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Full title: In vivo Lesion Index (LSI) validation in percutaneous radiofrequency catheter ablation

Running title: In vivo Lesion Index (LSI) validation

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jce.15442.

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Word count: 4923

ABSTRACT

Introduction: Lesion Index (LSI) has been developed to predict lesion efficacy during radiofrequency (RF) catheter ablation. However, its value in predicting lesions size has still to be established. The aim of our study was to assess the lesions size reproducibility for pre-specified values of LSI reached during RF delivery in an *in vivo* beating heart.

Methods: Ablation lesions were created with different values of LSI in 7 domestic pigs by means of a contact force sensing catheter (TactiCath™, Abbott). Lesions were identified during RF delivery by means of a 3D mapping system (EnSite™ Precision, Abbott) and measured after heart explantation. Histology was carried out after gross examination on the first 3 lesions to confirm the accuracy of the macroscopic evaluation.

Results: A total of 64 myocardial lesions were created. Thirty-nine lesions were excluded from the analysis for the following reasons: histological confirmation of macroscopic lesion measurement (n=3), transmurality (n=24), unfavorable anatomic position (n=10), not macroscopically identifiable (n=2).

In a final set of 25 non-transmural lesions, injury width and depth were respectively 4.6±0.6 mm and 2.6±0.8 mm for LSI=4, 7.3±0.8 mm and 4.7±0.6 mm for LSI=5, and 8.6±1.2 mm and 7.2±1.1 mm for LSI=6. A strong linear correlation was observed between LSI and lesion width ($r=0.87$, $p<0.00001$) and depth ($r=0.89$, $p<0.00001$). Multiple linear regression analysis identified LSI as

the only ablation parameter that significantly predicted lesion width ($p<0.001$) and depth ($p<0.001$).

Conclusion: In our *in vivo* study, LSI proved highly predictive of lesion size and depth.

Keywords: arrhythmias, radiofrequency energy, myocardial lesions, Lesion Index, contact force, *in vivo* model

Introduction:

Radiofrequency energy (RF) is commonly used for catheter ablation to treat several cardiac arrhythmias. The efficacy and safety of ablation are strictly related to proper RF delivery, and conventional parameters such as RF power, catheter tip temperature and impedance are regularly monitored during RF application. However, extensive assessment of these parameters in the past has revealed that their accuracy is limited.[1-3] Recently, real-time monitoring of catheter-tissue contact force (CF) has been introduced, and parameters including Force-Time Integral (FTI) and Lesion Index (LSI) have been developed in the attempt to predict safe and effective lesion formation.[4-8] LSI is a novel multi-parametric index which incorporates time, CF and electrical current recorded during RF ablation, and has been introduced to predict the extent of myocardial tissue lesions more precisely. Although LSI has been tested in previous clinical studies,[9,10] the correlation between LSI value and lesion size has never been systematically studied. In a recent i*n vitro* study, LSI was found to be highly predictive of RF lesion size and, at similar LSI values, lesions obtained by using lower power settings were similar in magnitude, but showed a superior safety profile.[11] The aim of our study was to evaluate the predictive value of LSI in terms of lesion width and depth in an *in vivo* setting.

Methods:

Study protocol

Seven female domestic pigs (Landrace X Large White) weighing 80±5 Kg were placed in the supine position after general anesthesia induced by an association of ketamine (10 mg/Kg) and midazolam (1 mg/Kg). Pigs were intubated with an endotracheal tube (size 9) and mechanically ventilated with a tidal volume of 15 ml/Kg by inhalation of oxygen and isoflurane. All the animals were treated with amiodarone (50 mg) and lidocaine (2 mg/Kg) and curarized with atracurium (2 mg/Kg initially + 0.12 mg/Kg/min). Two vascular accesses were created via a modified Seldinger technique or surgical cut-down. A 10-pole diagnostic catheter (Response™ CSL, Abbott) was positioned in the coronary sinus via the jugular vein under fluoroscopy and was used as a reference; a CF-sensing irrigated ablation catheter (TactiCath™, Abbott) was inserted into a femoral vein and advanced under fluoroscopy through the inferior vena cava toward the right atrium (RA). A 3D cardiac mapping system (EnSite™ Precision, Abbott) was used to map the chamber geometry and identify sites of lesions for matching after heart explantation. The left chambers were mapped via a trans-septal approach. RF power was fixed to 30 W and RF delivery was set in the temperature-control mode (42°C upper limit) with saline irrigation of 17 ml/min, according to the manufacturer's instructions for use (IFUs).

To better recognize the lesions in the *ex vivo* analysis, we performed them at a minimal distance of about 2cm. A few number of RF delivery in the ventricles per animal was imposed to avoid the ventricular fibrillation (VF).

Ablation lesions were created in all cardiac chambers focusing on non-transmural lesions to obtain a complete measurement of lesion depth. Transmural lesions were excluded from the analysis because transmurality prohibited measurement of lesion depth. During RF delivery, an average CF ranging from 10 g to 30 g was targeted, with the aim of one

of the following LSI values: less than 4, 4, 5 and 6. RF was delivered only when 3 stability criteria were simultaneously met, according to the IFUs of the mapping system: Average Contact Force, Constant Contact and Stable Contact Force. The number of lesions created was planned in such a way that the distance from one lesion to the next would be sufficient to allow accurate lesion identification after heart explantation. The pigs were sacrificed soon after the procedures.

This study was an investigator-driven study and Abbott provided only financial support. An executive steering committee composed by S. Themistoclakis, C. Tondo and V. Calzolari was responsible for the design and oversight of the study. All the authors had the access to analyzed and interpreted the data and reviewed and approved the report. The study was approved (authorization number 333/2017-PR) by the competent Italian authorities (Ministry of Health and National Institute of Health) and all the animal procedures (including housing, health monitoring, restrain, dosing, etc.) were performed in accordance with European and national rules on the protection of animals used for experimental purposes.[12, 13]

Lesion size measurement

Once the heart had been explanted and the blood evacuated, the positions of the ablation lesions were matched with the geometry created by the 3D mapping system (fig. 1) for lesion identification and labeling. Each lesion was labeled with the following information in the data-collection form: Animal ID, Lesion ID/number, cardiac chamber, ablation parameters (Power, RF duration, Impedance, Temperature, Energy, CF, FTI, LSI).

Each ablation lesion was cut through at its center, perpendicularly to the surface. Measurements of width and depth were taken at the border of necrotic cell tissue by

means of a caliper with a 0.1 mm resolution; a photograph was taken (fig. 2) of each lesion. At least 3 investigators independently measured the lesions, and, after agreement among themselves, entered the values into the dataset. Three ablated tissue samples were stored in 4% phosphate buffered formaldehyde and submitted for gross and histological examination to verify the accuracy of the above method of lesion measurement.

Histological examination

Myocardium samples with lesions, after fixation in 4% formaldehyde in phosphate buffer 0.1M at pH 7.2 and dehydration in ethanol crescent series were embedded in paraffin; 4-5 μm thick sections were stained with hematoxylin-eosin to detect cells and Azan Mallory, Heidenhein modified stain to detect both cells and extracellular matrix. Lesion dimensions were measured both on gross sections and micro-sections (modified Azan Mallory-Heidenhein stained sections) by means of a calibration bar. Measurements were assessed by an image-analysis system consisting of a Zeiss Axioplan 2 optical microscope (Carl Zeiss, Oberkochen, Germany) equipped with an AxioVision digital camera (Carl Zeiss, Oberkochen, Germany) and Image PRO-Plus 5.1 morphometrical image-analyzing software (Media Cybernetics, Silver Spring, MA, USA).

Statistical Analysis

One-way analysis of variance (ANOVA) used width or depth measurements of lesions as the dependent variable and the nominal LSI value as a factor. The differences between nominal LSI groups of measurements were tested by means of Bonferroni's post-hoc test. Pearson's linear regression analysis was used to estimate the quantitative relationship between LSI as predictor variable and width or depth as dependent variable.

Multiple linear regression analysis by means of the forward stepwise approach, with width or depth as the dependent variable, was used to select the best predictors from among the ablation parameters: LSI, FTI, RF duration, Impedance Drop, Average Temperature, Energy, Average Contact Force and Average Power.

Multiple interactions among the various ablation parameters were tested; if an interaction was significant, to limit the covariate number only the most significant one was entered.

All analyzes were made by means of IBM-SPSS Statistics v23 (Chicago, IL, USA) and STATISTICA v12 (StatSoft Inc. Tulsa, OK, USA).

Results:

A total of 64 lesions were created. Twenty-six lesions were excluded from the final analysis because either transmural (n=24) or not macroscopically identifiable (n=2). The transmural lesions were mainly detected in the atria (n=22, 92%) with LSI ≥ 4 and only 2 were obtained in the RV with higher LSI value (LSI 5 and 6, respectively). None of the lesions performed in the atria were non-transmural and then excluded from the analysis. Of the 38 non-transmural lesions (24 performed in the RV and 14 in the LV), the first 3 were used for the histology analysis and were therefore excluded from the analysis. Other 10 non-transmural lesions were excluded because it was impossible to measure their width and/or depth accurately owing to their unfavorable anatomical position (lesions performed close to papillary muscles, inside the trabeculations or under the valvular leaflet). Finally, a set of 25 non-transmural lesions were considered for the analysis.

No steam-pops occurred during the procedures and no char formation was observed afterward. In 3 pigs, VF occurred during RF delivery in the RV or LV; in one case, sinus rhythm was restored by external cardioversion (CVE), while in the other 2 cases VF was

not reversible; in these 2 cases, the procedure was prematurely interrupted and only the lesions created up to the cardiac arrest were considered.

Lesion size and relationship with LSI

In the final set of 25 non-transmural lesions, lesion width observed for nominal LSI=4 was 4.6±0.6 mm; for LSI=5 it was 7.3±0.8 mm and for LSI=6 it was 8.6±1.2 mm (fig. 3). On ANOVA, the effect of LSI on the resulting width was statistically significant ($F(2.22) = 43.09$, $p<0.00001$). Lesion depth was 2.6±0.8 mm for nominal LSI=4, 4.7±0.6 mm for LSI=5 and 7.2±1.1 mm for LSI=6 (fig. 3). On ANOVA, the effect of LSI on depth was statistically significant ($F(2.22) = 59.79$, $p<0.00001$). The post-hoc Bonferroni tests comparing increasing LSI values from 4 to 6 showed significant increments in lesion width and depth (fig. 3).

A strong, linear regression was observed between LSI and lesion width (fig. 4; $r=0.87$; $p<0.00001$) and depth (fig. 4; $r=0.89$; $p<0.00001$); the width (W) and depth (D) of the resulting lesions appear to be well predicted by the formulas W (mm) = - 4.21 + 2.17 * LSI, and D (mm) = - 7.54 + 2.54 * LSI. Figure 5 summarizes the relationships between the width and depth of lesions obtained by means of the 3 different LSI levels.

Each of the other experimental measurements recorded (FTI, RF duration, Impedance Drop, Average Temperature, Energy, Average Contact Force, Average Power) appeared to affect lesion width and depth. However, their contributions were overshadowed by LSI. Indeed, multiple linear regression models with a forward stepwise approach selected only LSI as a significant predictor of lesion width (R Square=0.75; F=68.7; B coefficient 2.17 (95% C.I. 1.63-2.71); $p<0.001$) and depth (R Square=0.80; F=94.9; B coefficient

2.43 (95% C.I. 1.92-2.95); $p<0.001$), even after checking for multiple interactions. The results of univariate and multivariate linear regression analysis were reported in the table. About transmural lesions, to which the analysis of depth is not applicable, a significant, linear regression between lesion width and LSI was observed ($r=0.43$, $p<0.05$). Lesion width was 6.0±1.7 mm for LSI=4, 7.4±1.7 mm for LSI=5 and 8.1±2.8 mm for LSI=6; these values were not significantly different from those of non-transmural lesions ($p=0.12$).

Histological examination

In one of the 3 samples that were sent for histological examination, the lesion could not be assessed, as it was located within a trabecular space. The other two ablation samples showed an acute injury of the myocardium, consisting of coagulative necrosis in the inner zone surrounded by a border zone in the middle (fig. 6 and 7) with wavy fibers and contraction bands of the myocytes and an outer zone with hyperemic vital myocardium. The endocardium showed no signs of alteration or mural thrombosis, except for the denudation of endothelium. Comparison between the gross and the micro measurements of necrotic areas revealed an average difference of 0.23 mm (range 0.16-0.3 mm), confirming the accuracy of the methods used for gross measurements.

Discussion:

Main Findings

In this *in vivo* study, LSI demonstrated a high value in predicting the size of ablation lesions. Indeed, among the ablation parameters considered, LSI was the only significant predictor of lesion width and depth. LSI-guided ablation proved safe and effective, given the linear correlation between LSI and lesion width and depth and the absence of charring

or thrombus formation on the ablation lesions or on the catheter tip. Moreover, the absence of tissue overheating - i.e. steam pops - confirmed the safety profile of LSI-guided ablation.

Comparison with other indexes

Traditionally, in addition to RF power and ablation duration, electrophysiologists used conventional parameters, including impedance, signal amplitude, catheter tip temperature and tactile feedback, in order to control RF ablation lesions. Previous studies have already shown their low reliability in predicting lesion width and/or depth.[21] The contact force (CF) between the catheter tip and the myocardium has been identified as an important determinant of lesion size,[22-28] and several studies have shown the safety and efficacy of ablation procedures in which this index is taken into account.[6,7,29] Lesion formation depends not only on CF, but also on several other parameters,[3] which can combine in a complex way, including RF power and RF delivery time. Consequently, other indices have been developed in the attempt to assess the quality of the lesion. The Force-Time-Integral (FTI), defined as the total CF integrated over the time of RF delivery,[25] has proved to be inversely associated with gap formation, and has shown a positive correlation with better outcome at 12 months after the ablation procedure in patients with paroxysmal atrial fibrillation.[4, 5, 30, 31] However, FTI does not take into account the important role played by RF power. The Lesion Index (LSI) was therefore developed[32] as a function of 3 independent variables: force (F), time (t) and electrical current (I), as described by the formula:

$$LSI(F,I,t) =$$

$$k1 * \left(f2\left(1 - e^{\frac{-F}{f1}}\right) + f0\right) * i2\left(1 - e^{-\left(\frac{I}{i1}\right)2}\right) * \left\{(1 - k0) + k0\left[\left(1 - e^{\frac{-t}{\tau}}\right)/\left(1 - e^{\frac{-60}{\tau}}\right)\right]\right\}$$

where f0, f1, and f2 are force parameter coefficients, i1 and i2 are electrical current coefficients, k0 is a diffusive heating coefficient, and τ is a characteristic time value, the coefficients being derived from the best curve fit with experimental data acquired during preclinical studies and the power proportional to the square of the electrical current. The Ablation Index (AI) also takes into account CF, RF time and RF power, though with a different formula.[17] AI and LSI are not directly comparable, owing to the different technologies used for the contact force sensors in the ablation catheters. However, animal studies of AI have reported a good match between the predicted and the actual lesion depth, although these studies provide no information on lesion width.[17,18] Likewise, no information on lesion size is reported in recent clinical studies involving AI.[7, 19, 20]

LSI was clinically tested by invasively reassessing patients from the EFFICAS I study at 3 months post-ablation in search of reconnection gaps;[9] this study found a strong correlation between LSI and PV isolation. Similar results were replicated in an *in vivo* animal model.[10] LSI has recently been tested also *in vitro* and has shown a higher correlation with lesion width and depth than FTI.[11]

To the best of our knowledge, our study is the first to systematically investigate the relationship between lesion size and LSI value in an *in vivo* setting. Our experience confirmed the *in vitro* findings: lesion size (both width and depth) appeared to be predicted by LSI. This was also the case when high FTI values were reached for several reasons, including automatic raising of RF power due to temperature control and subsequent increase in RF delivery times. Even in the case of low CF, LSI seemed to be a reliable index of lesion size. This finding could be of relevance to clinical practice, as the need to ablate in positions in which a good CF cannot be achieved is not infrequent.

Further confirmation of the safety of RF therapy and, in particular, of LSI is provided by the fact that we detected no damage to the tissues close to the cardiac walls in which transmural lesions were created. The acute pathologic substrates following endocavitary ablation consisted of a wavy front injury, from the endocardium to the epicardium, with an inner zone of coagulation necrosis, a mid-wall border zone of contraction bands necrosis and an outer sub-epicardial zone with surviving cardiomyocytes and intense hyperemia, with intact mesothelial cells; no complications occurred, like cardiac rupture or mural thrombosis. Moreover, the use of LSI enabled lesions - including ventricular lesions - to be reliably reproduced, even in the presence of cardiac wall thickness ≥ 5mm, without any pop and/or charring.

According to our results, lesion width and depth can be reliably predicted at different values of LSI. Although our results provide no information on the "drag" ablation technique, they indicate that LSI could play an important role in predicting lesion width during ablation by means of the point-by-point technique, and suggest the maximum distance that the catheter tip can be shifted to ensure a continue ablation line with no gaps. In that sense, a recent work in an *in vivo* model showed indeed that LSI guided ablation can facilitate continuous lesions.[33]

Moreover, in the POWER-FAST PILOT study, lower average LSI was correlated with higher reconnections lesions.[34] Similarly, the use of LSI to predict lesion depth could play an important role in obtaining transmurality in the tissue portion to be treated, thereby avoiding the risks related to unnecessary, excessive RF delivery. A direct comparison with humans cannot be made; however, the weight (~80Kg) of the pigs used was chosen to be as close as possible to that of patients in clinical practice, and the impedances

detected during RF ablation in these animals (max 140±14 Ω; min 93±8 Ω) were extremely close to those reported in human beings.[14] The transmural lesions were excluded from the analysis in order to obtain a valuable information about the depth. All lesions performed in the atria were excluded for the analysis because transmural in chambers apparently thinner than human[15] (average depth of 1.9±1.1 mm in the RA and 2.1±0.9 mm in the LA) and a complete measurement of lesion depth was prohibited. Therefore, our findings are related specifically to ventricular chambers and should not be directly translated to LA and RA chambers due to differences as chamber contractility, anatomy, catheter orientation and blood flow. According to the data collected in our study and the reported measurements of human heart wall thickness,[15] it may be only hypothesized that a minimum value of LSI enables transmural lesions to be created in typical anatomical sites of interest in humans; for example, the posterior wall of the left atrium (average thickness = 4.1 mm, for which an LSI $\geq$ 4.8 should be reached), the circumferential muscle between left pulmonary veins and left atrial appendage (average thickness = 5.0 mm, for which an LSI $\geq$ 5.2 should be reached) or the interatrial septum (average thickness = 5.5 mm, for which an LSI $\geq$ 5.4 should be reached). Although lesser atrial wall thicknesses have recently been reported in MRI studies[16] and our results need to be confirmed in chronic assessments and in human trials, these LSI values appear to be in line with those suggested to obtain longstanding lesions during PV isolation in previous clinical studies.[9, 10]

Study limitations:

Although we tried to maximize the number of RF applications, the total number of lesions was rather low, and the final number of analyzable lesions was further limited by

several factors. Moreover, none of the atrial lesions were considered for the final analysis because transmural in this animal model, therefore the hypothesis of minimum LSI value for transmural lesion in the human atria need to be verified in further studies. Although the greatest attention was paid during lesion measurement, the mechanical caliper used for the macroscopic evaluation of the lesions has limited accuracy, and involuntary tissue stretch or compression could have occurred. Only one RF power setting was investigated; this choice was prompted by the previous *in vitro* experience,[11] which did not show a significant impact of RF power on the size of the lesions obtained with the same LSI. However, given the small sample size and that important variables such as RF power and CF were either fixed or varied over a narrow range, the lack of statistical significance could then not necessarily correspond to the lack of an important effect that each ablation parameter other than the LSI could have on the lesion size. Finally, as catheter orientation in the beating heart could not be controlled, it was not considered in the analysis, although LSI appeared to include the effects of this real-life phenomenon.

Conclusions:

In this study, LSI proved to be highly predictive of lesion size in an *in vivo* model. This tool could be very useful to guide catheter ablation in term of efficacy and safety. Further clinical studies are necessary to confirm the potential benefits of LSI-guided ablation procedures in humans.

Acknowledgments:

The authors would like to thank Franco Noventa (Senior Scientist, Department of Molecular Medicine, University of Padova, Padova, Italy) for performing the statistical analysis.

Sources of Funding:

This study is funded by Abbott that provided an unrestricted grant for this investigation.

Data availability statement:

The data that support the findings of this study are available on reasonable request from the corresponding author.

Disclosures:

Igor Caporaso and Stefano Indiani are Abbott employees. Claudio Tondo serves as Scientific Consultant and Proctor for Abbott and receives lecture fees from Abbott, Medtronic, Boston Scientific and Biosense Webster. Gaetano Fassini serves as proctor for Abbott. Alessandro Addis is the Chief Executive Officer for the CRABCC Animal Facility and has an ongoing collaboration agreement with Abbott. Gaetano Thiene is the President of the Association for Research of Arrhythmic Cardiac Diseases (ARCA) based in University of Padova; ARCA received an unrestricted grant from Abbott. The other authors report no conflicts of interest to declare.

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Figures legends:

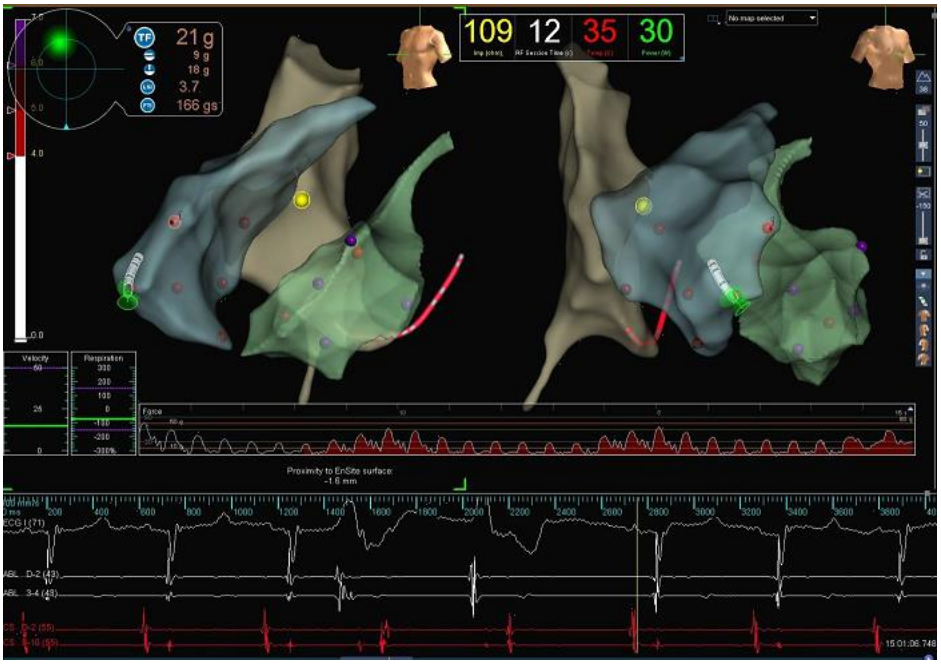

Figure 1 – 3D anatomy, lesions map and ablation parameters while creating a lesion

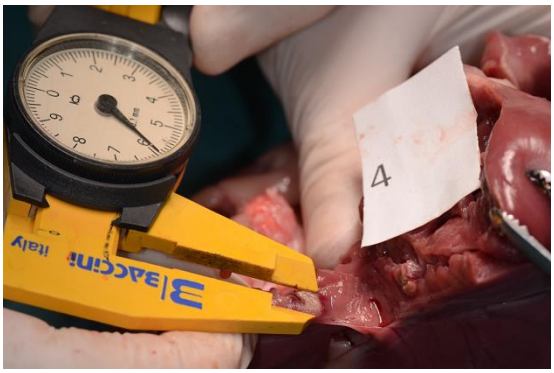

Figure 2 - Lesion measurement

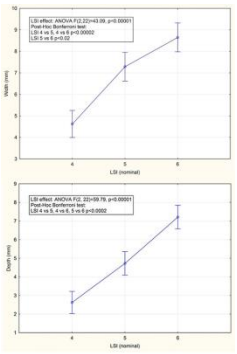

Figure 3 – Relationship between lesion width and depth and LSI

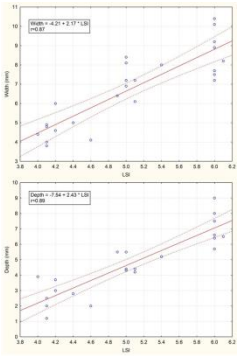

Figure 4 – Linear regression between LSI and lesion width ($p<0.00001$) and lesion depth ($p<0.00001$)

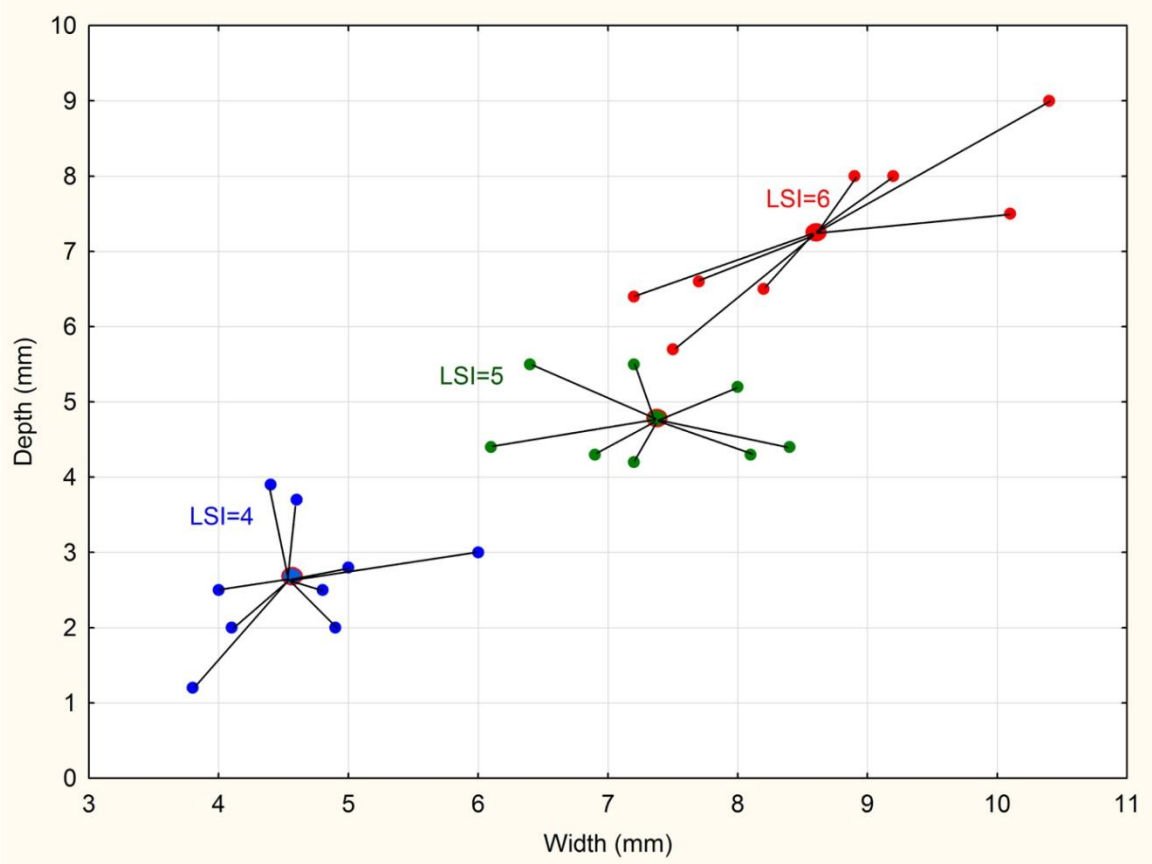

Figure 5 - Schematic representation of the "spread" of lesion size obtained by targeting LSI values of 4 (blue dots), 5 (green dots) and 6 (red dots) from average size. Central illustration

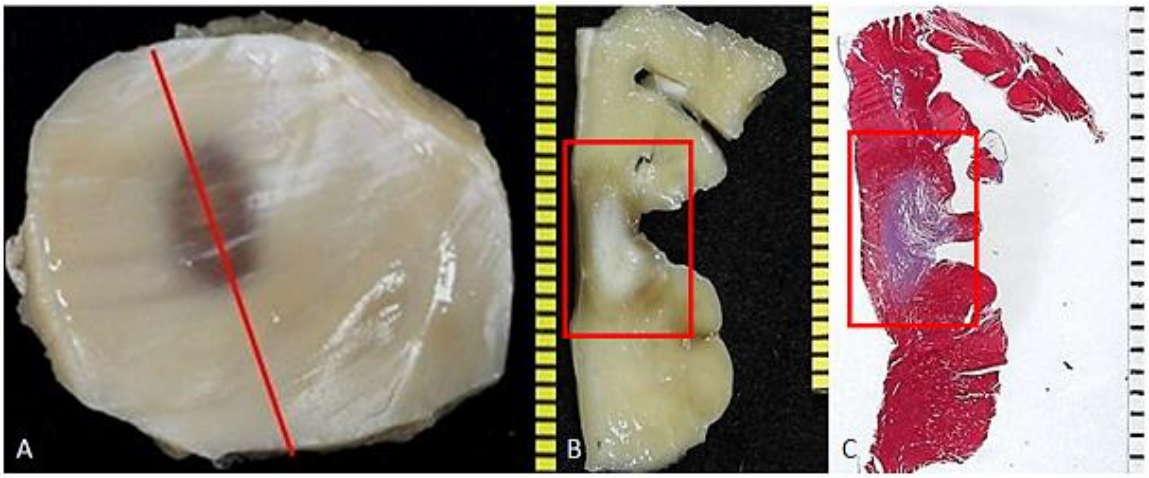

Figure 6 – Histological assessment, endocardial ablation on RV. A) Epicardial view with brown spot; transmural section is indicated (red line); B) transmural gross section with yellow injury surrounded by brown area (red square); C) histology section of B (Azan Mallory Heidenhein modified staining)

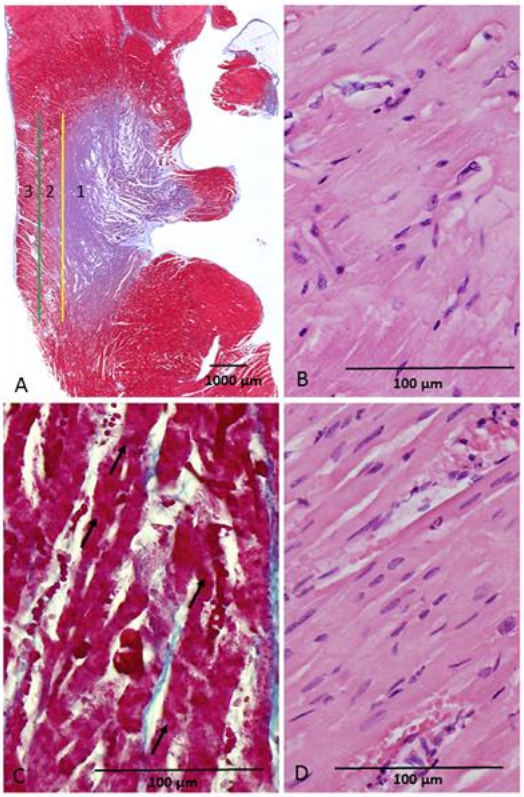

Figure 7 - Close-up of Fig. 6. A) Inner (1), Middle (2) and Outer injury zones (3). B) Coagulative necrosis (inner region); C) contraction bands necrosis (arrows) in the middle region; D) hyperemic vital myocardium of the outer region. A, C) Azan Mallory Heidenhein modified staining; B, D) haematoxylin-eosin staining. A) 5x original magnification, B-D) 200x original magnification.

Table: Results of univariate and multivariate linear regression analysis

	Dependent: Width			Dependent: Depth		
	Univariate		Multivariate	Univariate		Multivariate
Covariates	r	p=	p=	r	p=	p=
FTI	0.248	0.223	X	0.392	0.048	0.838
LSI	0.869	<0.001	0.001	0.889	<0.001	0.001
Impedence_Drop	0.369	0.063	0.825	0.270	0.182	X
Avg_Temperature	0.513	0.007	0.142	0.529	0.005	*
Energy	0.495	0.010	*	0.620	0.001	0.218
Avg_Power	0.015	0.941	X	0.099	0.632	X
Avg_Contact Force	0.379	0.056	0.948	0.370	0.063	X

* Because of the high interaction between Average Temperature and Energy, we entered the most significant one.
Avg: Average; FTI: Force-Time Integral; LSI: Lesion Index.