Electrophysiological pulmonary vein isolation (PVI) is the cornerstone of paroxysmal atrial fibrillation (AF) ablation. However, the incidence of AF recurrence remains high and mostly because of PV reconnection, emphasizing the need for more understanding of PVI durability and associated factors.

In this regard, it has been elegantly shown in a swine model that elimination of the negative component of the unipolar atrial electrogram during radiofrequency applications reflects transmural lesions creation. Subsequently, it has been clinically suggested that such a transmularity can be either irreversible or reversible. The present study is aimed to determine, at the histological level, whether transmural lesions, assessed by R morphology completion, might indeed be reversible in some circumstances or not.

Methods and Results—In 6 Mongrel hound dogs, superior and inferior vena cavae were isolated and individual lesions were created in the right atrium using radiofrequency energy (30 W/48°C/17 mL/min as presettings and 10 g of force in average) under CARTO guidance. Five types of lesions were created; R+0: termination of ablation at the time of R morphology completion; R+5, R+10, or R+20: extension of ablation for 5, 10, or 20 seconds, respectively, after R morphology achievement; and conventional: radiofrequency applications lasting 30 seconds irrespective of the atrial electrogram modification. All conventional, R+5, R+10, and R+20 lesions were necrotic and transmural, whereas some R+0 lesions were not (comprising a part of necrosis and a part of reversible cell damage). Interestingly, surrounding organ injuries were observed after conventional, R+10, and R+20 radiofrequency applications but were not observed after R+0 and R+5 applications.

Conclusions—Elimination of the negative component of the unipolar atrial electrogram reflects, in general, irreversible transmural necrosis creation. In some cases, however, it translates transmural lesion only (with potential reversibility) likely related to transient cell damage creation.

Key Words: animals ■ atrial fibrillation ■ catheter ablation ■ radio frequency

Electrophysiological pulmonary vein isolation (PVI) is the cornerstone of paroxysmal atrial fibrillation (AF) ablation. However, the incidence of AF recurrence remains high and mostly because of PV reconnection, emphasizing the need for more understanding of PVI durability and associated factors.

In this regard, it has been elegantly shown in a swine model that elimination of the negative component of the unipolar atrial electrogram during radiofrequency applications reflects transmural lesions creation, whereas the persistence of such a negative component constantly corresponds to non-transmural lesions.

We have subsequently reported, in patients affected by paroxysmal AF episodes, the relevance of the atrial unipolar modification analysis as a local ablative end-point while performing PVI and its positive impact in terms of mid-term SR maintenance rate. However and interestingly, this study also has strongly suggested that the elimination of the negative component of the atrial electrogram, although it did not provide histological evidence because it was conducted in humans, may correspond either to transmural but reversible lesions (likely related to edema or transient cell damage) or to transmural and irreversible lesions (likely corresponding to transmural necrosis).

The aim of the present study is to determine, at the histological level, whether the elimination of the negative component of the atrial electrogram during radiofrequency energy applications might in some circumstances reflect transmural but potentially reversible lesions or it might correspond to transmural and irreversible (necrotic) lesions in others.

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**WHAT IS KNOWN**

- It has been experimentally shown that elimination of the negative component of the unipolar atrial electrogram during radiofrequency energy applications reflects transmural lesion creation.
- Subsequently, it has been clinically suggested that such transmurality can be either irreversible or reversible.

**WHAT THE STUDY ADDS**

- The present study confirms that although the elimination of the negative component of the unipolar atrial electrogram reflects in general irreversible transmural necrosis creation, it may also translate, in some cases, to reversible transmural lesion creation related to transient cell damage.

**Methods**

**Study Animals**

A total of 7 Mongrel hound dogs were obtained from 2 separate class A–approved vendor (Marshall BioResources, North Rose, NY) for potential enrollment in the study. Six of these animals were enrolled and completed all phases of the study according to the protocol. One of 7 animals was not needed as a replacement and, therefore, was subsequently released from the population of potential study animals at the end of in-life study phase. All 6 animals enrolled were castrated males aged ≈10.4 months and weighing on average 25.3 kg at the time of enrollment.

The study was conducted in Saint Joseph’s Translational Research Institute, Atlanta, GA, from November 27 to November 29, 2012. The local Animal Research Ethics Committee approved animal handling procedures. On arrival at the institute, animals received general health examination by trained veterinary staff and were allowed to acclimate before start of the procedure. Quarantine was not required. Health examination included evaluation of the heart rate, body temperature, respiratory rate, and animals’ overall condition and attitude. All animals were considered healthy and suitable for enrollment.

**Animal Preparation**

After acclimation, study animals were sedated and prepared for the study as follows: a peripheral ear vein was cannulated for infusion of intravenous fluids and medications during the surgical procedure. The animal was intubated for administration of anesthetic gases and care was taken to thoroughly shave the area to be operated on. The animal was intubated for administration of anesthetic gases and monitored continuously throughout the procedure. Body temperature was also monitored. In addition, external defibrillator pads were placed on animal’s chest and verified before procedure started.

Animal monitoring was performed to determine the depth of anesthesia and physiological status. Specifically, heart rate, respiration rate, blood pressure (systolic, diastolic, and mean), body temperature, blood oxygen saturation, and percent inhalant anesthetic administered were recorded on the procedure record approximately every 15 minutes throughout the procedure. Active clotting time levels were maintained >350 seconds by intravenous heparin administration.

**Ablation Protocol and Subsequent Animal Management**

In all animals, a reference 6F nondeflectable hexapolar 2.5-2.5-2.5-2.5-2.5-2.5 mm interspacing electrodes catheter (Curve: A-Josephson type; Biosense Webster, Diamond Bar, CA) was placed in the RA, a 7F circular decapolar catheter (Lasso) was used to monitor superior vena cava (SVC) and inferior vena cava (IVC) isolation and a 3.5-mm 7F externally irrigated-tip contact force sensing catheter (SmartTouch) permitted radiofrequency applications in the RA. Intracardiac navigation was guided by the CARTO 3 electroanatomic system. Surface ECG and bipolar endocardial electrograms were continuously monitored and stored on a computer-based digital amplifier/recorder system (Prucka Cardiolab, GE Healthcare, Milwaukee, WI). Intracardiac electrograms were filtered from 30 to 500 Hz and measured with online callipers at 25 to 200 mm/s. Unipolar signal was recorded from the 3.5-mm distal electrode of the ablation catheter, with an indifferent electrode as cathode located in the IVC and was filtered on the CARTO 3 system with a [0.5; 120] Hz band pass filter and displayed in the CARTO 3 annotation viewer and analyzed on each ablation site before and after ablation at a sweep speed of 200 mm/s.

Five types of lesions were created. On one hand, radiofrequency applications lasted until elimination of the negative component of the unipolar atrial electrogram (ie, R morphology achieved; Figure 1). R+0 denoted termination of ablation at the time of R morphology achievement, whereas R+5, R+10, or R+20 denoted extension of ablation for 5, 10, or 20 seconds, respectively, after R morphology achievement. On the other hand, conventional lesions corresponded to radiofrequency applications lasting 30 seconds without monitoring the unipolar atrial electrogram nor the impedance drop or the bipolar atrial electrogram abatement. All lesions were created with the aid of a Stockert radiofrequency Generator ( Biosense Webster) in a power control mode, 30 W/48°C/17 ml/min as presetstings and a minimal force of 10 g through the entire radiofrequency applications.

In each animal, the SVC and the IVC were isolated using contiguous point-by-point radiofrequency applications around each vessel, and bidirectional block completion was systematically confirmed by the use of the Lasso catheter. In animal 1, both the SVC and the IVC were isolated after R+0 lesion creation. In animal 2, the SVC was isolated after R+0 lesion creation, whereas the IVC was isolated with conventional lesions. In animal 3, the SCV was isolated with conventional lesions, whereas the IVC was isolated with R+0 lesions. In animal 4, SVC and IVC were isolated using R+0 and R+5 lesions, respectively. In animal 5, the SVC and the IVC were isolated with R+10 and R+0 lesions, respectively. Finally, about animal 6, the SVC was isolated with the aid of R+0 lesions, whereas the IVC was isolated with R+20 lesions.

After the SVC and the IVC were isolated in each animal, 6 to 8 individual radiofrequency applications (2–4 conventional and 4 unipolar monitored –R+0, R+5, R+10, and R+20–) were performed in nontrabeculated areas of the RA (Figure 2).

Individual lesions were adequately spaced (>1 cm) to enable visual identification and harvest of lesions at necropsy (Figure 3). In each animal, lesion creation was performed in a random sequence to avoid any bias on histology patterns. Table 1 summarizes the lesions created in the study animals.

Thirty minutes after the last radiofrequency application, all study animals were humanely euthanized via intravenous injection of KCl (20 mEq IV) and were transferred for necropsy, tissue harvest, and macroscopic ablation lesion assessment. A necropsy was performed to grossly inspect the thoracic organs for adverse events because of ablations, including thorough inspection of the pericardial sac, lungs, trachea, and esophagus for any thermal injuries. The heart was...
excised and dissected for visual inspection of cardiac structures and was examined for any adverse events such as char, thrombus, and perforation. Subsequently, after proper fixation in 10% natural buffered formalin, excised hearts were transferred for histopathologic analysis. Hearts were trimmed and ablation sites excised and processed and embedded in paraffin. All paraffin blocks of radiofrequency sites were microtomed twice serially at ≈5 μm and stained with hematoxylin and eosin.

Statistical Analysis
Continuous normally distributed data were presented as mean (SD), continuous not normally distributed data were presented as medians [range], and categorical data were presented as n (%). The effect of different ablation strategies was assessed using a mixed model approach, to account for a potential random animal effect. Statistical analysis was performed using XLSTAT Pro 5.7.2 (Addinsoft, New York, NY). Significant differences were determined at \( P \leq 0.05 \).

Results

Ablation Results
The mean total procedure time was 109±8 minutes. All individual radiofrequency applications were achieved successfully. Furthermore, SVC and IVC could be acutely isolated in all animals with bidirectional block (entrance and exit) validation. Of note, in animal 2, electric reconnection of the SVC was observed 10.7 minutes after documented isolation, whereas in animal 3, IVC reconnection occurred 8.9 minutes after. No supplemental radiofrequency applications were

Figure 1. Animal 3. Unipolar atrial electrogram before and after an individual radiofrequency (RF) application has been performed in the right atrium (RA). A. Before the RF application, the unipolar atrial electrogram (MAP 1) demonstrates positive-negative morphology. B. Approximately 7 seconds after the RF application, the negative component of the unipolar atrial electrogram (MAP 1) has been eliminated and the unipolar atrial electrogram became complete positive signal. The II surface ECG lead is shown demonstrating sinus rhythm (in white), the unipolar (MAP 1 in light blue), and bipolar (MAP 1–2 in yellow) electrograms recorded by the end-tip of the ablation catheter, and the distal low RA signal recorded by the hexapolar catheter (RA 1–2 in pink).

Figure 2. Animal 4. Electroanatomic reconstruction of the right atrium. A, Modified posteroanterior view and (B) modified anteroposterior view. R+0 corresponds to an individual radiofrequency (RF) application having led to the elimination of the negative component of the unipolar atrial electrogram. R+5, R+10, and R+20 correspond to extension of ablation for 5, 10, or 20 seconds, respectively, after the elimination of the negative component of the unipolar atrial electrogram. Control spot ablations (light blue dots) correspond to 2 individual conventional RF applications (30 seconds). The orange dot denotes the His bundle location. The superior vena cava (SVC) and the inferior vena cava (IVC) were isolated by means of circular lesions created after the R+0 and the R+5 point-by-point ablation approach, respectively.
delivered in these 2 cases (no reisolation was done). Significantly, in no case char, steam pop or cardiac perforation was observed.

Gross Necropsy

There were no early mortalities in this study, all 6 dogs enrolled were euthanized and subjected to scheduled necropsy procedure per protocol. In agreement with acute ablation results, there were no events of pericardial effusion; thrombus or perforation noted could be associated with the treatment or procedural complications. All thoracic organs examined including pericardial sac, lungs, trachea, and esophagus were grossly unremarkable in all study animals regardless of treatment with exception of some limited collateral tissue injury associated with radiofrequency procedures. Accordingly, in animals 2 and 6 and in animals 3 and 5, pleural and lung lesions were, respectively, associated with radiofrequency energy delivery to IVC and SVC (Figure 4A; Figure I in the Data Supplement).

Histopathologic Results

Table 2 summarizes the radiofrequency applications made and their impact at the histological level. Of note, no significant animal effect was observed in the mixed model. Table 3 depicts the differences between transmural and nontransmural necrotic lesions achieved while following the R+0 unipolar-guided ablation approach.

Overall, all conventional lesions, R+ 5 lesions, R+10 lesions, and R+20 lesions were necrotic and transmural (Figure 4A), whereas some R+0 lesions did not correspond to transmural necrosis (Figure 4B–4D). However, surrounding organ injuries (in particular the lungs; Figure I in the Data Supplement) were observed after conventional radiofrequency applications and after R+10 and R+20 radiofrequency applications, whereas R+0 and R+5 radiofrequency applications were not associated with lesion creation beyond the heart.

Of note, as shown in Table 3, there is no evident difference between the radiofrequency R+0 applications having led to transmural and nontransmural necrosis in terms of force applied against the RA wall, impedance drop, energy delivered, and radiofrequency application time.

Discussion

The principal findings of the present work are the following: (1) elimination of the negative component of the unipolar atrial electrogram (R+0) while delivering radiofrequency energy in the RA of canines reflected, in the vast majority of cases, transmural necrosis completion; (2) in some cases, however, R+0 did not reflect transmural necrosis but transmural lesion only (which comprises a certain proportion of tissue necrosis and a remaining proportion of reversible tissue damage); (3) extension of ablation for 5, 10, or 20 seconds after R morphology achievement and

Table 1. Unipolar and Conventional Treatment Site Distribution

<table>
<thead>
<tr>
<th>Animal</th>
<th>Ablation Procedure Date</th>
<th>SVC Isolation</th>
<th>IVC Isolation</th>
<th>Unipolar Monitored Individual Ablations (4 Spot-Like Lesions)</th>
<th>Conventional Individual Ablations (2–4 Spot-Like Lesions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11/27/2012</td>
<td>R+0</td>
<td>R+0</td>
<td>R+0, +5, +10, and +20</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>11/27/2012</td>
<td>R+0</td>
<td>Conventional</td>
<td>R+0, +5, +10, and +20</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>11/28/2012</td>
<td>Conventional</td>
<td>R+0</td>
<td>R+0, +5, +10, and +20</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>11/28/2012</td>
<td>R+0</td>
<td>R+5</td>
<td>R+0, +5, +10, and +20</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>11/28/2012</td>
<td>R+10</td>
<td>R+0</td>
<td>R+0, +5, +10, and +20</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>11/29/2012</td>
<td>R+0</td>
<td>R+20</td>
<td>R+0, +5, +10, and +20</td>
<td>Yes</td>
</tr>
</tbody>
</table>

IVC indicates inferior vena cava; and SVC, superior vena cava.
Background

Electrophysiological PVI is the ablative strategy used in the daily clinical practice worldwide to treat patient afflicted by paroxysmal AF. However, there is a great concern about PVI durability as the PV reconnection phenomenon remains...
frequent and is in direct relationship with paroxysmal AF recurrence.2,3

It has been shown that PVI could be electrophysiologically achieved even if the circular lesion created around PV lacks perfect contiguity and transmurality related to edema and reversible cell damage.7,8 When this occurs, PV reconnection is practically unavoidable.2,3,0

Creation of the circular lesions around PV can be achieved by a one-fits-all device or following a point-by-point approach. In both cases, however, there is no currently defined local ablative end point in addition to PVI assessed by a circular multielectrode catheter, which is a global end point. In support of this, the local radiofrequency application time is currently either empirically defined or based on parameters, providing inaccurate lesion creation feedback (impedance drop, bipolar electrogram abatement, temperature).10,11 The individual radiofrequency application time, therefore, may be either too long and the source of lesion creation beyond the heart or too short and the source of nontransmural as well as noncontiguous lesions (leading to transmural and necrotic lesions without leading to lesions beyond animals’ heart). Whether these findings could be superimposed to human beings deserves particular attention but must be considered with caution. Indeed, both stable maintenance of the ablation catheter and continuous force application (beyond 10g) are not always easy tasks during PVI completion in humans. Furthermore, tissue thickness and its vascularization characteristics rendering difficult transmural lesion creation at certain regions may differ between canines and humans.

Comparison With Previous Works

In their seminal work conducted in a swine model, Otomo et al4 did not distinguish between transmural non-necrotic and transmural necrotic lesions. All transmural lesions assessed in this study were likely necrotic in nature. It must be underlined, however, that radiofrequency applications lasted 30 seconds in all cases, which should have contributed to necrotic and transmural lesions completion even if the ablation catheters used were not irrigated. In support of this, our present study shows that, when radiofrequency applications lasted 12 seconds and beyond, all lesions created were transmural and necrotic, whereas shorter radiofrequency applications led to transmural but non-necrotic lesions despite complete elimination of the negative component of the unipolar atrial electrogram.

Limitations

The present study has the following limitations: (1) the number of lesions created by applying the R+5 method was relatively low. Had the number of R+5 lesion created been higher, perhaps nontransmural necrotic lesions could have been evidenced or at the opposite surrounding organ injuries diagnosed. (2) Adding 5, 10, or 20 seconds after R morphology completion, in an attempt to find the method allowing to achieve transmural and irreversible lesions (necrosis) without leading to lesions

---

<table>
<thead>
<tr>
<th>R+0 Lesions</th>
<th>Transmural Necrosis</th>
<th>Nontransmural Necrosis</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of RF applications</td>
<td>128</td>
<td>7</td>
<td>…</td>
</tr>
<tr>
<td>RF applications duration, s</td>
<td>7 (5–10)</td>
<td>7 (6–10)</td>
<td>0.32</td>
</tr>
<tr>
<td>Impedance drop (Ω) during individual RF applications</td>
<td>12 (10–20)</td>
<td>13 (10–17)</td>
<td>0.38</td>
</tr>
<tr>
<td>Energy delivered (kJ) per individual RF applications</td>
<td>210 (150–300)</td>
<td>210 (180–300)</td>
<td>0.32</td>
</tr>
<tr>
<td>Mean force applied by the ablation catheter, g</td>
<td>10 (7–19)</td>
<td>11 (9–13)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Data are expressed as median (range). RF indicates radiofrequency.
creation beyond the heart, is somewhat simplistic because lesion formation is the result of the association of various parameters, including, inter alia, force, catheter stability, energy delivered, ablation irrigation conditions, and tissue thickness and its vascularization. Further studies are, therefore, needed to identify predictive markers of transmural and irreversible lesion creation, which could be safely and efficiently applied in humans. (3) Although some noise is inevitably present within the unipolar channel during radiofrequency applications, it does not preclude reproducible real-time monitoring of the unipolar atrial electrogram modification as the Movies I and II in the Data Supplement Bortone et al clearly illustrate. This is mainly the result of the specific and tailored CARTO settings detailed in the Methods section. (4) In the present study, pacing capture could have been used as an alternative method for transmural lesions creation assessment.12,13 In our opinion, however, immediately after radiofrequency applications have been made (acute state), the inability to capture may correspond either to a complete necrotic lesion (irreversible) or to a partial necrotic lesion (with a certain proportion of stunned cells that may subsequently totally or at least partially recover their conductive properties and lead, for example, to PV reconnection in the human clinical setting). By contrast, in the chronic state,5 the absence of capture may universally translate a necrotic state while capture may either translate normal naïve tissue (never ablated) or the recovery of the capability to be excited in prior stunned but not necrotic cells.5,9 (5) Bipolar atrial signal attenuation was not used to assess transmural lesions creation. It has been previously shown, however, that bipolar signal attenuation does not reliably assess transmural lesion creation owing to its lack of easy interpretability and reproducibility, which is not the case of the unipolar atrial signal modification (corresponding to an ON: negative component OFF: elimination of the negative component phenomenon). (6) Because our research is mainly exploratory, with the primary goal to provide histopathologic details associated with R+0, the number of alternative applications varied among lesion types and from dog to dog. (7) Finally, although no surrounding organ injuries were observed in the setting of R+0 and R+5 treatment, the upper bound of the 95% confidence interval does not rule out a low frequency of injury.

Conclusions
Elimination of the negative component of the unipolar atrial electrogram (R morphology achievement) led in the vast majority of cases to irreversible transmural necrosis creation. In some cases, however, R morphology did not translate transmural necrosis but transmural lesion only (with potential for reversibility) likely related to transient cell damage creation.

In the present animal model, our findings suggest that the extension of ablation for 5 seconds after R morphology completion (R+5) may be the most interesting approach because it was constantly associated with transmural and necrotic cardiac lesion creation, without lesion creation beyond animals’ heart.

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Disclosures
Dr Bortone is a consultant for Biosense-Webster, Inc. A. Appetiti is an employee of Biosense-Webster, Inc. The other authors report no conflicts.

References
Elimination of the Negative Component of the Unipolar Atrial Electrogram as an In Vivo Marker of Transmural Lesion Creation: Acute Study in Canines
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Supplemental Figure 1. Animal 3. Gross view of the trachea and the lungs. Please note the impact (thermal injury) of spot conventional lesions created in the RA and during SVC isolation on the animal’s right lung (white arrows).