SUPPLEMENTARY APPENDIX

Symptom Burden, Coagulopathy and Heart Disease after Acute SARS-CoV-2 Infection in Primary Practice - Results from the Study of HEarT DiseAse and ImmuNiTy After COVID-19 in Ireland (SETANTA)

Running head: *Heart disease after SARS-CoV-2 Infection*

Authors: Roisin Colleran MB, BCh, PhD [a,b]; Sean Fitzgerald MB, BCh, PhD [a]; Himanshu Rai MSc, PhD [a,b]; Laurna McGovern MB, BCh [a]; Roger J. Byrne MB, BCh, MD [c]; Ahmed Mansur MBBS [c]; Andrea Cradock MSc, MRSO [d]; Ros Lavery MB, BCh [c]; James Bisset BSc Hons [c]; Shane McKeogh MB, BCh [e]; Gordon Cantwell MB, BCh [f]; Darach O'Ciardha MB, BCh [g]; Hannah Wilson MSc [a]; Nicoletta Begossi MSc [a]; Nial Blake MB, BCh [a]; Maria Fitzgibbon MB, BCh [h]; Jonathan McNulty BSc, PhD, PG Dip [d]; Gábor Széplaki MD, PhD [a]; Emma Heffernan MSc [h]; Margaret Hannan MB, BCh [h]; James P. O'Donnell MB, BCh, PhD [i], Robert A. Byrne MB, BCh, PhD [a,b]

[a] Cardiovascular Research Institute (CVRI) Dublin, Mater Private Network, Dublin, Ireland; [b] School of Pharmacy and Biomolecular Sciences, RCSI University of Medicine and Health Sciences, Dublin, Ireland; [c] Mater Private Network, Dublin, Ireland; [d] Department of Medicine, University College Dublin, Ireland; [e] Solas Medical Centre, Dublin, Ireland; [f] Drs Cantwell & Spillane Practice, Family and General Medicine, Dublin; [g] Institute of Population Health, Trinity College Dublin, Ireland; [h] Department of Pathology, Mater Private Network, Dublin, Ireland; [i] Irish Centre for Vascular Biology, RCSI University of Medicine and Health Sciences, Dublin, Ireland

Figures: 5; Tables: 1; Spellcheck: English (UK)

Supplementary methods:

Data collection

Eligible patients, who provided written informed consent, were invited to attend a baseline visit over two consecutive days at a hospital clinic. The first visit comprised of consent and collation of baseline demographics, medical history, current medications, and self-reported SARS-CoV-2 symptoms. Assessments included vital signs, electrocardiogram (ECG), 24-hour ambulatory ECG recording and blood sampling by a dedicated study nurse. Bloods were drawn for C-reactive protein (CRP), ferritin, lactate dehydrogenase, von Willebrand factor antigen, fibrinogen, D-dimer, NT-proBNP-type natriuretic peptide, soluble suppression of tumorigenicity (ST2) and galectin-3. Roche Diagnostics (Basel, Switzerland) Elecsys anti-SARS-CoV-2 spike protein (S) and nucleocapsid protein (NCP) total antibody assays were used to confirm serological evidence of prior SARS-CoV-2 infection. Ambient samples were shipped within 2 hours for analysis at a centralized pathology laboratory. Further testing for specialized biomarkers was carried out at collaborating university laboratories. Samples were aliquoted and stored at -20°C within 4 hours of sample collection, and then transported at a later date to the concerned university laboratories. Patients were asked to complete two quality of life (QOL) questionnaires, the EQ-5D-5L (EuroQol 5 Dimension 5 Level) and a modified version of the SAQ7 (Seattle Angina Questionnaire 7), removing the item referring to nitroglycerine use, given that the patients assessed did not have pre-existing diagnoses of ischemic heart disease and were not prescribed nitroglycerine at the point of inclusion in the study. The patient returned to the hospital clinic after 24 hours and underwent CMR imaging. Incidence of major adverse cardiac events (MACE) were followed up via telephone at 1, 6 and 12 months following the baseline visit, with changes in QOL indices reviewed at months 6 and 12.

Cardiac magnetic resonance imaging

CMR imaging was performed on a 1.5T whole body scanner (Magnetom Sola, Siemens Healthcare Sector, Erlangen, Germany). The CMR protocol consisted of static T2 weighted in all three planes and T2 weighted imaging with fat saturation in the short axis plane. Cine imaging was carried out in the standard vertical long axis (VLA), horizontal long axis (HLA), left ventricular outflow tract (LVOT), right ventricular outflow tract (RVOT) and short axis. Phase contrast flow imaging was carried out in the aorta and pulmonary artery, along with pulmonary vein assessment. T2 mapping, native T1 mapping and post contrast T1 mapping was carried out to establish T2, T1 and the extracellular volume of the myocardium. Perfusion imaging was carried out after the administration of 0.4mg of Regadenoson as a rapid bolus over 10 seconds. This was followed by 0.2mmol/kg of the gadolinium-based contrast agent gadobutrol (Gadovist; Bayer Phama, Berlin, Germany) several minutes later. Standard delayed gadolinium enhancement was carried out in the HLA, VLA, LVOT and short axis with cross-cut imaging over any apparent abnormality.

Electrocardiogram assessment

Normal ranges for ECG parameters were defined as PR interval (0.12-0.20 sec), QTc (350-450 milliseconds (ms) for males and (360-460ms) for women, QRS (0.08-0.12 sec). T wave inversion was defined as "negative T-wave of ≥ 1mm in depth in two or more continuous leads, with exclusion of leads aVR, III and V1. Lifecard® CF was used for 24-hour ambulatory ECG monitoring (Spacelabs Healthcare, WA, USA). Standard published definitions were used for ambulatory ECG parameters.(1) SDNN was defined as standard deviation of NN (normal-to-normal RR) intervals over a 24-hour period, a measure which reflects total heart rate

variability (HRV); rMSSD, root mean square of differences between successive NN intervals; SDSD, standard deviation of the successive NN differences; HRV Index, mean of standard deviation of NN over 24 hours period; TINN, baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of all NN intervals.

Statistical analysis

Categorical variables are presented as numbers (percentages, %), while continuous data are presented as mean±SD or median (Interquartile range, IQR). Differences between categorical variables were delineated using the χ^2 test or Fisher's exact test. Continuous variables were compared using independent Student's T-test or Mann-Whitney U test, depending on the distribution of the analysed variable. Inter-and intra-observer variability was tested for CMR data in ≈10% of a randomly selected sample. Cohen's Kappa coefficient was used for categorical variables, with inter-class coefficient (ICC) used for continuous variables. An observed two-sided p value of <0.05 was considered statistically significant for all tests. All statistical analyses were performed using Prism (GraphPad Prism version 9.1.0, GraphPad software, San Diego, California, USA, www.graphpad.com).

Electrocardiography analysis

At the time of ECG and ambulatory ECG monitoring, 99% of patients were in sinus rhythm. Electrocardiographic abnormalities, such as T-wave inversion and ST-changes, were detected in 9% and 3% of subjects, respectively. Median 24-hour SDNN was 143.7 (122.7, 175.9) ms, while 24-hour HRV index was 41.9 (33.5, 51.4) ms (**Supplementary Table 1.**). Patients with SDNN and HRV values in the 3rd and 4th quartiles were found to have higher median NT-proBNP levels [67.0 (33.0, 140.5) versus 38.0 (20.0, 76.0), p= 0.008] and [53.0

(31.0, 155.0) versus 41.0 (18.0, 81.0), p= 0.047] for SDNN and HRV, respectively (**Supplementary Figure 4.**).

In our study, patients with SDNN and HRV values in the 3rd and 4th quartiles were found to have higher median NT-proBNP levels [67.0 (33.0, 140.5) versus 38.0 (20.0, 76.0), p= 0.008] and [53.0 (31.0, 155.0) versus 41.0 (18.0, 81.0), p = 0.047] for SDNN and HRV, respectively. Thus, although not quite as powerful an association as that with mortality, this association is noteworthy and deserves further dedicated study.

Supplementary Table 1. Electrocardiographic parameters

	results
Electrocardiographic parameters	
Sinus rhythm	99 (99)
PR interval (ms)	156 (136, 169.5)
QTc (ms)	396 (384.7, 405)
QRS (ms)	92 (83, 100)
T-wave inversion	9 (9)
Normal axis	95 (95)
ST-changes	3 (3)
Ambulatory ECG parameters	
Mean heart rate	73 (68, 81)
Minimum heart rate	53 (48, 58)
Maximum heart rate	126 (114, 137)
SDNN total record (ms)	143.7 (122.7, 175.9)
RMSSD total record (ms)	34.8 (24.2, 50.5)
SDSD total record (ms)	25.5 (17.5, 41.0)
HRV index total record (ms)	41.9 (33.5, 51.4)
TINN total record (ms)	644.5 (511.7, 800.8)

Data expressed as number (%), mean±SD or median (interquartile range). Abbreviations: SDNN, standard deviation of NN (normal-to-normal RR) intervals over a 24-hour period-reflects total HRV; rMSSD, root mean square of differences between successive NN intervals; SDSD, standard deviation of the successive NN differences; HRV Index, mean of standard deviation of NN over 24 hours period; TINN, baseline width of the minimum

square difference triangular interpolation of the highest peak of the histogram of all NN intervals

Supplementary Figure Legends

Supplementary Figure 1. **Study flow chart.** This figure highlights the enrolment criteria, primary and follow-up investigations and outcomes of interest for this trial.

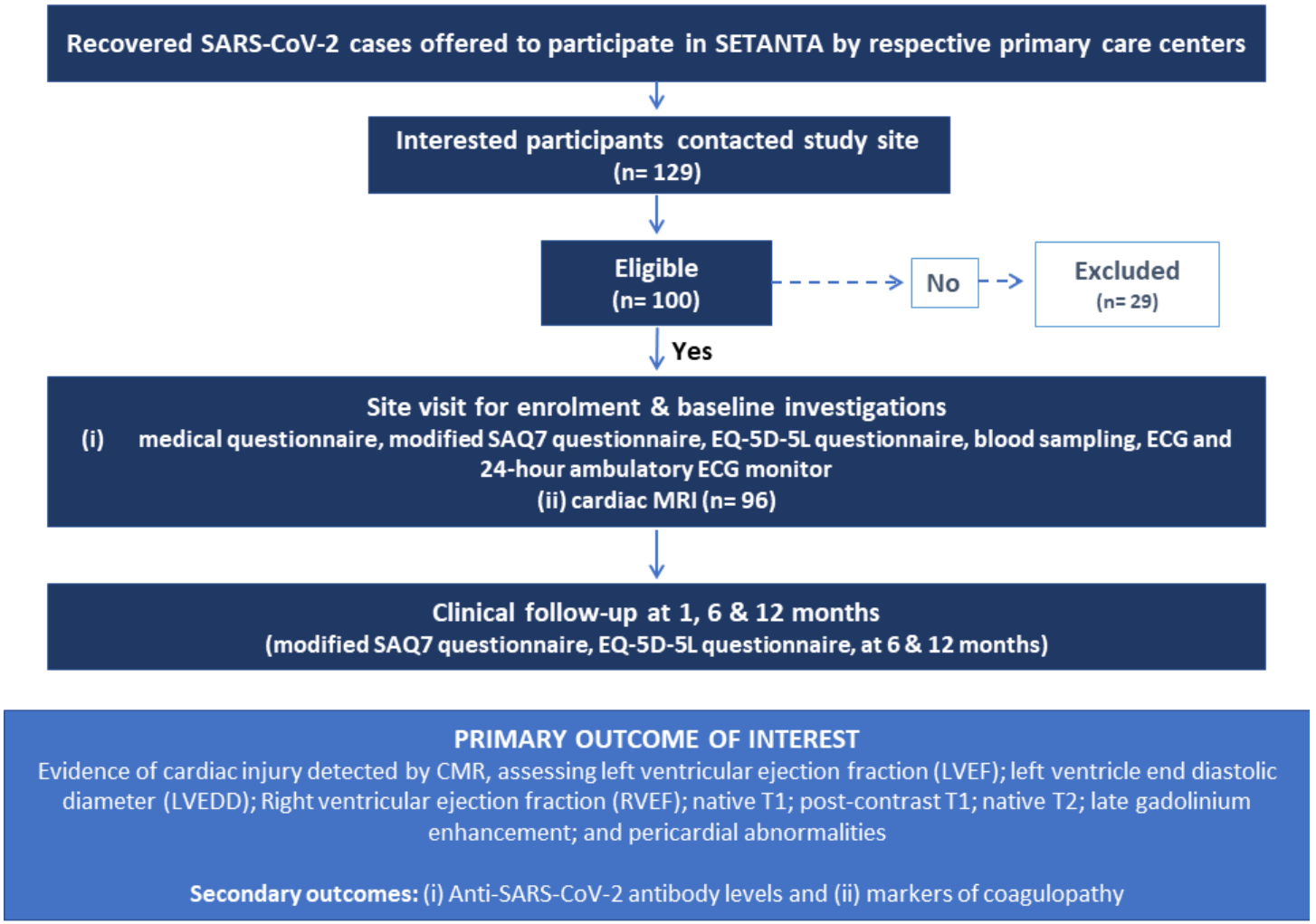
Supplementary Figure 2. **Markers of coagulation and immunity.** Panels A-F highlight serum levels of von Willebrand factor-antigen, fibrinogen, D-dimer, NT-proB-type natriuretic peptide, soluble suppression of tumorgenicity 2 (ST2) and galectin-3, respectively. Black dots represent levels below the upper reference limit, while red dots represent levels above this value. Median and interquartile range are superimposed on the data as blue bars.

Supplementary Figure 3. **Markers of inflammation.** Panels A-C highlight serum levels of C-reactive protein, ferritin and lactate dehydrogenase, respectively. Black dots represent levels below the upper reference limit, while red dots represent levels above this value. Median and interquartile range are superimposed on the data as blue bars.

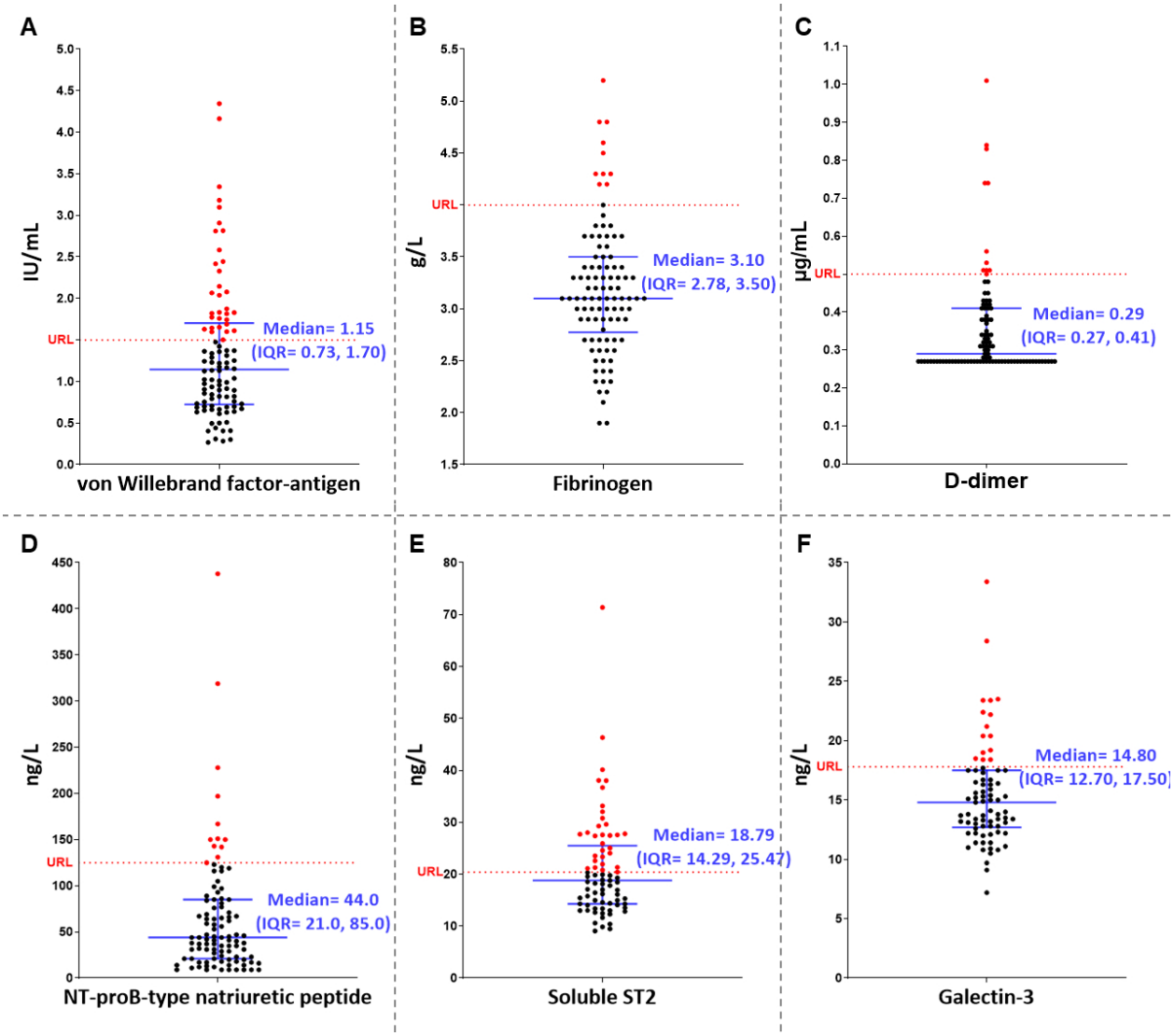
Supplementary Figure 4. **Representative cardiac magnetic resonance imaging findings in patients with COVID-19.** Panel (A) highlights late gadolinium enhancement present in 2 patients. Representative images of patients recently recovered from COVID-19 with pericardial effusion are shown in Panel (B). Mean LVEF (short axis) overall was 60.82±5.69%, with a LVEF below the normal reference limit in 17.4% of patients (Panel C).

Supplementary Figure 5. **Heart rate variability and NT-pro BNP.** This figure highlights the difference in NT-pro BNP levels between patients with SDNN (standard deviation of NN [normal-to-normal] RR intervals over a 24-hour period) and HRV (heart rate variability) values in top two quartiles versus lower two quartiles, respectively.

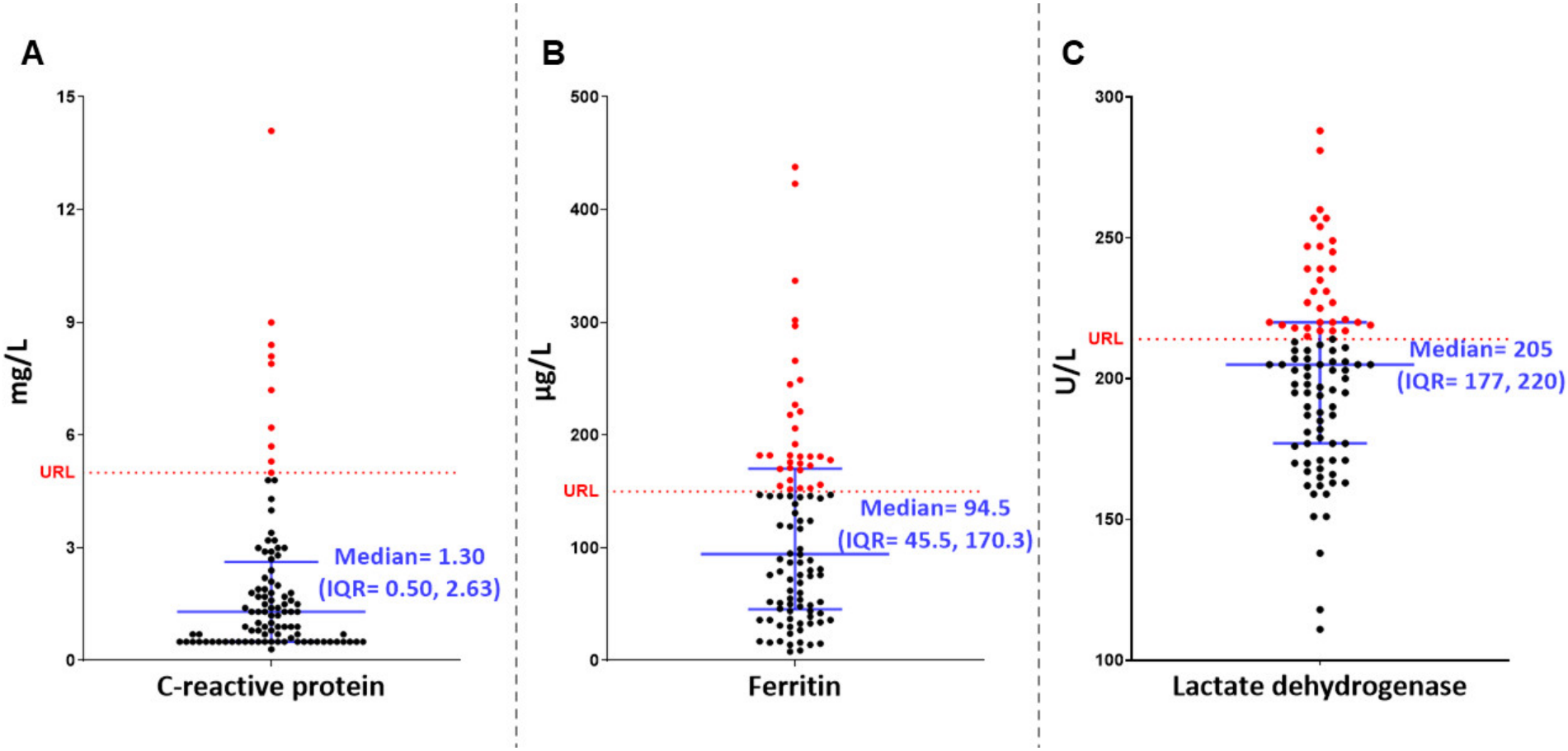
Supplementary Figure 1.



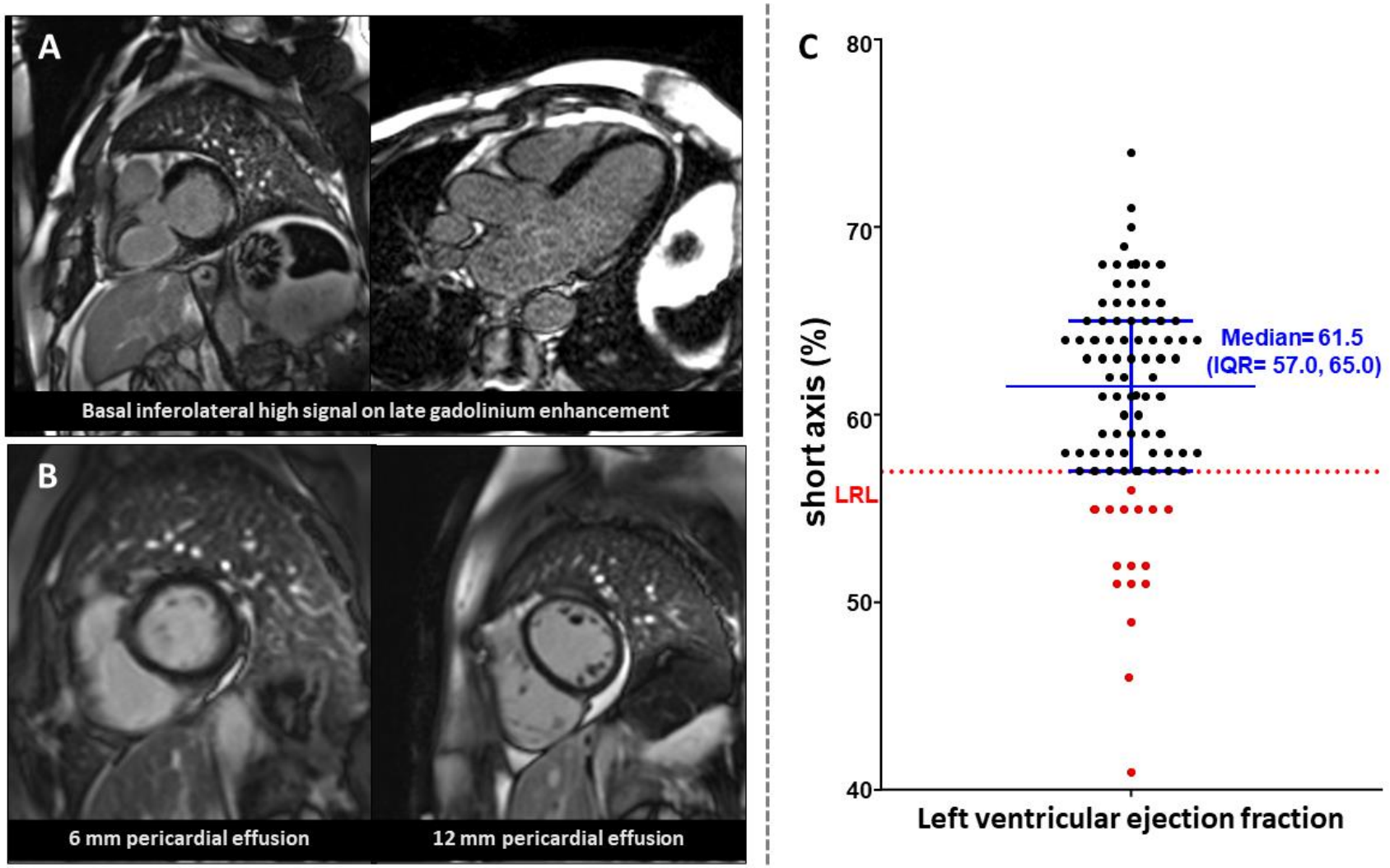
Supplementary Figure 2.



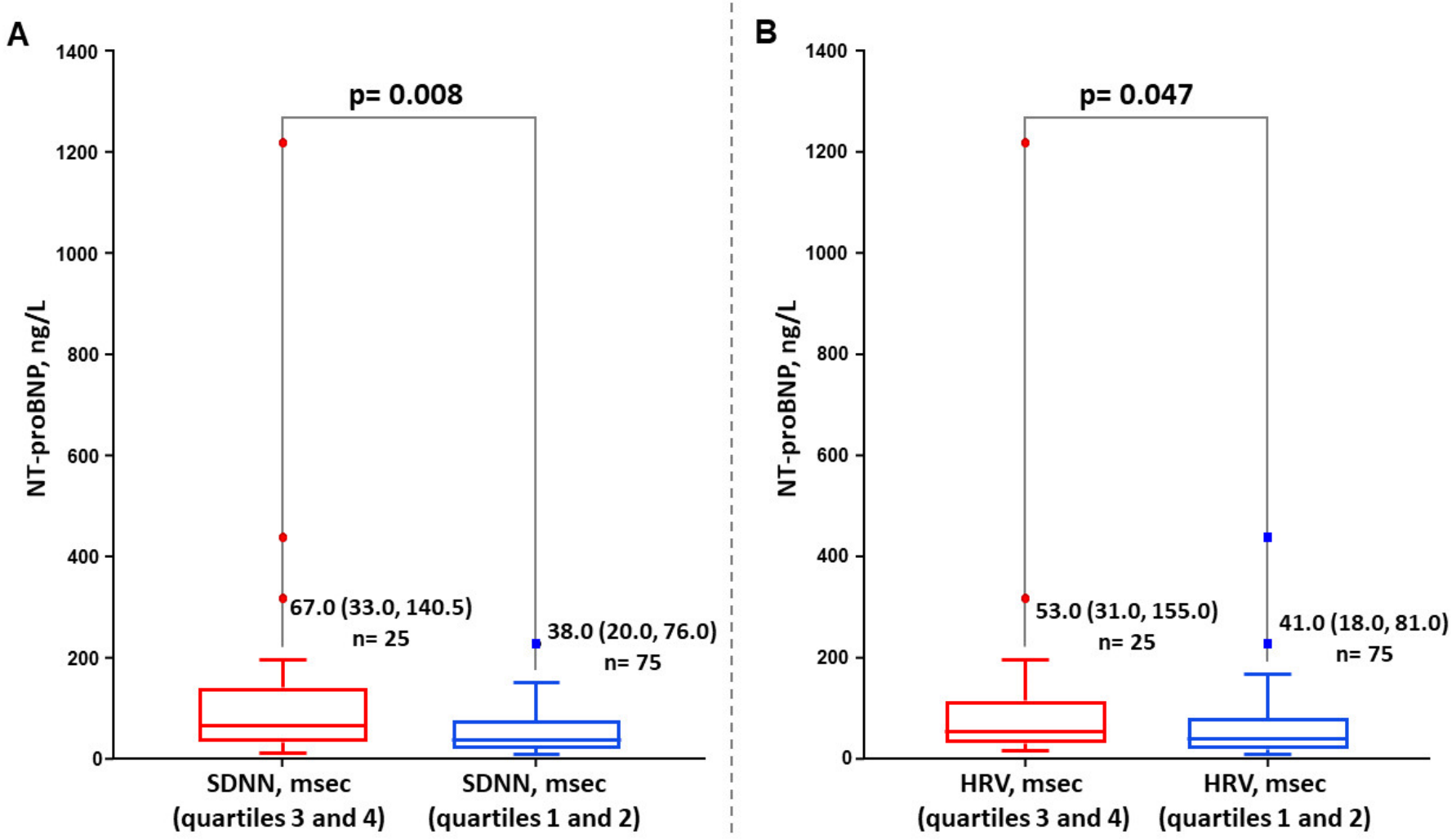
Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure 5.



REFERENCES:

1. Electrophysiology TFotESoCtNA. Heart Rate Variability. Circulation. 1996;93(5):1043-65.