SUPPLEMENTARY APPENDIX

Symptom Burden, Coagulopathy and Heart Disease after Acute SARS-CoV-2 Infection in Primary Practice

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

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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. Blood samples were drawn for C-reactive protein (CRP), ferritin, lactate dehydrogenase, von Willebrand factor antigen, fibrinogen, D-dimer, NT-proBNP , 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 nitroglycerin use, given that the patients assessed did not have pre-existing diagnoses of ischemic heart disease and were not prescribed nitroglycerin 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

Cardiac magnetic resonance (CMR) imaging was performed on a 1.5T whole body scanner (Magnetom Sola, Siemens Healthcare Sector, Erlangen, Germany). The CMR protocol consisted of localisers followed by single shot T2 weighted turbo spin echo (TSE) black blood image stacks for anatomical assessment in the trans-axial, sagittal and coronal planes. Base steady state free precession (bSSFP) cine imaging was carried out in the vertical long axis (VLA), horizontal long axis (HLA), left ventricular outflow tract (LVOT), right ventricular outflow tract (RVOT) and short axis. T2 weighted TSE dark blood sequences with fat suppression were acquired in the short axis of the RV and LV to detect myocardial oedema. Slices had an 8 mm thickness with a 1.6mm slice gap. Phase contrast flow imaging was carried out through the aortic root and pulmonary artery, along with pulmonary vein assessment. T2 mapping, native T1 mapping and post contrast T1 mapping were carried out to establish T2 recovery, T1 relaxation and the extracellular volume (ECV) of the myocardium. The post contrast T1 mapping sequence used was a MOLLI (modified Look-Locker inversion recovery) 4 (1) 3 (1) 2 (Myomaps, Siemens Healthineers, Erlangen, Germany) and acquired 8 minutes post contrast. Three slices were acquired through the left ventricle at the base, midventricular and apical level. Native T1 and post contrast T1 were measured by contouring epicardial and endocardial borders, defining the superior RV/LV insertion point, placing a region of interest (ROI) in the septum and blood pool of each slice. The series was registered and ECV maps were automatically generated comparing pre and post contrast T1, also acknowledging blood haematocrit, which was required to correct volume of distribution of contrast in blood. Each patient had a serum full blood count (FBC) taken prior to their CMR to acquire this information. T2 mapping was a similar process to T1 mapping. All mapping values were recorded as global and individual slice averages.

Perfusion imaging was carried out after the administration of 0.4mg of regadenoson as a rapid bolus over 10 seconds. This was followed by 0.1 mmol/kg of the gadolinium-based contrast agent gadobutrol (Gadovist; Bayer Phama, Berlin, Germany) infused 45 to 60 seconds after. Late gadolinium enhancement (LGE) imaging was carried out in HLA, VLA, LVOT and short axis views with cross-cut imaging over any apparent LGE, 10 minutes after administration of gadolinium.

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 (N, %)	99 (99)
PR interval (ms)	156 (136, 169.5)
QTc (ms)	396 (384.7, 405)
QRS (ms)	92 (83, 100)
T-wave inversion (N, %)	9 (9)
Normal axis (N, %)	95 (95)
ST-changes (N, %)	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.

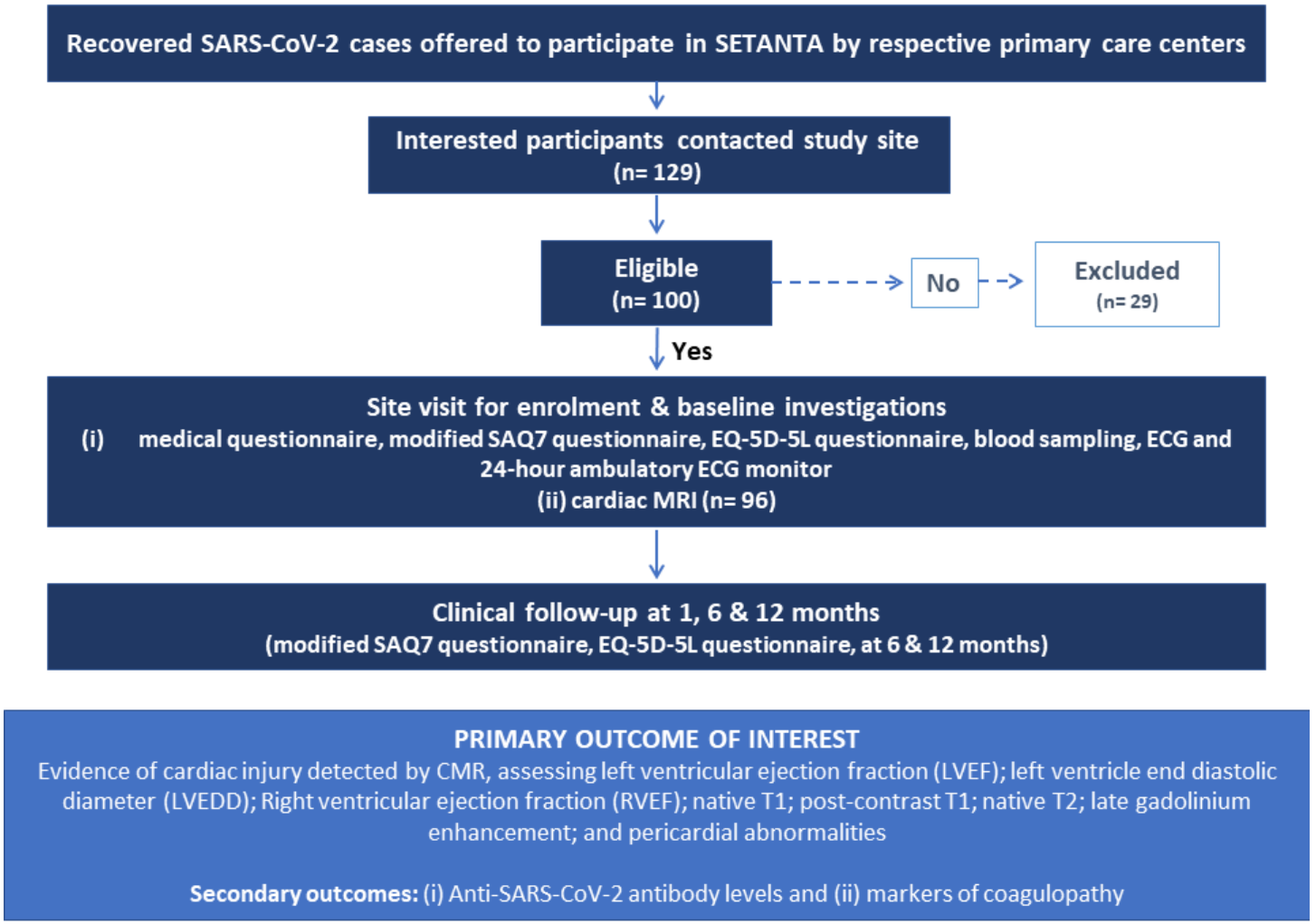
Supplementary Figure 2. **Markers of coagulation and immunity.** Panels A-F highlight serum levels of von Willebrand factor-antigen, fibrinogen, D-dimer, NT-proB-type natriuretic peptide, soluble suppression of tumorgenicity 2 (ST2) and galectin-3, respectively. Black dots represent levels below the 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.

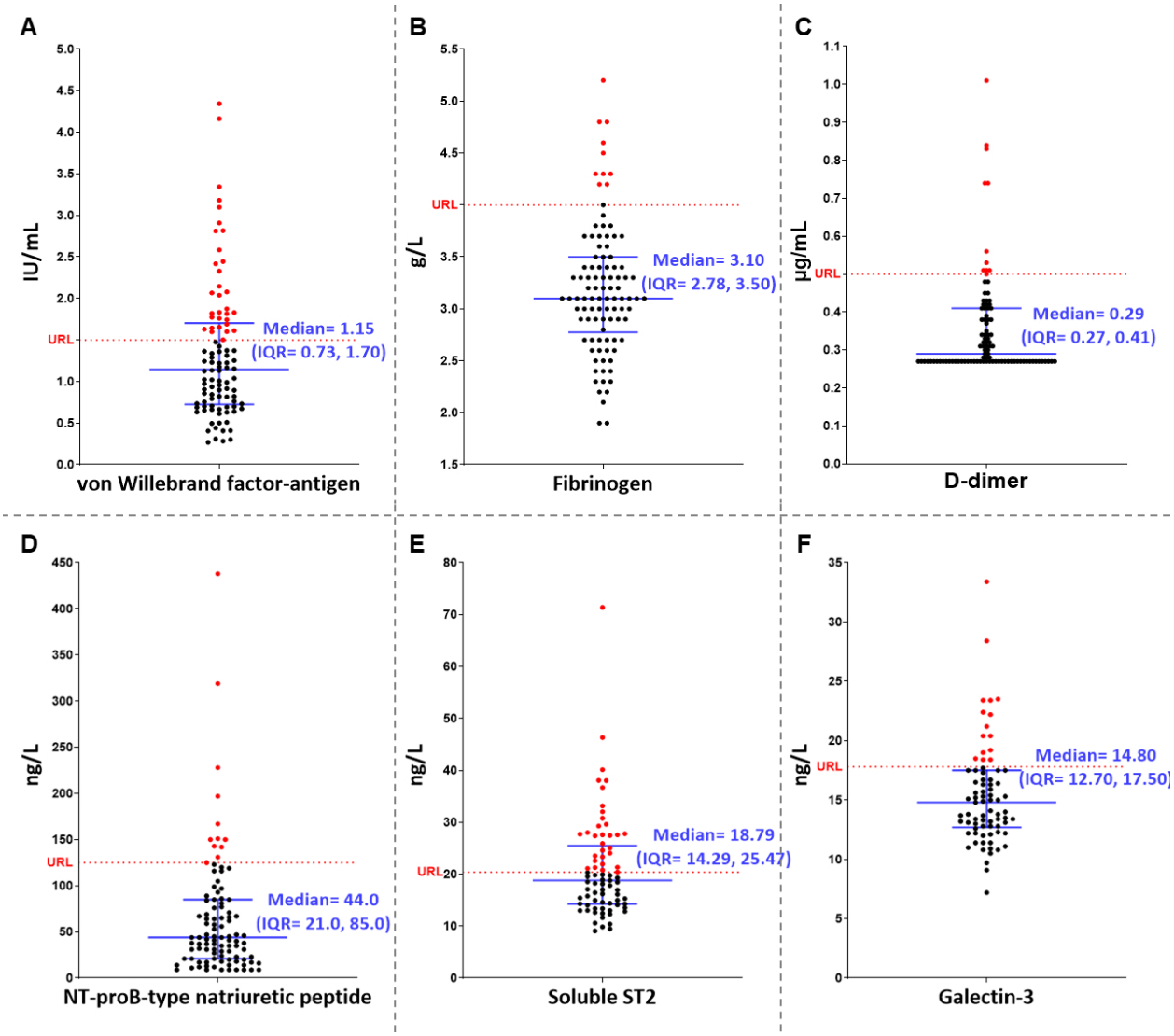
Supplementary Figure 4. **Representative cardiac magnetic resonance imaging findings in patients with COVID-19.** Panel (A) highlights late gadolinium enhancement present in 2 patients. Representative images of patients recently recovered from COVID-19 with pericardial effusion are shown in Panel (B). 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.

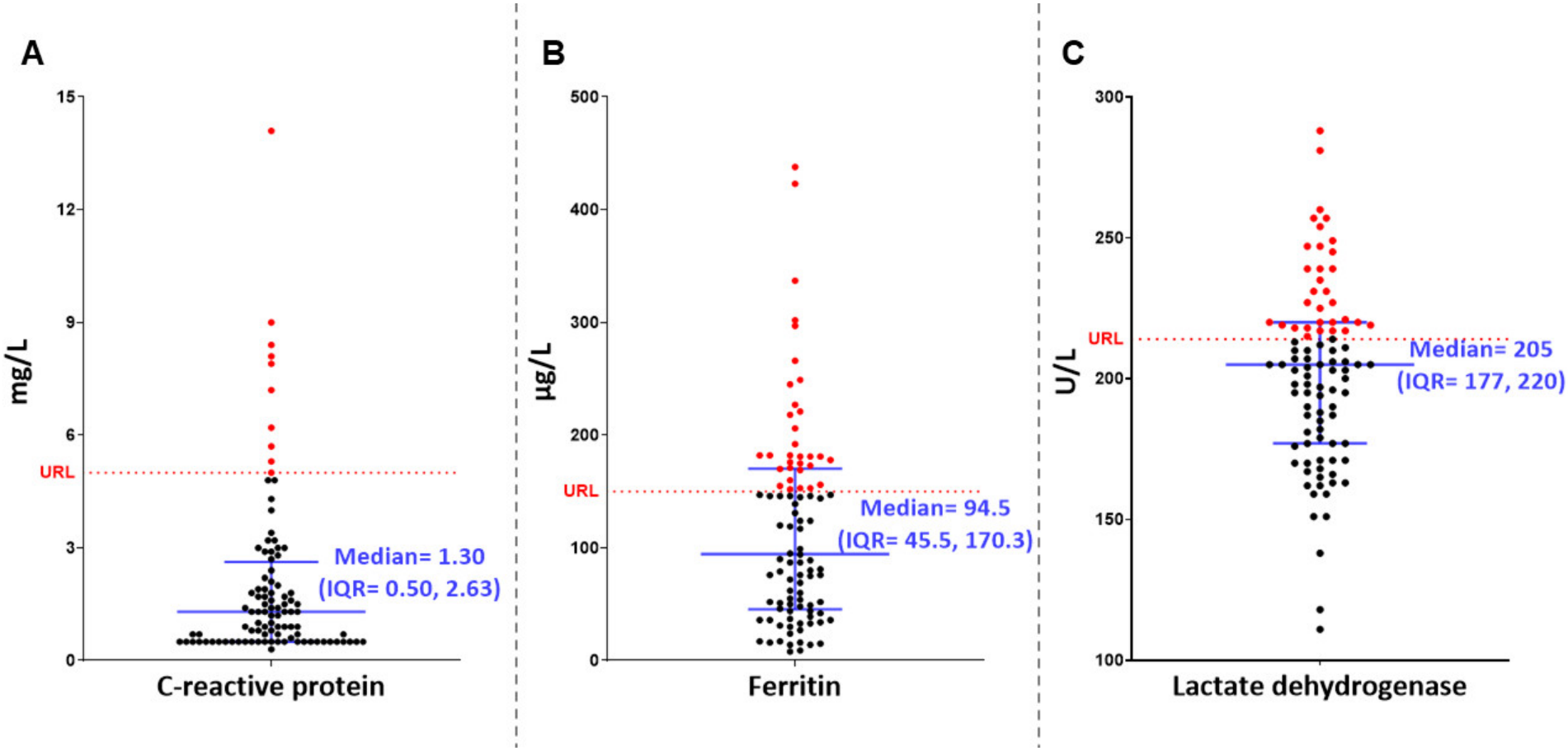
Supplementary Figure 1.



Supplementary Figure 2.



Supplementary Figure 3.



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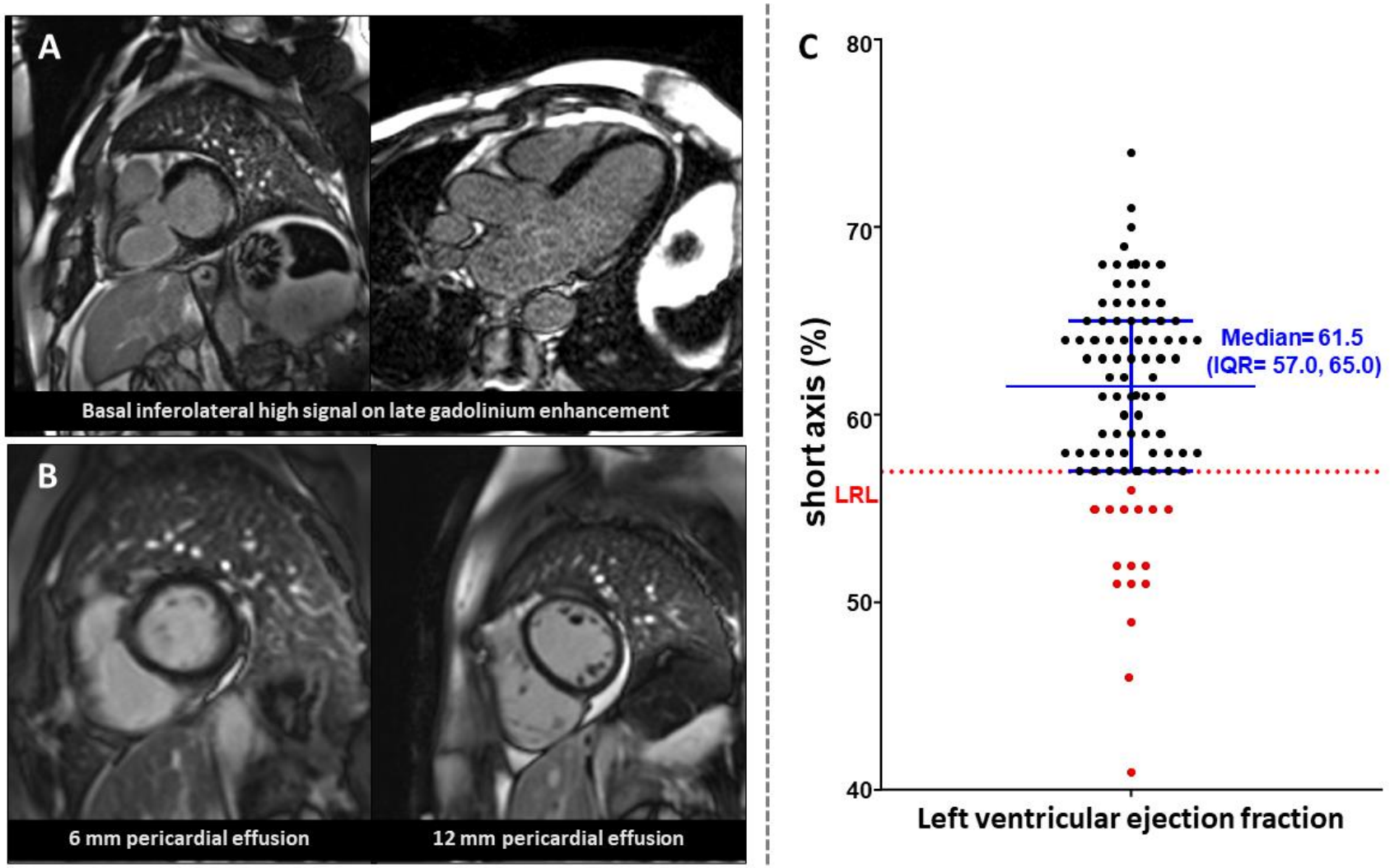

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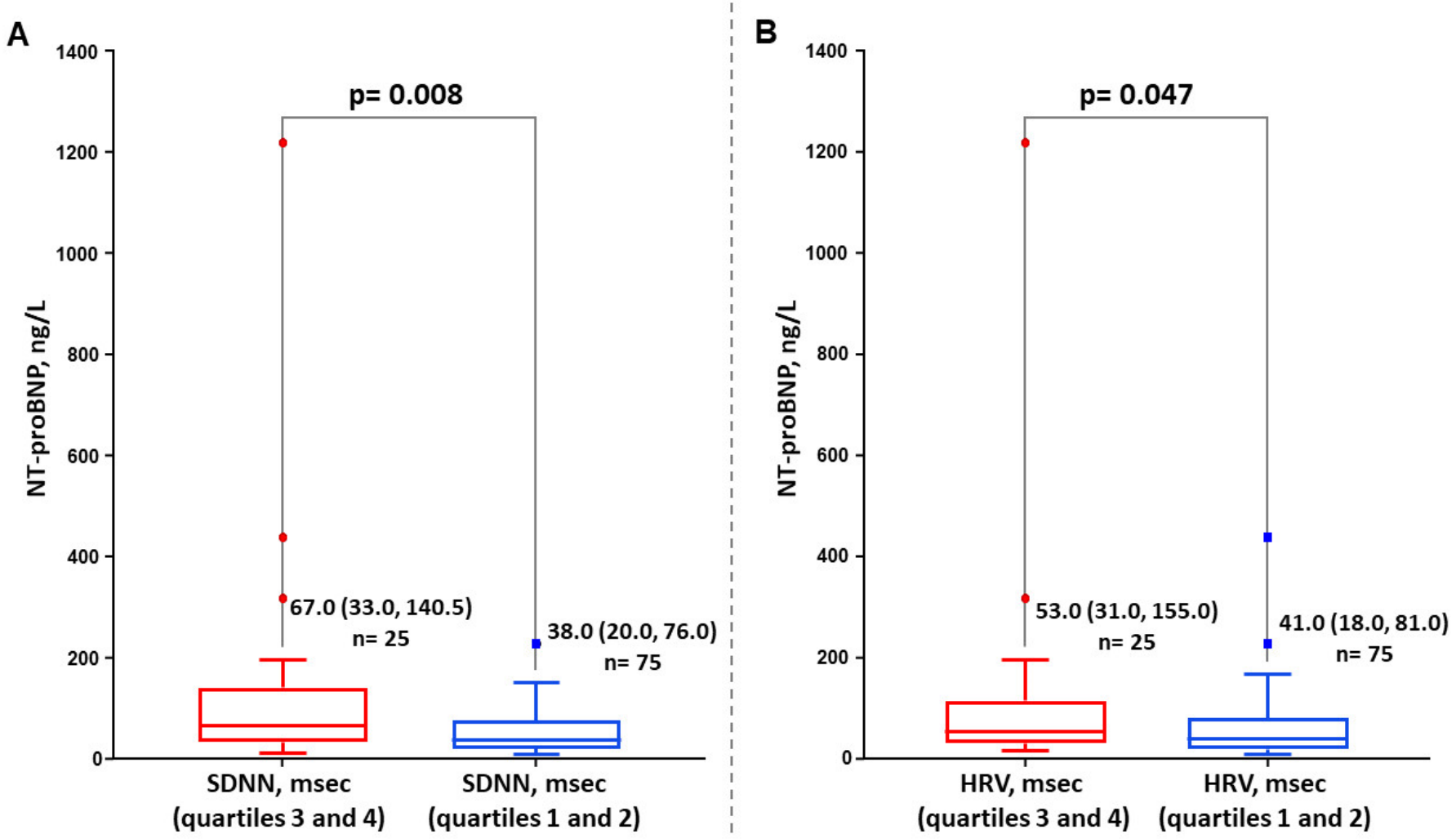

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