged lesion depths observed in the three histological sections; due to the absence of a continuous lesion we could not calculate a lesion width in this lesion.

Lesion depth: Mean depth [range] of the 50J, the 100J and the 200J lesions was 5.0 ± 2.1 [range 2.8-7.4] mm, 7.0 ± 2.0 [range 4.4-9.3] mm and 11.9 ± 1.5 [range 10.0-13.9] mm, respectively (Figure 5). Mean depth of all lesions combined was 8.0 ± 3.4 mm. In the 100J, and especially in the 200J lesions, significant shrinkage due to scar contracture was obvious. The difference in depth between the respective lesions was statistically highly significant (p<0.001). In more detail, the differences in depth between the 50J vs. the 200J lesions and the 100J vs. the 200J lesions were statistically significant (p<0.001 and p=0.003, respectively). The difference in depth between the 50J and 100J lesions was not statistically significant (p=0.18). Transmurality of the lesion was seen in 20% of the 100J lesions and in 80% of the 200J lesions.

Lesion width: Mean maximal width [range] of the 50J lesions, the 100J lesions and the 200J lesions was 16.6 ± 1.1 [range 15.2-17.7] mm, 18.1 ± 1.0 [range 16.6-18.9] mm and 19.8 ± 1.8 [range 17.4-21.3] mm, respectively. Mean maximal width of all lesions combined was 18.2 ± 1.9 mm. The differences in width between the 50J vs. the 200J lesions were statistically significant (p=0.007) (Figure 6).

**Coronary angiography**

All pre-ablation coronary angiograms were normal. Post-ablation coronary angiography
demonstrated short-lasting (<30 minutes) luminal narrowing with subsequent normalization in the targeted area, suggestive of coronary spasm. After 3 months survival, there was no significant narrowing of any coronary artery. Visually, all coronary angiograms resembled the pre-ablation coronary angiograms. No significant intimal hyperplasia of coronary arteries was seen in any histological section.

**Discussion:**

Epicardial radiofrequency ablation has a long history. From the first publication by Sosa et al. in 1996, percutaneous epicardial radiofrequency ablation is nowadays being performed in many electrophysiologic centers and for a variety of indications, mainly ablation of ventricular tachycardia. However, severe complications, such as damage to the coronary arteries and the phrenic nerve, were described soon after the introduction of epicardial radiofrequency ablation. Since then, many techniques have been introduced with varying success in order to prevent severe complications, such as coronary angiography, endocardial ablation with an ablation catheter equipped with a retractable needle, endocardial ablation to reduce or obviate epicardial ablation, and injection of air or saline into the pericardium.

Recently, Wittkampf et al. and Du Pré et al. have demonstrated that epicardial ablation using electroporation in pigs is feasible, safe and causes only minimal damage to the coronary arteries. These ablations were performed in an open-chest model, where the epicardium was surgically exposed. This is not the regular, clinical approach when performing epicardial ablation in humans. Therefore, we recently demonstrated that closed-chest epicardial catheter ablation using electroporation is feasible, safe and able to create deep myocardial lesions. Ablation with 200J electroporation applications on top of main coronary arteries only caused temporary spasm and a median of 4 ± 10% luminal stenosis.
Electroporation pulse and catheter model

Between 1980 and 1990, direct current catheter ablation was the only energy source available for catheter ablation. At the end of that era, low energy direct current ablation was developed, but very soon thereafter it was replaced by the more elegant radiofrequency ablation technique. In the present and earlier studies, we used a readily available monophasic defibrillator to deliver the electroporation pulse. However, instead of using a single electrode, a circular arrangement of 8 electrically connected electrodes was used in the present study for application of electroporation energy.

In general, a circular electrode catheter has an important advantage over a single ablation electrode for the application of electrical current. Whilst the latter creates an exponential decay in current density with increasing distance from the ablation electrode, a circular arrangement of electrically connected electrodes creates a thorus-shaped electrical field near to and around the ablation hoop, at least in a first approximation. The surface area of a thorus relates linearly to the thickness of the thorus. Therefore, current density will initially decrease linearly with increasing distance from the ablation hoop and not exponentially. Penetration depth of an electroporation pulse will therefore be greater with such an ablation hoop than with a single ablation electrode.

In addition, a circular multi-electrode arrangement will allow for faster electrophysiological mapping than a linear electrode catheter, because it can be used to determine not only the timing, but also the direction of the activation wavefront. Finally, the 8 ring electrodes of 2 mm in length have a total surface area of 115 mm², which definitively allows for a higher arcing threshold than a single electrode; even an 8 mm 7F electrode only has a total surface area of 58 mm² and such a large electrode has poor mapping characteristics.
Lesion size

In this study we were able to create deep, wide and continuous lesions. There was a significant relationship between magnitude of the electroporation application and lesion depth and width: with a larger magnitude of the application a larger myocardial lesion could be created. Where a single 50J application was able to create myocardial lesions up to 7.4 mm deep and 17.7 mm wide, single 200J applications created myocardial lesions up to 13.9 mm deep and 21.3 mm wide. These 200J electroporation applications are sufficiently powerful to create transmural ventricular lesions. There were no adverse effects associated with these applications.\textsuperscript{27, 28}

Mean width of all lesions was 18.2 ± 1.9 mm. The widest 200J lesion was 21.3 mm; this is almost 2 times the width of the diameter of the ablation catheter itself. Whilst up to 21 mm wide lesions might be undesirable for the ablation of a focal epicardial source of arrhythmia, lesions this wide might be useful and effective when homogenization of a large area of epicardial scar tissue is required. With future catheter design and further titration of energy settings it should be possible to find the optimal energy settings for clinical applications.

Effect on coronary arteries

The coronary arteries were not specifically targeted in this study, but they were also not purposly avoided. Apart from short-lasting (<30 minutes) coronary spasm, no long-term luminal narrowing was seen. After 3 months follow-up, the luminal diameters of the main coronary arteries were similar to the baseline luminal diameters. These data support the findings of Du Pré et al. and our previous findings: similar results were found after a follow-up period of 3 weeks and 3 months, respectively.\textsuperscript{15, 16}

Clinical implications

Data from this study and previous studies suggest that epicardial electroporation ablation is a
very safe and effective ablation modality.\textsuperscript{15,16} It is able to create large and deep myocardial lesions whilst sparing the coronary arteries, electroporation ablation is the first and only ablation modality capable of doing so. Using this new technology, safe and effective epicardial ablation on or near coronary arteries can become a real possibility for the treatment of ventricular tachycardias which would otherwise not be treatable with catheter ablation.

Also, deep intramural foci of ventricular arrhythmia will become within reach of catheter ablation using electroporation, because a single 200J electroporation application is able to safely create very deep, continuous and transmural myocardial lesions. This desirable feature is also unique for electroporation ablation.

The clinical introduction of epicardial electroporation ablation could possibly significantly change the way epicardial ablation is being performed.

Limitations

We investigated only 3 energy settings: 50J, 100J and 200J. We have no information on lesion size or adverse effects when using other energy settings.

Significant shrinkage due to scar contracture of the 100J and especially the 200J lesions definitively caused underestimation of lesion size. Also, transmurality of the lesion could have caused underestimation of lesion depth.

Although we did not record any arrhythmia following the ablation, nor experienced sudden death in the animals during follow-up, we cannot exclude occurrence of proarrhythmic effects following electroporation ablation.

We did not investigate the possible negative influence of (the amount of) epicardial fat or preexisting myocardial fibrosis on lesion size.

In this study, the electroporation ablation catheter was placed on the left ventricular
epicardium to investigate the effects of certain energy settings on lesion size. Although we might expect a similar outcome, we do not have information about lesion size created by epicardial electroporation ablation in atrial tissue.

Lesion size and form with electroporation ablation will depend on the ultimate catheter/device design and measures to ensure electrode-tissue contact. Given the experimental character of this study, extrapolation of the results of this study to final catheter/device design should be performed with great caution.

Conclusions:
Epicardial electroporation ablation following subxiphoid pericardial puncture can create deep, wide and transmural ventricular myocardial lesions. There is a significant relationship between the amounts of electroporation energy delivered epicardially and lesion size in the absence of major adverse events.

Acknowledgments: The authors wish to thank the staff of the Department of Experimental Cardiology of the University Medical Center Utrecht for technical assistance during the experiments.

Conflict of Interest Disclosures: Fred Wittkampf is a consultant for St. Jude Medical, Atrial Fibrillation division. Both Fred Wittkampf and Harry van Wessel are co-inventors of circular electroporation. The other authors have no conflicts of interest to disclose.

References:


**Table:** Relationship between magnitude of application, output and lesion size.

<table>
<thead>
<tr>
<th>Energy (J)</th>
<th>Peak power (V)</th>
<th>Peak current (A)</th>
<th>Peak resistance (Ω)</th>
<th>Lesion depth (mm)</th>
<th>Lesion width (mm)</th>
<th>Transmural lesion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1220 ± 46</td>
<td>11.6 ± 1.4</td>
<td>107 ± 14</td>
<td>5.0 ± 2.1</td>
<td>16.6 ± 1.1</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>1670 ± 74</td>
<td>19.0 ± 1.5</td>
<td>91 ± 14</td>
<td>7.0 ± 2.0</td>
<td>18.1 ± 1.0</td>
<td>20</td>
</tr>
<tr>
<td>200</td>
<td>2305 ± 54</td>
<td>27.1 ± 0.7</td>
<td>85 ± 4</td>
<td>11.9 ± 1.5</td>
<td>19.8 ± 1.8</td>
<td>80</td>
</tr>
</tbody>
</table>

Ablations were performed with 50J, 100J, and 200J. Lesion depth and width were measured in all histological cross-sections. Lesion transmurality was determined per lesion. Data are given as the mean ± standard deviation.

**Figure Legends:**

**Figure 1.** Subxiphoid pericardial puncture and ablation catheter. The left panel shows the black guidewire after subxiphoid pericardial puncture and the distal end of the steerable pericardial sheath with the dilator inside. The right panel shows the custom circular electroporation ablation catheter that was used for this study. The distal circular, 12 mm diameter segment of the
deflectable 7 French catheter contains 8 electrodes of 2 mm in length.

**Figure 2.** Schematic drawing of a lesion and the positions of the 3 segments. The first 4.0-mm wide lesion segment (A) was taken aside from the middle of what visibly appeared to be the center of the circular lesion (grey circle); two other lesion segments (B and C) were dissected perpendicular to that first segment, again aside from the apparent center of the lesion. The histological sections were taken from the side of the segment which went through the center of the lesion. Maximal lesion depth was measured in lesion segment A, lesion width was calculated as the average value of: lesion width in lesion segment A and the sum of lesion widths in lesions segments B and C plus 4.0 mm.

**Figure 3.** Histologic sections of epicardial lesions. Histological elastic–van Gieson–stained sections through the center of epicardial lesions created with 50J (panel A), 100J (panel B) and 200J (panel C). Each section was taken perpendicular to the epicardial surface and over the course of the diameter of the lesion. The endocardial side is at the bottom and the epicardial side is at the top of the picture. Note that there is no heat sink effect of the coronary arteries: the lesion includes the coronary artery completely and continues on the endocardial side of the coronary artery. Also note the shrinkage due to scar contracture in panel C. The inset in the lower right corner of panel C shows a magnification of the coronary artery shown in panel C.

#: coronary artery; J: joule

**Figure 4.** Histologic section of epicardial lesion after 50J electroporation application.

Histological elastic–van Gieson–stained section through the center of an epicardial lesion created
with 50J. The section was taken perpendicular to the epicardial surface and over the course of the diameter of the lesion. The endocardial side is at the bottom and the epicardial side is at the top of the picture. There are two separate, whitish lesions visible beneath the epicardial surface with vital myocardium in-between. The location of the cross-section relative to the circular lesion is sketched in the inset in the lower right corner.

#: coronary artery; J: joule

**Figure 5.** Magnitude of electroporation application and maximal lesion depth. Relationship between magnitude of electroporation application and maximal lesion depth per lesion. The horizontal bar shows the mean value of the lesion depths per energy setting. The differences in depth between the 50J vs. the 200J lesions and the 100J vs. the 200J lesions are statistically significant.

J: joule

**Figure 6.** Magnitude of electroporation application and mean maximal lesion width. Relationship between magnitude of electroporation application and mean maximal width per lesion. The horizontal bar shows the mean value of the lesion widths per energy setting. The differences in width between the 50J vs. the 200J lesions are statistically significant.

J: joule.
Lesion

Lesion segments

Histological sections
A scatter plot showing the relationship between lesion depth (mm) and application energy setting (joules). The plot includes data points for 50 joules, 100 joules, and 200 joules. The statistical significance is indicated with $P < 0.001$, $P = 0.18$, and $P = 0.003$. The graph is labeled with "Circulation: Arrhythmia and Electrophysiology" and "Journal of the American Heart Association".
Myocardial Lesion Size after Epicardial Electroporation Catheter Ablation following Subxiphoid Puncture
Kars Neven, Vincent van Driel, Harry van Wessel, René van Es, Pieter A. Doevendans and Fred Wittkampf

_Circ Arrhythm Electrophysiol._ published online July 11, 2014;
_Circulation: Arrhythmia and Electrophysiology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/early/2014/07/11/CIRCEP.114.001659

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Arrhythmia and Electrophysiology_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Arrhythmia and Electrophysiology_ is online at: http://circep.ahajournals.org//subscriptions/