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1 Tissue Characteristics Underlying Endocardial Local Impedance Subtypes 2 in Chronic myocardial Infarction

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Short title: Local impedance underlying tissue composition

Short tweet: Local impedance subtypes associated with distinct tissue composition by means of CMR and histology. #Epeeps #AblateVT

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3 ABSTRACT

4 **BACKGROUND** Local impedance (LI) mapping is feasible and provides additional tissue
5 characterization of the ventricular tachycardia substrate. Data on tissue composition underlying
6 the LI spectrum are lacking.

7 **OBJECTIVE** To describe the tissue composition underlying different LI subtypes in a chronic
8 myocardial infarction (MI) swine model.

9 **METHODS** One month after non-reperused anterior MI, eighteen Landrace Large White pigs
10 underwent delayed-enhancement cardiac magnetic resonance (DE-CMR) and endocardial left
11 ventricular (LV) LI mapping. DE-CMR images were post-processed off-line to obtain LV wall
12 thickness, scar subtypes, and border-zone (BZ) corridors, and were co-registered with LI maps.
13 Tissue samples were obtained from abnormal LI sites.

14 **RESULTS** Low LI zones exhibited more pronounced wall thinning compared to intermediate LI
15 tissue (2.8 ± 0.7 vs 3.8 ± 0.9 mm; $P < 0.001$) and correlated with DE-CMR dense endocardial
16 scarring (91.4%) and with epicardial scarring (75% dense and 24% BZ tissue). Intermediate LI
17 tissue exhibited predominantly subendocardial scarring, with more heterogeneous distribution
18 (45% dense, 47% BZ, and 8% healthy tissue) and less epicardial involvement (73% healthy tissue).
19 Most DE-CMR BZ corridors (75.6%) co-localized with intermediate LI tissue. Histologically, tissue
20 from intermediate LI zones displayed less collagen I ($P = 0.008$), collagen III ($P = 0.053$), and
21 collagen volume fraction ($P = 0.021$), and greater vascular density ($P = 0.075$), compared to low LI
22 zones.

23 **CONCLUSIONS** Areas of low LI had higher proportion of dense, transmural scar and wall thinning
24 compared to intermediate LI areas. DE-CMR BZ corridors colocalized with intermediate LI in
25 most cases. LI subtypes showed distinctive histological composition.

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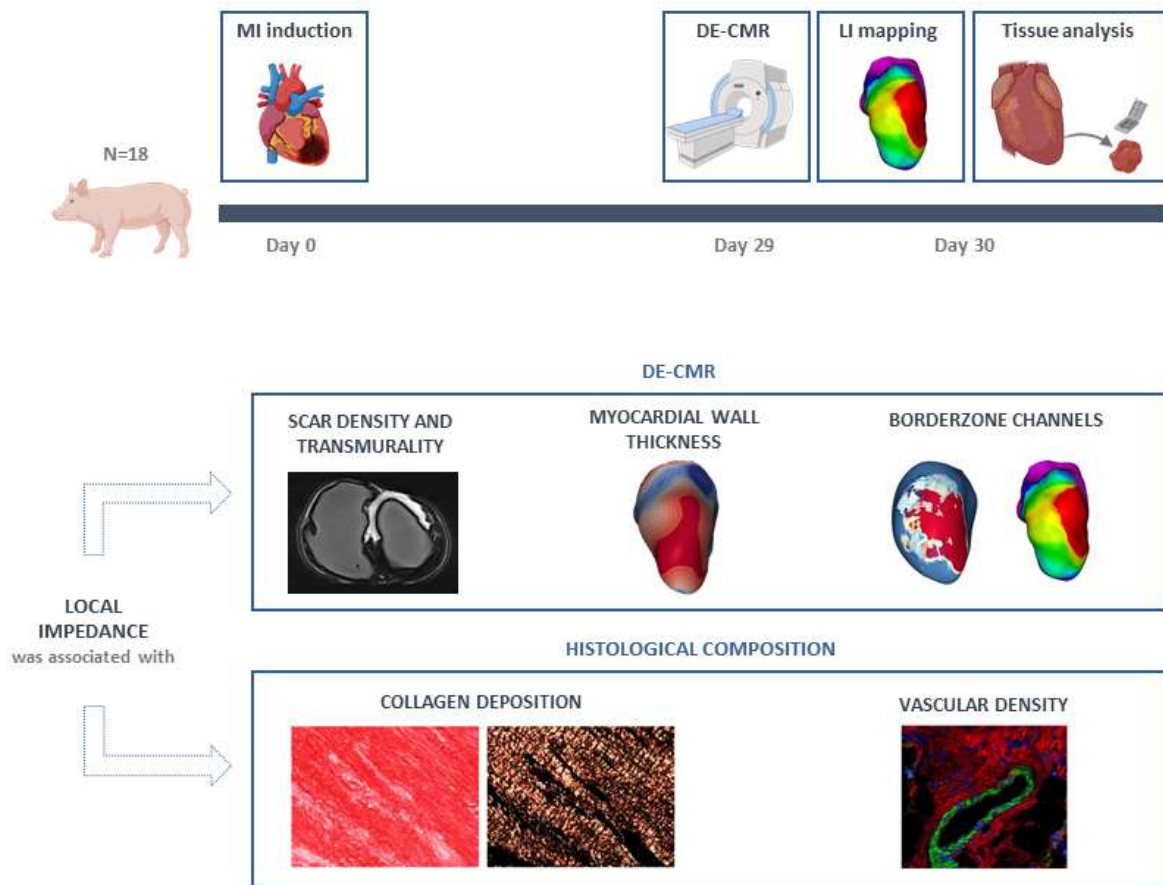
28 **KEYWORDS:** local impedance, ventricular tachycardia, catheter ablation, substrate mapping,
29 tissue characterization

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30 GRAPHICAL ABSTRACT



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32 **ABBREVIATIONS AND ACRONYMS**

BP = blood pool

BZ = border zone

DE-CMR = delayed enhanced cardiac magnetic resonance

LI = local impedance

LV = left ventricular

LVWT = left ventricular wall thickness

MI = myocardial infarction

PSI = pixel signal intensity

VT = ventricular tachycardia

Col I = collagen type I

Col III = collagen type III

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34 INTRODUCTION

35 Local impedance (LI), a biophysical property, is defined as the passive opposition that tissue
36 exerts to electric current, and is determined by the resistance and capacitance of intracellular
37 and extracellular compartments. LI measurement is a useful tool for characterizing the intrinsic
38 structural composition of tissue. In myocardium, LI can distinguish scar tissue from ischemic or
39 healthy tissue, since LI is lower in scars.¹⁻² This difference may be related to greater fibrotic
40 content and extracellular matrix in the infarcted scar.³ There remains a need for more
41 information about the distinctive tissue composition underlying different LI subtypes.

42 Myocardial LI measurements were initially performed using cell preparations or animal
43 models, due to the prolonged time and invasive techniques required. Currently, commercially
44 available catheters that provide real-time LI measurement have allowed for new and easier LI-
45 based mapping and ablation techniques.⁴ New tools are particularly required for ventricular
46 tachycardia (VT) ablation procedures, where arrhythmogenic substrate characterization can be
47 particularly challenging. The clinical application of LI substrate mapping was first described in the
48 atrium, where LI could detect native fibrosis and scarred tissue from previous ablation
49 procedures.⁵ A recent pre-clinical study demonstrated that LI mapping is also feasible in the
50 ventricle, providing valuable substrate information. Specific LI patterns demonstrated to predict
51 the location of critical components of scar-related VT, providing potential new targets for
52 ablation in substrate-based procedures.⁶

53 LI-based technology has provided the opportunity to better characterize VT
54 arrhythmogenic substrate. However, there are no available data regarding the tissue
55 composition underlying different LI values. Thus, in the present study, we aimed to describe the
56 tissue composition underlying different LI subtypes, by performing cardiac magnetic resonance
57 imaging (CMR) and histopathology in a chronic myocardial infarction (MI) swine model.

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59 **METHODS**

60 **MYOCARDIAL INFARCTION PORCINE MODEL.** Twenty-one Landrace X Large White pigs
61 underwent a non-reperfused anterior MI procedure, as previously described.⁷ Under general
62 anesthesia, MI was induced by percutaneous selective coil embolization (VortX-18 Diamond 3
63 mm/3.3 mm coil, Boston Scientific/Target, Natick, MA, 13 USA) of the left anterior descending
64 coronary artery, after the first diagonal branch. Complete thrombotic occlusion was confirmed
65 by persistent ST-segment elevation, and by repeating coronary angiography 10 minutes after coil
66 implantation (TIMI flow score = 0). The animals were recovered and survived for 30 days after MI
67 induction.

68

69 **CARDIAC MAGNETIC RESONANCE.** On day 29 post-MI (before LI mapping), delayed contrast-
70 enhanced cardiac magnetic resonance (DE-CMR) was performed using the 3T MRI scanner
71 (Canon Vantage Galan 3T, Canon Medical Systems) under general anesthesia, during apnea, and
72 synchronized with ECG. At 10 minutes after administration of intravenous gadolinium contrast
73 (0.2 mmol/kg gadobutrol; Gadovist; Bayer, Berlin, Germany), conventional delayed enhanced
74 images were obtained using a well-established segmented 3D inversion recovery gradient-echo
75 sequence.

76 DE-CMR images were post-processed using ADAS 3DTM software (Galgo Medical SL,
77 Barcelona, Spain). Left ventricular (LV) endocardial and epicardial contours were manually
78 traced for segmentation and 3D reconstruction of the ventricle. Ten concentric equally spaced
79 surface layers, from 10-90% of the LV wall thickness, were automatically created from the
80 endocardium to the epicardium of the LV. The pixel signal intensity (PSI) of the DE-CMR
81 sequence was projected in each layer of the shell, and a maximum-pixel-intensity-based
82 algorithm was used to define core, border zone (BZ), and healthy tissue (>60%, 40-60%, and
83 <40% of maximum pixel intensity, respectively). LV wall thickness (LVWT) was automatically

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84 calculated. BZ corridors were automatically detected according to the standard definition: a
85 continuous corridor of BZ surrounded by dense scar or by core area and an anatomic barrier,
86 connecting two areas of healthy myocardium.⁸

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88 **LOCAL IMPEDANCE MAPPING.** At 30 days post-MI, the animals underwent invasive LI mapping.
89 A point-by-point endocardial LI map of the LV was performed in sinus rhythm via retrograde
90 aortic access, using the Rhythmia HDx 3D mapping system and Intellanav MiFi OI™ ablation
91 catheter (Boston Scientific, Massachusetts, USA), as previously described.⁶ Briefly, LI map was
92 created by manual annotation of the LI value (in ohms; Ω) obtained at each endocardial point, on
93 a local activation time map as activation time in milliseconds (filling threshold of 6 millimeters). A
94 three-dimensional map depicting the LI measurements (false activation map) was obtained,
95 being the “earliest” activation equivalent to the lowest LI. Since LI is lower in scar than in blood,
96 baseline blood pool (BP) was used as the inferior cut-off threshold, and a 10- Ω scale range was
97 established to define low ($<BP - 1\Omega$), intermediate ($>BP - 1$ and $\leq BP + 9\Omega$), and high tissue
98 resistance ($>BP + 9\Omega$).¹¹ The LI corridor was defined as a myocardial tract with higher resistivity,
99 connecting two healthy areas and surrounded by lower LI tissue.⁷ A standard cut-off was used to
100 define the LI corridor. LI corridors after cut-off adjustment could not be detected due to
101 limitations of the ADAS 3D software. LI gradient was identified by an iso-impedance map as
102 previously described.⁶ For inter-animal comparison, LI values were standardized by calculating
103 the LI ratio (LIR = tissue LI/BP LI) for each point. According to the LIR distribution, LIR values of <1
104 and >1.15 were consistently associated with low and high tissue resistance, respectively
105 **(Supplemental Figure 1).**

106 Within a subgroup of five animals, a navigated needle-catheter (MyoStar injection catheter
107 (BioSense Webster) was used to inject Evans blue at sites with abnormal LI (low and
108 intermediate). This provided an anatomical reference to guide ex vivo tissue sampling. Each
109 animal received four 0.2-mL injections (2 in low LI, and 2 in intermediate LI).

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110 **VENTRICULAR TACHYCARDIA INDUCIBILITY AND MAPPING.** Programmed ventricular
111 stimulation protocol was performed from right and left ventricle with S1 trains of 400, 350, and
112 300 ms up to S5 coupled at 20 ms above the ventricular effective refractory period. If an
113 hemodynamically tolerated sustained monomorphic VT was induced, a high-density activation
114 map was performed. Activation maps were accepted if they accounted for at least 80% of
115 tachycardia cycle length. VT isthmus was defined as the central part of the diastolic pathway
116 exhibiting mid-diastolic potentials. Corregistration between LI, VT activation maps and DE-CMR
117 data was performed off-line.

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119 **CARDIAC MAGNETIC RESONANCE AND LI MAPPING CORRELATION.** The LI maps (*visualization*
120 *toolkit* -VTK- files) were imported in ADAS 3D software, and the LI maps and DE-CMR
121 were coregistered using landmarks. Manual adjustments (*manual alignment* tool) were
122 allowed to ensure adequate structure superposition. LI points were projected on all DE-
123 CMR layers (from 10% to 90%) (**Figure 1**). LI (Ω) was compared with PSI (%) and LVWT
124 (millimeters) using the *statistical correlation* tool. For statistical correlation, a maximum
125 2-mm match tissue radius was allowed. Correlation with PSI was conducted using the
126 20% and 80% layers to obtain endocardial and epicardial data, respectively; 10% and
127 90% were discarded due to a higher likelihood of error. The distance of the LI points to
128 DE-CMR BZ corridors was calculated using points <2 mm away from a BZ corridor.
129 Correlation between DE-CMR BZ corridors and standard LI corridors was analyzed.

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131 **TISSUE COLLECTION AND HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS.**
132 Histological and immunohistochemical analyses were conducted on low and intermediate LI
133 samples from a subgroup of five animals. These animals were pharmacologically euthanized
134 with an overdose of pentobarbital sodium (200 mg/kg). Next, the hearts were explanted, and
135 the LV dissected. Endocardial Evans-blue tags were identified, and guided sample extraction

(**Supplemental Figure 2**). Five-mm tissue samples were collected, which were fixed in 10% formalin and embedded in paraffin for histological analysis, or frozen in optimal cutting temperature (OCT) for immunohistochemical analysis. Fibrosis and adipose deposition were evaluated using Masson's trichrome staining on 4- μ m-thick paraffin sections. Interstitial collagen type I (Col I) and type III (Col III), Col I/III ratio, and collagen volume fraction (CVF) were analyzed using PicroSirius Red staining. Images were acquired with an Olympus CKX41 and Leica DMI6000B microscope. Col I and Col III were quantified using Image-Pro Plus software (6.2.1 version). Vessel density was evaluated by immunostaining for biotinylated *Griffonia simplicifolia* Lectin I Isolectin B4 (IsoB4; 1:25; Vector Labs), and Streptavidin-Alexa 568 (1:500; Invitrogen). Images were acquired with a STEDYCON confocal microscope (Abberior). Cx43 and SERCA2 expressions were assessed within the infarct zone of the OCT-frozen tissue via immunofluorescence staining using the following antibodies: anti-Cx43 (1:200; Sigma-Aldrich), anti-SERCA2 (1:50; Santa Cruz), and anti-cTnI (1:100; Abcam). Images were acquired with an LSM 980 confocal microscope (Zeiss). Data are presented as Cx43 or SERCA2 expression relative to cTnI expression.

ETHICAL IMPLICATIONS. The study was approved by the Animal Experimentation Ethics Committee of Germans Trias i Pujol Health Research Institute (IGTP) and the relevant Government Authorities (Generalitat de Catalunya; Code: 11208). This study complied with all guidelines regarding animal use for research and education purposes, as defined by the Guide for the Care and Use of Laboratory Animals. Research was conducted at the Comparative Medicine and Bioimage Center of Catalonia (CMCiB).

STATISTICAL ANALYSIS. All analyses were performed using R software version 4.3.0. Categorical variables are described by the number of observations in each category, and the percentage by group. Continuous variables are described by the number of cases, mean, median, standard

deviation, or interquartile range depending on the distribution. The association between LI and tissue thickness was assessed using a linear mixed regression model to account for the pig cluster effect. The estimated model included the log transformation of tissue thickness as the dependent variable, local impedance as the independent variable, and pig identification as the cluster. We assessed the linearity of the LI effect by comparing the model's likelihood with a linear effect and polynomial effect. Regression coefficients are reported with 95% confidence intervals. All assumptions of the linear mixed effects model were validated. The same procedure was used to assess the association between LI and endo and epicardial PSI, and associations among LIR, tissue thickness, and endo-epi PSI. A Mantel-Haenszel chi-square test was performed to assess possible linear associations between LI level (low, intermediate, or high) and tissue category (core, BZ, or healthy). Kendall's Tau rank correlation coefficient was calculated to assess the strength and direction of the relationship between the above variables. Linear mixed models were used to compare histological and immunohistochemical measures between low and intermediate LI zones.

RESULTS

Twenty-one Landrace X Large White pigs underwent MI induction. Three animals died due to ventricular fibrillation during the procedure. The remaining 18 animals (12 male and 6 female) were recovered and survived for the 1-month post-MI period. **Table 1** summarizes the LI mapping and DE-CMR baseline characteristics.

LOCAL IMPEDANCE MAPPING. At 1 month post-MI, left ventricular LI mapping was successfully performed in 18 animals (mean weight: 45.2 ± 4.8 kg). A total of 1233 LI points were obtained in the 18 animals (median: 74 [55-82] per animal). The median LI-BP was 75 [72-79] Ω . Mapping revealed no association between BP and hematocrit ($29.8 \pm 2.0\%$, $P = 0.408$), leukocytes (24.0 ± 5.9 , $P = 0.834$), or temperature (35.3 ± 0.75 , $P = 0.428$). Most cases ($n = 17$) exhibited a large

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188 anterior area of abnormal LI (low or intermediate), with a median abnormal LI area of 20.0 [14.3-
189 23.2] cm². Low LI points (LIR < 1) were detected in 88% (n = 16). The median LIR at low,
190 intermediate, and high LI points was 0.95 [0.91-0.97], 1.06 [1.03-1.09], and 1.41 [1.24-1.60],
191 respectively. Using standard cut-offs, LI corridors were identified in six animals.

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193 **CARDIAC MAGNETIC RESONANCE AND LI MAPPING CORRELATION.** Cardiac magnetic
194 resonance imaging was performed in all 18 animals. One was excluded from DE-CMR analysis
195 due to poor image quality, and two were excluded from analysis of correlation with the
196 epicardial layer due to poor projection accuracy (n=18, n=17 and n=15 for LVWT, endocardial
197 and epicardial PSI correlation, respectively). In total, 1129 LI points were considered for analysis,
198 and projected on the 3D LV surface; 104 LI points (8.43%) were excluded due to incorrect
199 projection. **Figure 2** presents LI mapping and DE-CMR characteristics of each animal.

200 The animals with more low LI points (LIR < 1; animal numbers 2, 3, 5, 6, and 11) also had
201 more LVWT points <3.5 mm. Low LI was associated with myocardial thinning (**Figure 3**).
202 **Supplemental Figure 3** shows an example of this association. Mean LVWT was 2.8 ± 0.7 , $3.8 \pm$
203 0.9 , and 6.1 ± 1.5 mm for low, intermediate, and high LI, respectively. Comparison of the LVWT
204 and LI categories revealed statistically significant differences between low vs high LI ($P < 0.001$)
205 and intermediate vs high LI ($P < 0.001$) (**Figure 4**).

206 Animals with lower LIR had greater endocardial PSI on DE-CMR (animal numbers 2, 3, 6,
207 13, 14, 15, and 16), whereas the animal without abnormal LI (number 12) exhibited no delayed
208 enhancement (Figure 2). Most low LI points (91.4%) colocalized with core scarring, and only 8.2%
209 and 0.4% with BZ and healthy tissue, respectively. Intermediate LI points showed a more
210 heterogeneous distribution; 44.4%, 46.8%, and 8.8% colocalized with core, BZ, and healthy
211 tissue, respectively. Most normal LI points corresponded with healthy tissue on DE-CMR (97.5%).
212 Overall, point analysis results were statistically significant ($P < 0.001$).

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213 Most of the regions where DE-CMR revealed transmural scar (epicardial core scarring)
214 corresponded to low LI endocardial points (low in 79.5% and intermediate in 20%). Overall
215 analysis of LI points showed that low LI endocardial points corresponded with epicardial core
216 scarring in 75%, BZ in 24%, and healthy tissue in 1%. Intermediate LI corresponded with
217 epicardial core scarring in 10%, BZ in 16%, and healthy tissue in 73%. The odds ratio of epicardial
218 scarring (either core or BZ) was 3.7 [1.07-13.1] in low LI compared to intermediate LI ($P < 0.001$).

219 We identified 26 DE-CMR BZ corridors in 12 animals (2.1 ± 1.0 per animal). In 59% of
220 cases, BZ corridors were identified in the subendocardium (10-40% layers). LI points that co-
221 localized with DE-CMR BZ corridors were classified as intermediate LI in 75.6% ($n = 127$) of the
222 cases, whereas only 22% ($n = 37$) and 2.4% ($n = 4$) as low and normal LI, respectively (**Figure 5**).

223

224 **VENTRICULAR TACHYCARDIA INDUCIBILITY.** VT was inducible in 13 animals. Animals with
225 inducible VT had DE-CMR corridors more often (84.6% vs. 25%, $p=0.022$) and higher prevalence
226 of specific proarrhythmic LI patterns (LI corridor 61.5% vs. 0%, $p=0.031$ and LI gradient 92.3% vs.
227 25%, $p=0.006$). In 76.9% of the animals with inducible VT, DE-CMR corridors co-localized with
228 specific LI patterns (LI corridor or gradient). Figure 5 provides examples of this co-localization.
229 When DE-CMR colocalized with specific LI patterns, VT was inducible in 90.9% of cases (positive
230 predictive value 65%). VT isthmuses ($n=8$) colocalized to LI patterns in 100% and DE-CMR
231 corridors in 75% of the cases.

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233 **HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSES.** Compared to low LI zones,
234 intermediate LI tissue exhibited reduced Col I ($1.03 \pm 0.26\%$ vs $0.34 \pm 0.35\%$, $P = 0.008$), Col III
235 ($9.92 \pm 2.98\%$ vs $6.47 \pm 1.80\%$, $P = 0.053$), Col I/III ratio (0.12 ± 0.06 vs 0.05 ± 0.05 , $P = 0.055$), and
236 collagen volume fraction (sum of Col I and Col III; 10.92 ± 2.77 vs 6.81 ± 1.92 , $P = 0.021$), without
237 differences in overall fibrosis ($P = 0.436$) or adipocytic deposition ($P = 0.189$).
238 Immunohistochemical analyses showed that intermediate LI tissue tended to have more

239 Isolectin B4-positive blood vessels than low LI zones (2.42 ± 0.58 vs 1.88 ± 0.55 , $P = 0.075$) (**Figure**
240 **6**). Cx43/cTnI ($P = 0.693$) and SERCA2/cTnI ($P = 0.094$) expressions did not significantly differ
241 between groups, indicating similar proportions of viable cardiomyocytes and connexins.

242

243 **DISCUSSION**

244 The present study evaluated the tissue composition underlying different LI categories, using
245 non-invasive and invasive tissue characterization. Our findings were: 1) LI correlated with LVWT
246 (thinner myocardium at low LI sites); 2) abnormal LI correlated with endocardial dense scarring
247 on DE-CMR; 3) low LI areas co-localized with epicardial scarring (especially core scarring); 4) DE-
248 CMR BZ corridors colocalized with intermediate LI points; 5) LI subtypes showed differential
249 histological composition (**central illustration**).

250 Myocardial scar tissue has a lower electrical LI than healthy tissue.¹⁻² LI is primarily
251 determined by the tissue's electrical properties, particularly the resistance of intra- and extra-
252 cellular components.^{9,10} Cellular factors determining electrical impedance include the number
253 and disposition of membrane cells (comprising a phospholipid bilayer with dielectric properties),
254 intercellular connections (gap junction conductance), and intracellular components. Extra-
255 cellular resistance depends on interstitial space volume and properties. Tissues with greater
256 water content, e.g., blood or lymph, have lower electrical impedance than tissues with greater
257 cell components, e.g., muscles. Among muscles, LI may vary depending on fiber orientation
258 (anisotropy), since muscle resistance is higher in a transversal fiber orientation versus a
259 longitudinal one.¹¹

260 DE-CMR is the gold standard technique for assessing myocardial scar, and the
261 relationship between PSI and tissue composition has been demonstrated.¹² Gadolinium is
262 retained within myocardial fibrotic tissue, due to greater distribution in the extracellular matrix,
263 which slows washout kinetics. DE-CMR image post-processing enables direct quantification of

264 scarring and identification of potential VT circuits, associated with DE-CMR BZ corridors.¹³ In this
265 study, DE-CMR was used to analyze tissue composition underlying different LI subtypes, and
266 establish the arrhythmic propensity.

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268 **LOCAL IMPEDANCE AND POST-INFARCTION SCAR COMPOSITION.** In post-MI scars, muscle
269 fibers are replaced by fibroblasts and collagen fibers—predominantly type I collagen and, to a
270 lesser extent, type III—which are immersed in an amorphous substance comprising water and
271 glycoproteins.¹⁴ Collagen fibers typically deposits from the endocardium to the epicardium
272 concentrically, providing the scar with tension and deformation resistance. Collagen deposition
273 is associated with progressive tissue compaction and myocardial wall thinning.¹⁵ Cardiomyocytes
274 that survive ischemic injury exhibit a certain degree of dysplasia and metaplasia, and will remain
275 isolated by scarred tissue.¹⁶ Viable cardiomyocytes will remain supported by a well-organized
276 network of functional microvessels.¹⁷ Adipose infiltration is also key in scar proliferation and
277 maturation, and it is known to occur especially around surviving myocytes, providing structural
278 support. Post-MI scars are electrically active. Scar components behave differently following
279 electric current passage, which can be evaluated by LI.

280 In this study, low LI was associated with myocardial wall thinning, and with endocardial
281 and epicardial scarring on DE-CMR, and was histologically characterized by increased collagen.
282 This suggests that low LI translates into mature and transmural scars, defined by significant
283 fibrosis replacement and tissue compaction, and likely associated with greater cell decoupling.
284 Consistently, previous studies have shown that the degree of fibrosis is associated with LI.^{3,18}
285 Additionally, a previous clinical study showed that low intracardiac impedance was significantly
286 associated with ventricular wall thinning.¹⁹ Loss of gap junctions and a great amount of
287 extracellular matrix cause tissue to lose resistive electrical properties, and enable the passive
288 transmission of electrical current, creating a highly conductive medium with high current

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289 dispersion.⁹ This explains why LI is lower in this tissue, and precludes electrical current
290 propagation (extracellular current sink).

291 Intermediate LI zones were associated with subendocardial and largely heterogeneous
292 scarring on DE-CMR. Histologically, intermediate LI zones had less collagen and greater vascular
293 density. These could be a surrogate of less mature scars, oriented in a less organized manner,
294 and more likely to host surviving myocytes—as suggested by greater vascular density, which is
295 associated with viable cell persistence.¹⁷ Such tissue heterogeneity of intermediate LI is related
296 to its potential participation in VT circuits.

297

298 **LOCAL IMPEDANCE AND ARRHYTHMIC PROPENSITY.** BZ corridors on DE-CMR have
299 demonstrated to predict ventricular arrhythmia occurrence during follow-up, and identify
300 potential VT circuits.^{20,21} Therefore, detecting DE-CMR corridors has become useful in clinical
301 practice for planning ablation procedures and guiding substrate-based ablations. In the present
302 study, DE-CMR BZ corridors colocalized with intermediate LI points and specific LI patterns.

303 Re-entry is the primary mechanism of post-MI VT.²² It is associated with the presence of
304 surviving cardiomyocytes that have suffered intercellular remodeling—including ion channels
305 and gap junction—and that remain isolated within scarred tissue.²³ Re-entry requires a
306 conducting corridor and an anatomical or functional obstacle or conduction blockage point.
307 Source-sink and impedance mismatch have been suggested as potential causes of functional re-
308 entry.²⁴ In a previous study, comparison of LI data with VT activation maps demonstrated the
309 arrhythmogenic potential of intermediate LI, since intermediate LI areas predicted VT
310 inducibility, and VT isthmuses were consistently located at intermediate LI zones, especially
311 zones with LI gradients and LI corridors.⁶ LI corridors and gradients represent heterogeneous
312 tissue and regions of abrupt change in myocardial thickness or intercellular connections -regions
313 of mismatch-, favoring wavefront curvature functional blockage and re-entry. Intermediate LI
314 may suggest greater scar content of higher LI tissue, such as viable cells or adipose tissue.

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315 Lipomatous metaplasia has been demonstrated to display higher impedance than connective
316 tissue, and to promote VT, as DE-CMR BZ corridors participating in VT circuits were
317 predominantly traversing or adjacent to lipomatous metaplasia.²⁵ It is hypothesized that
318 lipomatous metaplasia increases the electrical resistance surrounding VT corridors, reducing
319 current loss during wavefront propagation along the critical isthmus. This reinforces the concept
320 that only scar tissue maintaining certain resistance properties are capable of reduce the
321 electrical consumption (or current loss) of the scar, propagating the wavefront among partially
322 decoupled myocytes and allowing re-entry, which is not the case for connective tissue.²⁶ Our
323 present study did not include computed tomography, precluding lipomatous metaplasia
324 assessment. Histological analysis did not reveal differences in the amount of adipose infiltration.
325 However, our sample size was relatively small, and intermediate LI tissue was sampled without
326 considering its participation in VT circuits.

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328 **CLINICAL IMPLICATIONS.** As previously demonstrated, tissue with intermediate LI exhibits
329 significant arrhythmic propensity. The extent of intermediate LI area was associated with risk of
330 VT inducibility, and consistently colocalized with DE-CMR BZ corridors and VT isthmuses.⁶
331 Accordingly, targeting areas of intermediate LI could become a new substrate-based ablation
332 strategy or, together with the traditional substrate-based strategies, could reduce ablation
333 requirements and improve outcomes. The previous study also showed that most of abnormal
334 endocardial EGMs (91%) were recorded at intermediate LI areas, reinforcing the arrhythmic
335 propensity of the intermediate LI tissue.⁶ Low LI points were consistently found in dense, very-
336 low voltage areas (100% of cases) while intermediate LI tissue could be found in both voltage
337 core (87%) and BZ (13%) areas, allowing for further discrimination of different tissue
338 compositions within voltage dense scar.

339 Tissue impedance also determines its thermodynamic properties. Clinical studies have
340 reported the potential benefit of radiofrequency energy titration based on LI changes.²⁷

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341 Evaluation of baseline/pre-ablation LI could be useful for predicting radiofrequency lesion
342 quality, which is directly associated with procedure success rates. Radiofrequency lesion
343 creation in scarred tissue is reportedly unpredictable, nonhomogeneous, and barely
344 identifiable.²⁸ In dense scar areas, thermal lesions produced collagen disruption and
345 fragmentation, with no clear relationship to the distance from the catheter. Connective tissue
346 damage occurs in islets both near the catheter-endocardial interface and further away,
347 interspersed with intact connective tissue. Similar to electric currents, when thermal energy
348 passes through collagen fibers, -low resistivity tissue- energy loss may occur, producing smaller
349 degrees of tissue heating. Low LI zones correspond to densely packed and transmural fibrosis,
350 rich in collagen content, hampering energy delivery and lesion creation. In contrast,
351 intermediate LI zones not only have the greatest arrhythmogenic potential, but also could be
352 more susceptible to effective tissue heating.

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354 **STUDY LIMITATIONS.** Tissues with different LI were stained with Evans blue during mapping. We
355 cannot exclude the possibility of slight displacement of the catheter upon injection or tissue
356 removal. The correlation of LI points with DE-CMR data was analyzed assuming a 2-mm error
357 distance. The sample size for histological analysis was relatively small. LI maps were acquired
358 with non-contact force catheters. Whether LI maps acquired with contact-force sensing
359 catheters or using different current characteristics would differ from those obtained in this study
360 is unknown and requires further investigation. Impact of wavefront direction on LI was not
361 tested in this study. Further accuracy and reproducibility testing is required before it can be
362 implemented in clinical practice. There remains a need for further investigation of LI distribution
363 in revascularized MI models and non-ischemic substrates.

364 **CONCLUSIONS**

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365 LI was associated with scar density, transmural, and wall thinning on DE-CMR. Compared to
366 intermediate LI areas, low LI areas exhibited higher proportions of dense transmural scarring and
367 wall thinning. DE-CMR BZ corridors colocalized with intermediate LI in most cases. Histological
368 composition differed between LI subtypes.

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374

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462 **TABLES**

463 **TABLE 1 Baseline Local Impedance and DE-CMR Parameters**

	n	Median [IQR]
LI parameters		
LI blood pool, Ω	18	76 [73-81]
Total abnormal LI area, cm^2	18	20.0 [14.3-23.2]
Low LI area, cm^2	18	5.6 [2.8-9.8]
Intermediate LI area, cm^2	18	15.1 [3.8-18.2]
DE-CMR parameters		
Total endocardial scar, cm^2	18	19.4 [17.8-21.3]
Endocardial dense scar, cm^2	18	12.6 [10.8-15.5]
Endocardial BZ scar, cm^2	18	6.3 [4.9-8.0]
Total epicardial scar, cm^2	18	18.5 [16.4-20.3]
Epicardial dense scar, cm^2	18	7.8 [4.2-13.0]
Epicardial BZ scar, cm^2	18	9.7 [5.9-12.2]
Left ventricle thickness, mm	18	4.7 [1.3-10.4]
Number of BZ channels	18	2 [0-2]

476 BZ = border zone; IQR = interquartile range; LI = local impedance.

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477 FIGURES

478 FIGURE 1: Delayed Enhancement Magnetic Resonance Imaging and Local Impedance Map

479 **Coregistration.** (Panel 1 and 2) Correlation between DE-CMR and LI map using the software

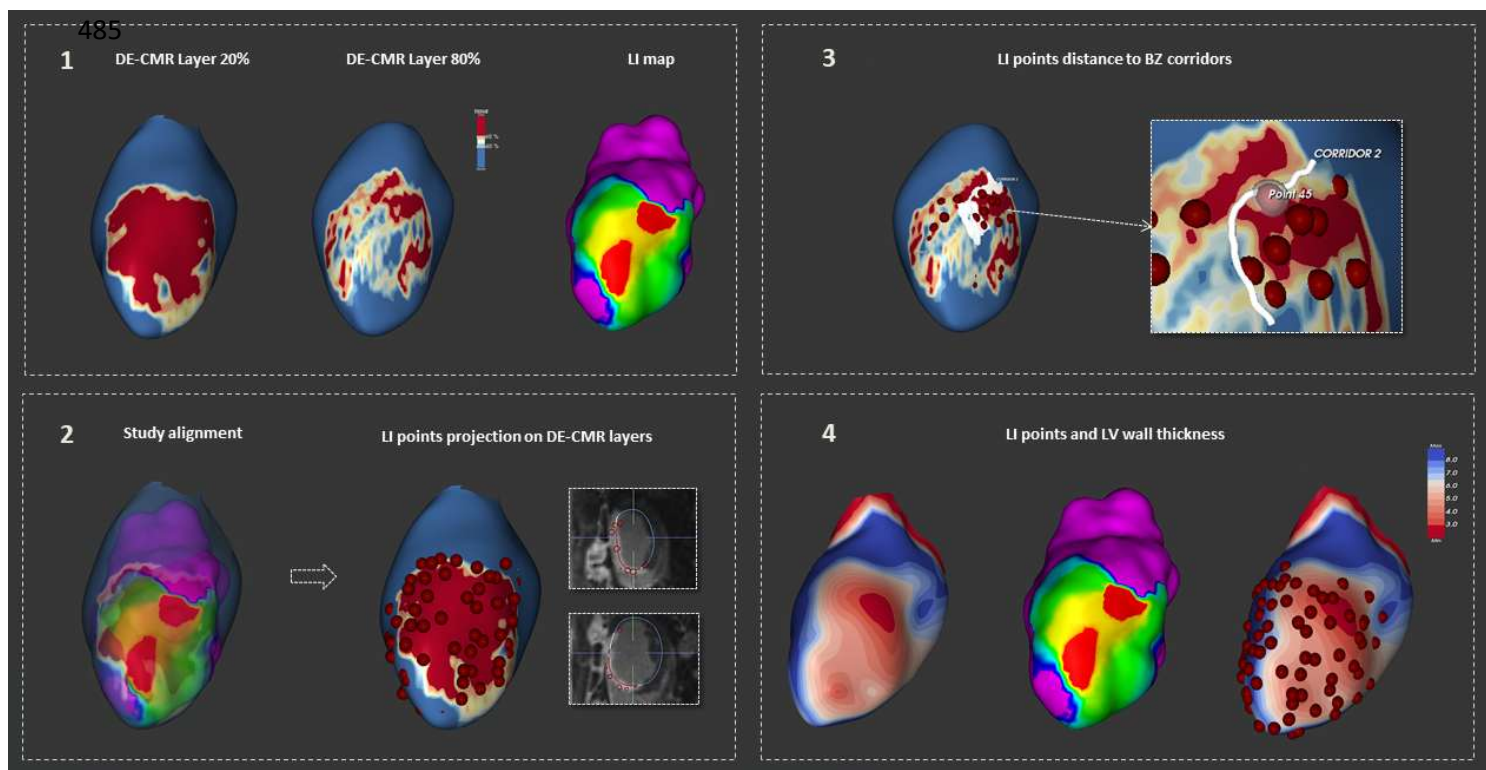
480 ADAS 3D™. Landmarks were used to align maps, and LI points were projected on DE-CMR 20%

481 and 80% layers. (Panel 3) Distance from LI points to DE-CMR BZ corridors was calculated to

482 determine LI values in BZ corridors. (Panel 4) Comparison between LI points and LVWT data. BZ =

483 border zone; DE-CMR = delayed enhanced cardiac magnetic resonance; LI = local impedance;

484 LVWT = left ventricular wall thickness.



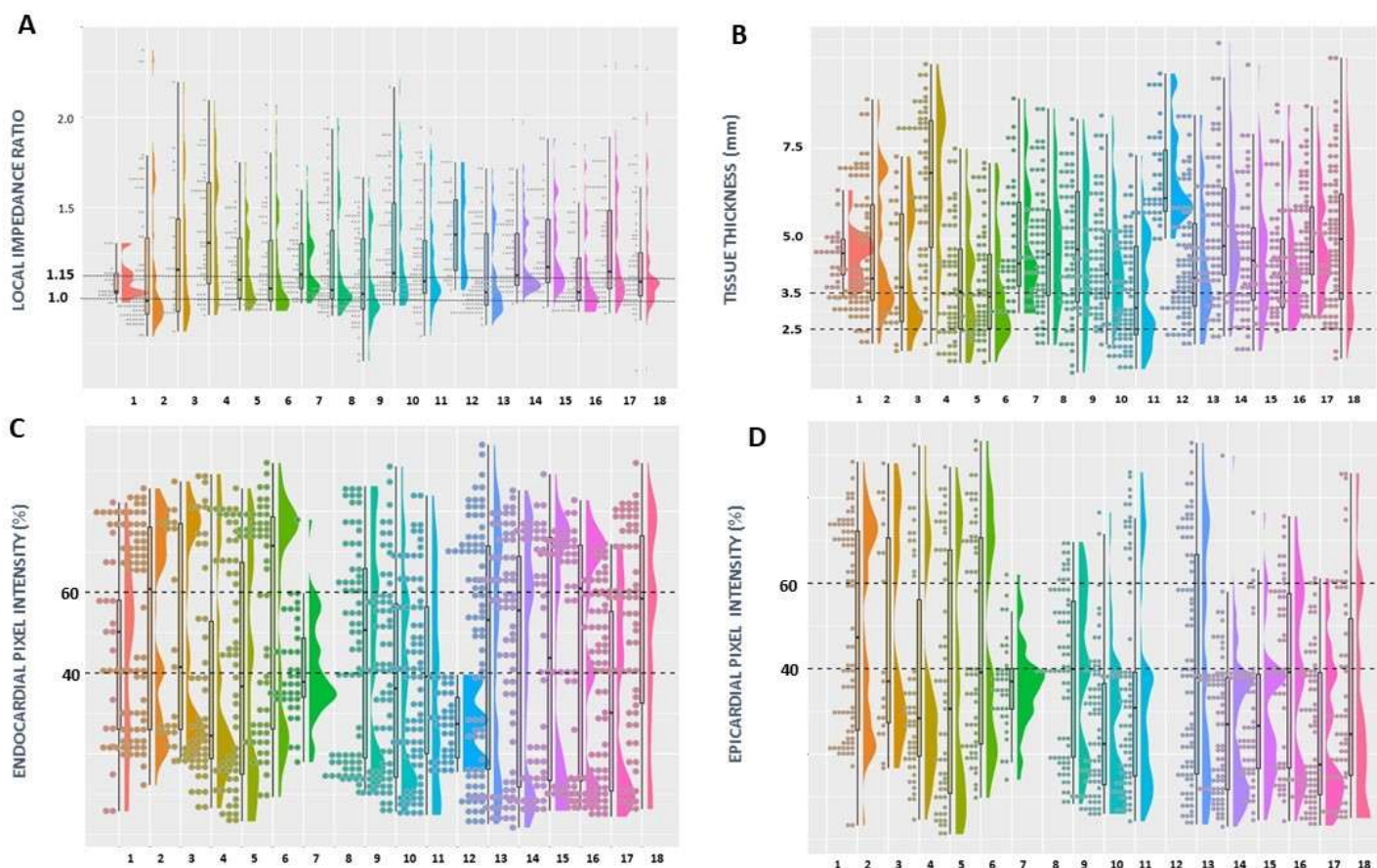
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486 **FIGURE 2: Local Impedance Mapping and DE-CMR Data (Ridgeline Plots of Densities).**

487 Horizontal axis shows 18 animals. Vertical axis shows distribution and density of LIR values (LI
488 value/BP) (panel A), LVWT (panel B), endocardial (panel C), and epicardial (panel D) pixel signal
489 intensity. **(Panel A)** Most animals exhibited abnormal LIR points (<1.15). Animal numbers 2, 3, 5,
490 6, 8, 9, 13, and 16 had the lowest LI values. Animal number 12 lacked abnormal LI values. **(Panel**
491 **B)** Animals with higher proportions of LV wall thinning (thickness < 3.5 mm) exhibited higher
492 proportions of low LI area (numbers 2, 3, 5, 6, and 11). **(Panels C and D)** Animals with more
493 endocardial and epicardial PSI exhibited higher proportions of low LI area ($LIR < 1$). Animal no. 12
494 lacked abnormal LIR values, and did not exhibit late gadolinium enhancement. DE-CMR =
495 delayed enhanced cardiac magnetic resonance; BP = blood pool; LI = local impedance; LIR = LI
496 ratio; LV = left ventricular; LVWT = left ventricular wall thickness.

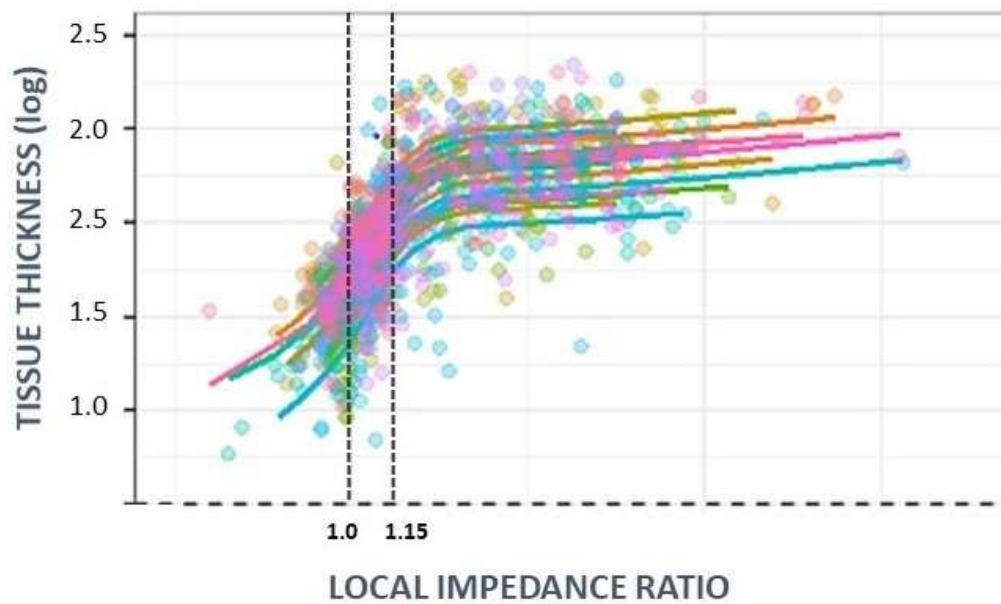
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498 **FIGURE 3: Correlation Between LIR Values and Myocardial Thickness (Logarithmic**
499 **Transformation).** A linear association between LI and myocardial wall thickness is observed at
500 abnormal LI values ($LIR < 1.15$). Low LI points ($LIR < 1.0$) exhibited more pronounced wall thinning
501 compared to intermediate LI tissue ($1.0 < LIR < 1.15$). LI = local impedance; LIR = LI ratio.



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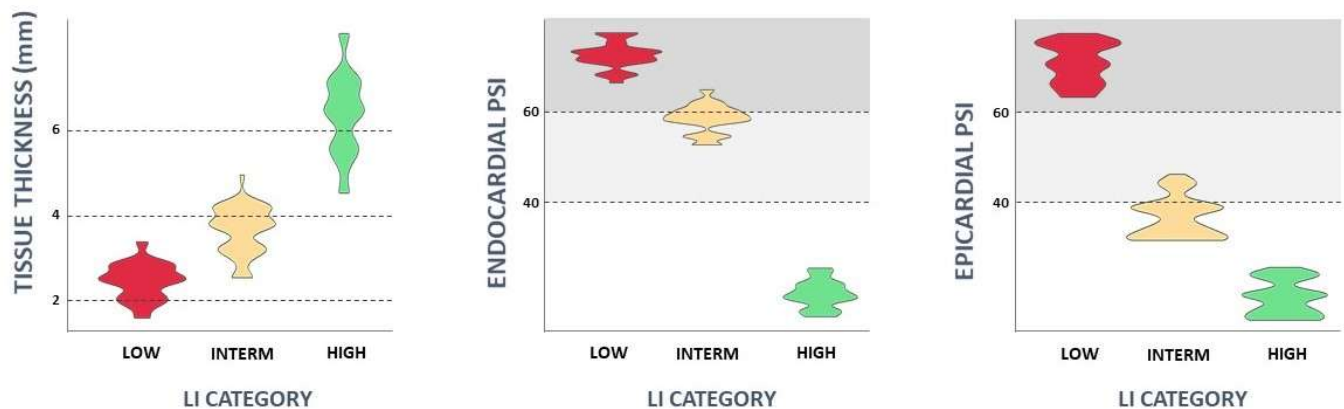
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503 **FIGURE 4: DE-CMR Predicted Values for Low, Intermediate, and High LI Tissue.** Predicted tissue
504 thickness (**panel A**), endocardial (**panel B**) and epicardial (**panel C**) pixel signal intensity for low,
505 intermediate, and high LI tissue. DE-CMR = delayed enhanced cardiac magnetic resonance; LI =
506 local impedance.

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PREDICTED VALUES FOR LOCAL IMPEDANCE CATEGORY

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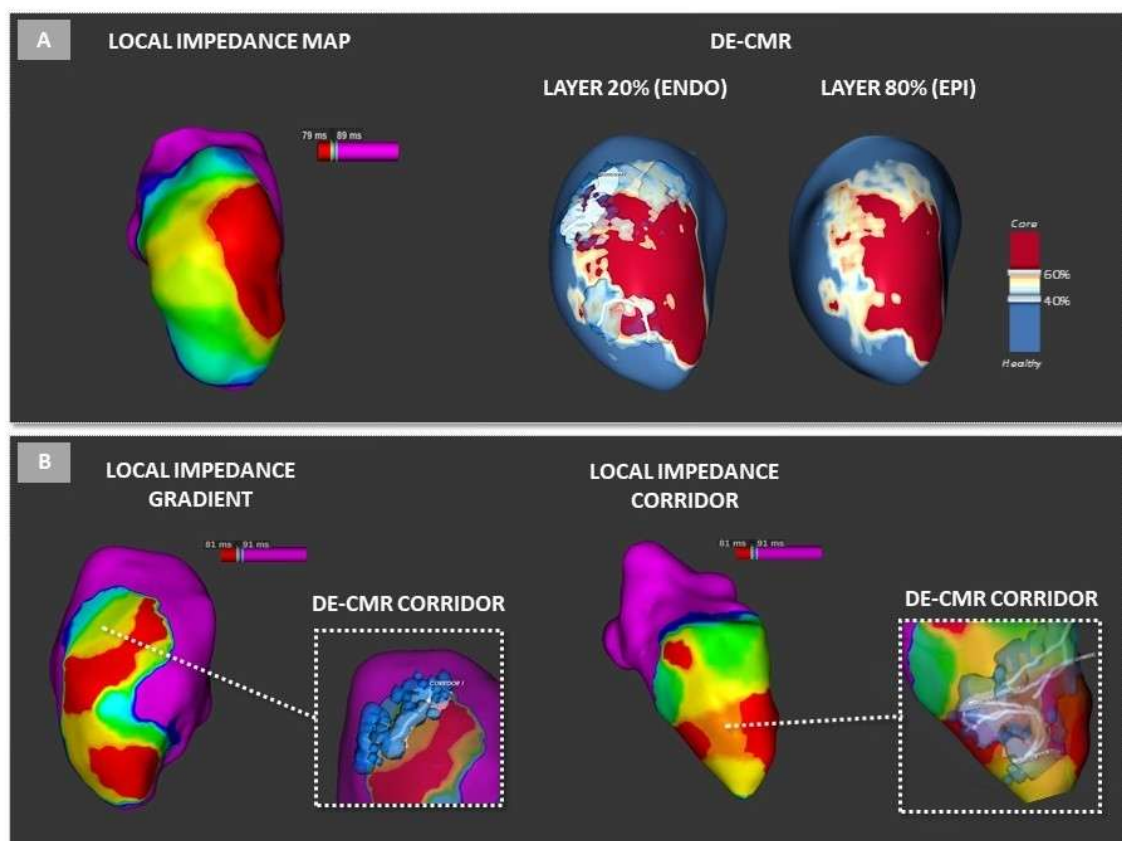


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509 **FIGURE 5: Panel A. Correlation between DE-CMR-Detected BZ Corridors and LI Map.** BZ scar
510 and BZ-corridors co-localize with intermediate LI areas. BZ = border zone; DE-CMR = delayed
511 enhanced cardiac magnetic resonance; LI = local impedance. **Panel B. Correlation between DE-**
512 **CMR-Detected BZ Corridors and specific LI patterns.** BZ scar and BZ-corridors co-localize with LI
513 gradient and corridor.

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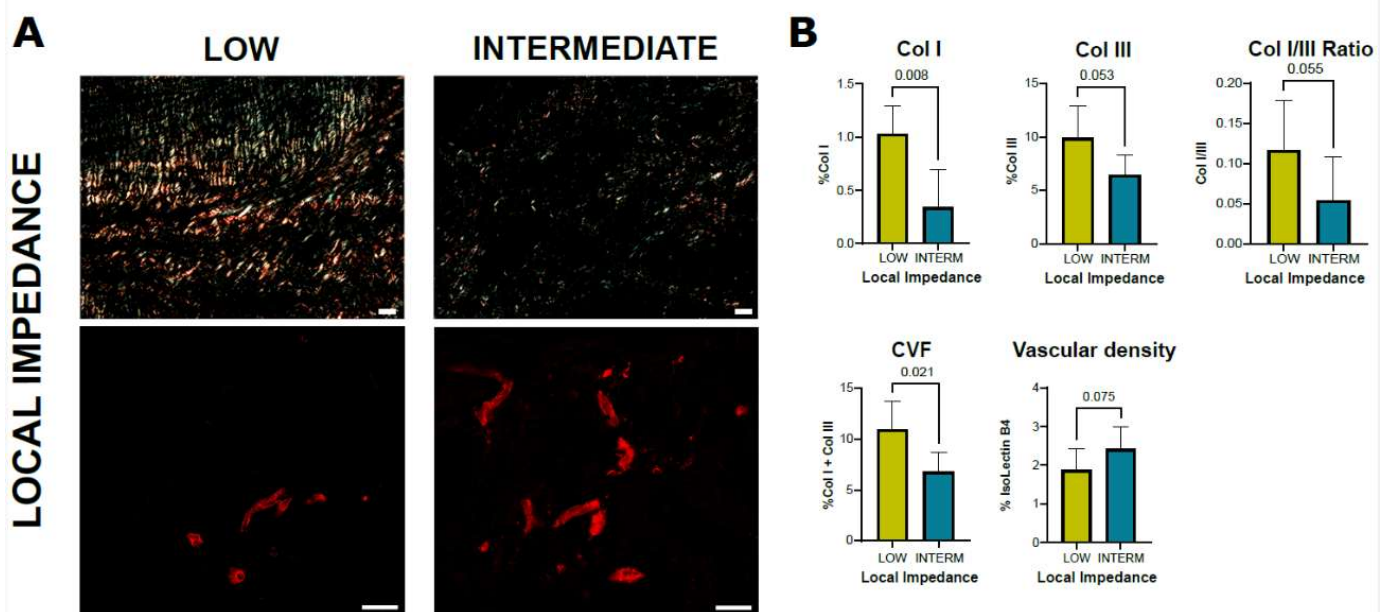


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115 **FIGURE 6: Histological Characteristics of Low and Intermediate LI.** (A) Top: Polarized light
116 microscopy images of Picrosirius red staining. Red/yellow, Col I fibrils; Green, Col III (scale bar =
117 25 μ m). Bottom: Images from isolectin B4 immunostaining. Red, vessels (scale bar = 25 μ m). (B)
118 Histograms displaying Col I and Col III percentages, Col I/Col III ratio, collagen volume fraction
119 (CVF), and vascular density, from low and intermediate LI samples.

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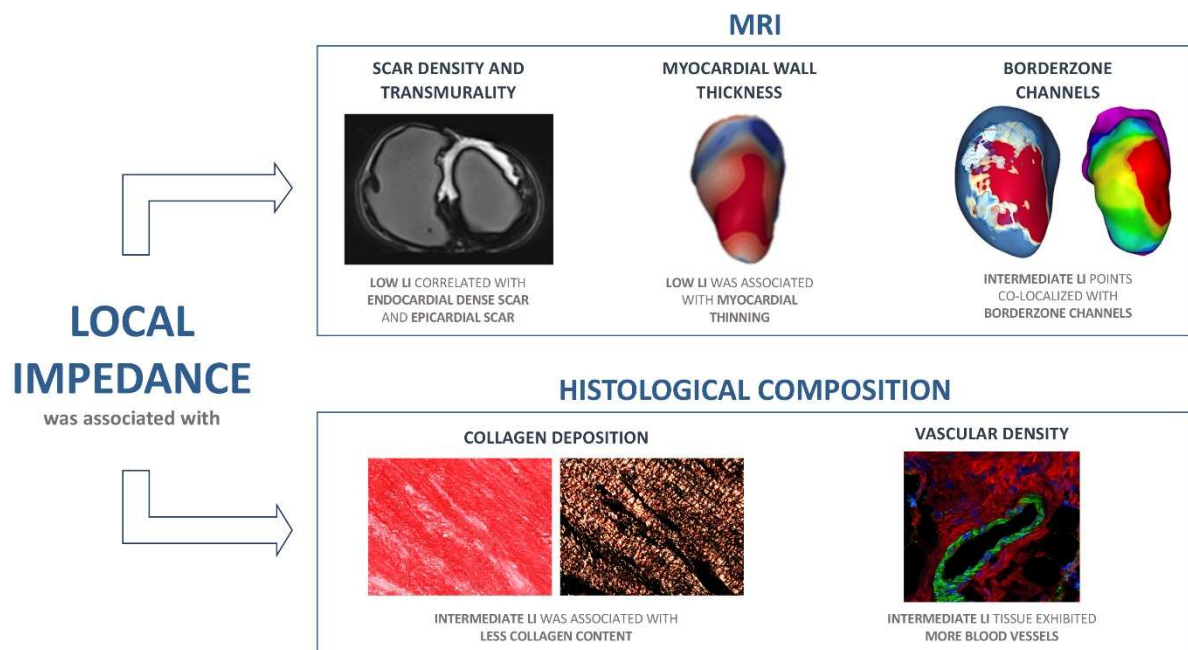


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521 **Central Illustration:**

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