

ORIGINAL RESEARCH

Ventricular Conduction Stability Noninvasively Identifies an Arrhythmic Substrate in Survivors of Idiopathic Ventricular Fibrillation

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BACKGROUND: Idiopathic ventricular fibrillation (VF) is a diagnosis of exclusion following normal cardiac investigations. We sought to determine if exercise-induced changes in electrical substrate could distinguish patient groups with various ventricular arrhythmic pathophysiological conditions and identify patients susceptible to VF.

METHODS AND RESULTS: Computed tomography and exercise testing in patients wearing a 252-electrode vest were combined to determine ventricular conduction stability between rest and peak exercise, as previously described. Using ventricular conduction stability, conduction heterogeneity in idiopathic VF survivors ($n=14$) was compared with those surviving VF during acute ischemia with preserved ventricular function following full revascularization ($n=10$), patients with benign ventricular ectopy ($n=11$), and patients with normal hearts, no arrhythmic history, and negative Ajmaline challenge during Brugada family screening (Brugada syndrome relatives; $n=11$). Activation patterns in normal subjects (Brugada syndrome relatives) are preserved following exercise, with mean ventricular conduction stability of $99.2\pm0.9\%$. Increased heterogeneity of activation occurred in the idiopathic VF survivors (ventricular conduction stability: $96.9\pm2.3\%$) compared with the other groups combined (versus $98.8\pm1.6\%$; $P=0.001$). All groups demonstrated periodic variation in activation heterogeneity (frequency, 0.3–1 Hz), but magnitude was greater in idiopathic VF survivors than Brugada syndrome relatives or patients with ventricular ectopy ($7.6\pm4.1\%$, $2.9\pm2.9\%$, and $2.8\pm1.2\%$, respectively). The cause of this periodicity is unknown and was not replicable by introducing exercise-induced noise at comparable frequencies.

CONCLUSIONS: In normal subjects, ventricular activation patterns change little with exercise. In contrast, patients with susceptibility to VF experience activation heterogeneity following exercise that requires further investigation as a testable manifestation of underlying myocardial abnormalities otherwise silent during routine testing.

Key Words: exercise testing ■ idiopathic ventricular fibrillation ■ myocardial ischemia ■ premature ventricular complex

Patients surviving cardiac arrest are usually investigated for underlying obstructive coronary disease, structural heart disease, or channelopathy. In

younger patients experiencing ventricular fibrillation (VF), standard tests, such as the ECG, echocardiogram, and exercise or drug provocation testing, can be normal in up

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CLINICAL PERSPECTIVE

What Is New?

- Without a clear substrate demonstrable on routine cardiac testing, patients at risk of idiopathic ventricular fibrillation provide a risk stratification challenge.
- Electrocardiographic imaging can noninvasively detect exercise-induced changes in cardiac conduction sufficient to differentiate idiopathic cardiac arrest survivors from controls.

What Are the Clinical Implications?

- This could be tested in future patients requiring intensive evaluation, such as relatives of idiopathic ventricular fibrillation survivors.

Nonstandard Abbreviations and Acronyms

BrS	Brugada syndrome
ECGi	electrocardiographic imaging
IHD	ischemic heart disease
V-CoS	ventricular conduction stability
VE	ventricular ectopy

to 20%.¹ Coronary angiography and cardiac magnetic resonance imaging are rarely diagnostic in this group of patients with normal standard tests; the term idiopathic VF is used as a diagnosis of exclusion.

Current clinical tools cannot adequately describe electrophysiological abnormalities that may be present in idiopathic VF survivors. Altered relationship between heart rate and action potential characteristics (ie, the loss of normal rate adaptation) has been shown to be proarrhythmic in invasive, in vitro, and in silico studies.^{2–6} We previously described a noninvasive method to compare activation maps during physiological stress as a surrogate for assessing rate adaptation.⁷ Applying the technique to activation maps reconstructed using electrograms from a CardiInsight vest (Medtronic Inc), we showed evidence of loss of normal rate adaptation in cardiac arrest survivors.⁸ However, this ventricular conduction stability (V-CoS) technique needed further development to overcome the risk of selection bias of the electrograms that were analyzed. Furthermore, the V-CoS test was previously only conducted on a small number of “normal” control patients. To determine if patients with idiopathic VF had evidence of loss of normal rate adaptation, we validated an enhanced version of the V-CoS technique that reduced the risk of selection bias and was compared against a broader range of control subjects.

METHODS

Patient Selection

Patients were recruited from Imperial College Healthcare National Health Service (NHS) Trust and were required to give informed consent, perform an exercise test, and safely cease any antiarrhythmic drugs to undergo the V-CoS test. Ethics were granted by the UK Health Research Authority and the Fulham Research Ethics Committee (London, UK) under references 14/LO/1318 and 17/LO/1660. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Four groups of patients were recruited. (1) Patients with idiopathic VF who had documented VF and had undergone comprehensive investigations that failed to determine an underlying pathology at our inherited cardiac conditions clinic. (2) Survivors of cardiac arrest from VF in the context of total single-vessel occlusion and ST-segment elevation or critical 3-vessel disease. To be eligible, these patients needed complete revascularization, normal left ventricular function by echocardiographic criteria, return to full exercise capacity, and asymptomatic status for >1 year. The purpose of this control group was to determine if VF events inherently cause myocardial injury detected by the V-CoS test as a post hoc finding. (3) Patients with frequent benign ventricular ectopy (VE) attending for clinically indicated ablation with CardiInsight guidance. These patients had normal echocardiography or magnetic resonance imaging, no family history of cardiac electrical disease, and no symptoms of cardiac ischemia. We selected these controls as their VEs are not a trigger for lethal ventricular arrhythmia. In contrast, VEs in idiopathic VF survivors are well described as triggers to VF. (4) Asymptomatic relatives of patients with Brugada syndrome who had been proven not to have the same condition by a negative Ajmaline challenge reaching dose end point of 1 mg/kg (maximum, 120mg): the gold standard for diagnostic testing in Brugada syndrome. Recruitment took place following confirmation of normal 12-lead ECG, Ajmaline test, and echocardiography. This control group was the closest to a “normal heart” cohort. As the V-CoS test requires a low-dose computed tomography (CT) scan, we excluded women who were pregnant or unable to rule out pregnancy by last menstrual period or highly effective contraceptive.

Exercise Electrocardiographic Imaging Testing and Epicardial Reconstruction

Electrocardiographic imaging (ECGi) combines body surface measurements with cross-sectional imaging to estimate epicardial electrophysiology. It has been used to demonstrate electrical changes in patients

with recurrent arrhythmia, patients with inherited cardiac conditions, and survivors of aborted sudden cardiac death.⁸⁻¹³ We used the commercially available CardiInsight system (Medtronic), which uses a 252-electrode vest with a CT image to reconstruct epicardial activation patterns.

Previously, we were able to show changes in rate adaptation that lead to activation heterogeneity in patients with hypertrophic cardiomyopathy and Brugada syndrome using ECGi.^{7,8} We used V-CoS to describe this phenomenon of preserved activation patterns at peak exercise.

The V-CoS process is graphically represented in Figure 1. Each volunteer was fitted with the appropriately sized 252-electrode CardiInsight vest based on his/her height and body shape (Figure 1A). Heart rate was monitored using standard exercise ECG limb lead configuration. Volunteers exercised to peak effort (defined as exhaustion and exceeding 85% of predicted maximum heart rate) using the treadmill Bruce protocol, then underwent 10 minutes of recording in a supine position. During this time, the CardiInsight vest records body surface ECG from each of the 252 electrodes. A noncontrast CT scan of the chest was performed to provide a spatial template for electrogram reconstruction (Figure 1B).

Epicardial electrogram (EGM) reconstruction has been previously described.¹⁴ The user draws a 3-dimensional mesh of the heart based on the CT scan (Figure 1C) and identifies the position of each electrode in the vest. The user selects a cardiac cycle of interest based on the body surface recordings (Figure 1D). Noisy body surface signals are identified by the user and selected for removal; the computer summarizes how many electrodes in the vest have been deselected (Figure 1E). If there was no evidence of isoelectric baseline on the recording of a particular body surface electrode, then the trace was discarded on the basis that evident noise was likely affecting the QRS morphology and therefore activation calculations. Using the clean body surface signals, CardiInsight then calculates epicardial EGMs by combining this information with the 3-dimensional coordinates from the CT-derived mesh, as well as rendering activation maps (Figure 1F). Approximately 1200 epicardial electrograms could be extracted per beat, per patient, and these were taken immediately after peak exertion, and at 10 minutes of recovery.

Extracted data files were loaded into software developed by our group. A region of interest containing the QRS is defined by the user for the cardiac cycles studied: 1 immediately following exercise and 1 at full recovery (Figure 1G). Local activation time was defined as the point corresponding to minimum first derivative of the QRS complex. Semiautomatic deselection of epicardial locations was performed by our software

based on the compared electrogram morphological features (Figure 1H). Relative change in electrogram local activation time between exercise and recovery was calculated across ~1000 to 2000 ventricular epicardial locations (Figure 1I). V-CoS is the percentage of the ventricle that showed no significant change in relative local activation time (<10ms).⁷ A lower score reflects more conduction heterogeneity.

Single Lowest Value V-CoS Score Versus V-CoS Matrix

The previously discussed studies suggest that ECGi could contribute to current risk-stratification algorithms. However, the process for analyzing data with ECGi has multiple steps, and it is not known whether the reproducibility of these measurements is adequate for risk stratification.

We previously described using the “single lowest value” as the representative measurement of V-CoS. Figure 2A describes the process.⁵ The graph originates from the time of maximal exertion in the Bruce treadmill test. Heart rate (blue trend line) and respiration rate (red trend line) are both elevated initially, with a fall toward resting values as 10 minutes of supine rest is reached. The rise and fall of the chest during respiration is a major contributor of bioelectrical noise in the recovering patient. In the original method, a selection of cardiac cycles were used to calculate the V-CoS against the reference comparator at the 10-minute phase. Preexercise cardiac cycles were not used as reference in case of shift of vest electrodes caused by vigorous exercise. The V-CoS process (Figure 1D–1F) is performed for each cardiac cycle selected at the operator’s discretion, giving a variety of scores per patient. This is represented in the figure with 4 example V-CoS maps, with the most intensely colored map (peak exercise in this patient) representing the lowest score. This lowest score was taken as the worst-case scenario of activation heterogeneity. However, there was also a risk that this method was more prone to selection bias and from artefactual outlier data.

During reproducibility studies, there was variation in V-CoS scores between operators and exercise tests as well as the beat-to-beat variation.⁷ We hypothesized that variation in reconstructed ECGi electrograms can arise from the following: (1) true differences in epicardial electrophysiology, (2) bioelectric noise from the body-electrode interface, and (3) nonuniform selection of both input and reconstructed electrograms by the operator. To characterize this variation, we developed the V-CoS matrix method (Figure 2B) to compare all beats and remove any selection bias.

Peak exercise and 10 minutes of recovery were bookmarked at the time of testing. In the revised method, the first 10 consecutive cardiac cycles

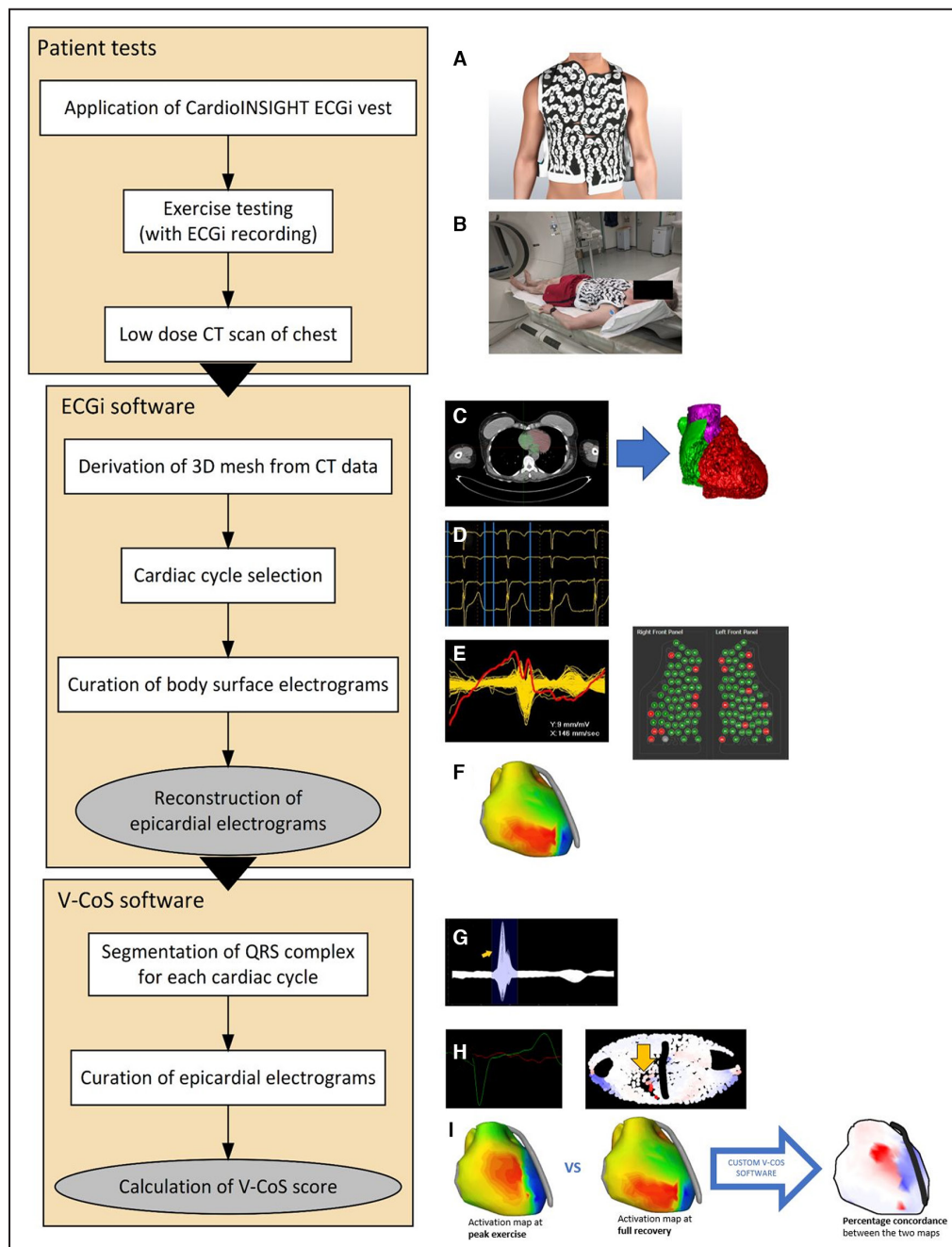


Figure 1. The V-CoS score process.

A, The 252-electrode ECGi sensor vest is applied to the patient undergoing maximal Bruce protocol exercise. **B**, Recordings are made during 10 minutes of supine recovery, which is followed by noncontrast CT scan of chest. **C**, The CT scan is segmented into a 3D mesh. **D**, Cardiac cycles from the recording are selected for analysis. **E**, Body surface signals from the vest too noisy for analysis are identified and removed from the vest recording. **F**, Activation maps are drawn from the reconstructions. **G**, In the V-CoS software, the QRS complex is identified. **H**, Reconstructed electrograms (compare with the body surface signals from **E**) are curated for excess artifact and removed from the analyzed map. The V-CoS process compares the 2 activation maps and calculates the percentage score of electrograms with median-relative LAT difference <10ms. 3D indicates 3-dimensional; CT, computed tomography; ECGi, electrocardiographic imaging; LAT, local activation time; and V-CoS, ventricular conduction stability.

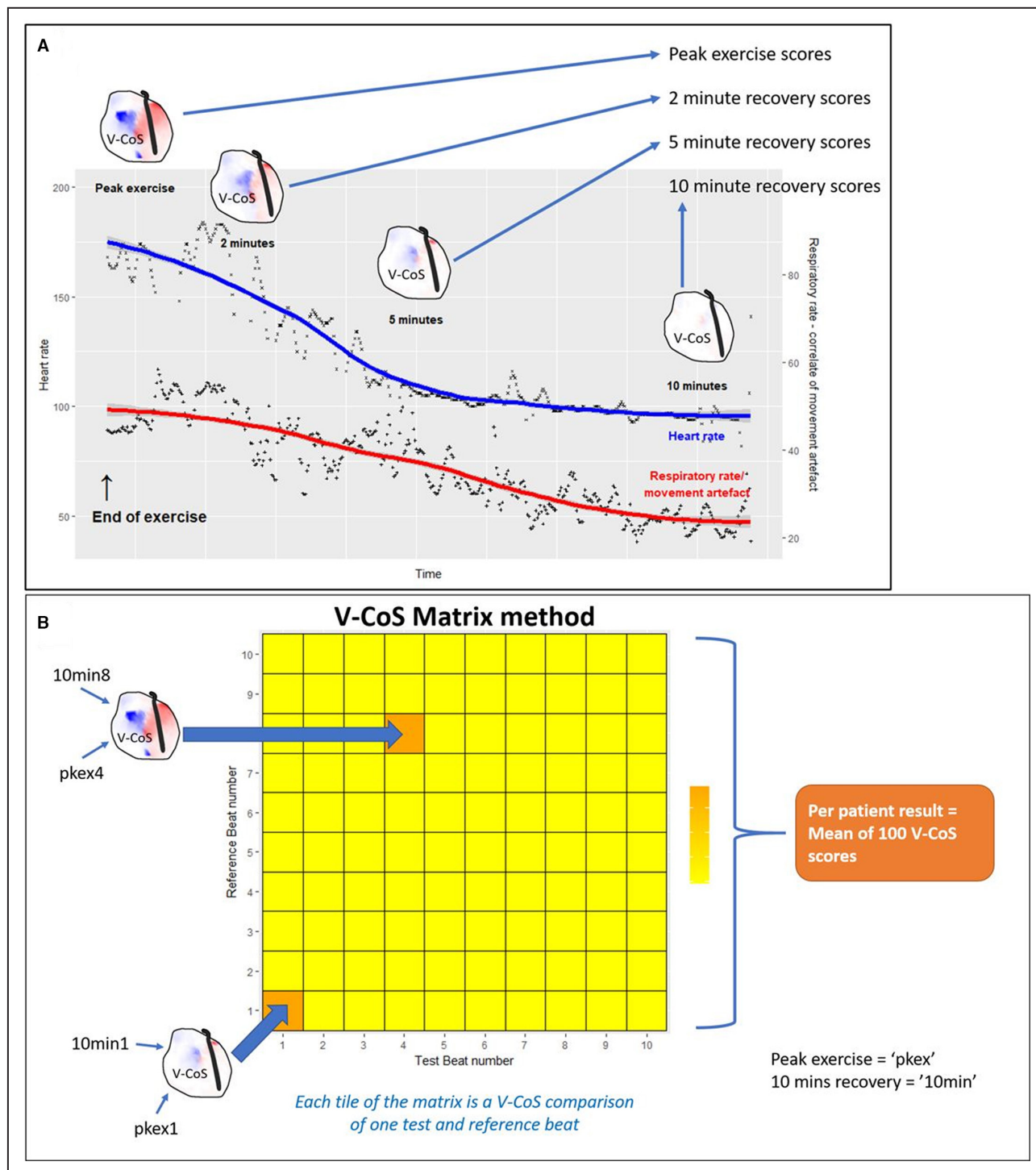


Figure 2. Methods of assigning a V-CoS score to a patient.

A, The “single lowest value” method: following peak exercise, patient heart rate (blue trendline) and respiration rate (red trendline, correlating with chest movement) fall toward resting values. Cardiac cycles selected immediately after exercise and 3 stages of recovery undergo V-CoS mapping (the 4 epicardial shells). The lowest calculated V-CoS is defined as the patient score. In this method, cardiac cycles are selected at the user’s discretion, and cardiac cycles are individually stripped of uninterpretable electrograms at user discretion. **B**, The V-CoS matrix: this study uses a new method, where the mean of a 10-by-10 comparison matrix of consecutive cardiac cycles from the peak and rest phases is taken as the patient score. By standardizing the curation of electrograms between cardiac cycles and forcing the selection of consecutive beats, user-induced variability is reduced, and temporal relationships are revealed. V-CoS indicates ventricular conduction stability.

Table 1. Comparison of Patient Characteristics Between Groups

Characteristic	Idiopathic VF	Ischemic VF	Ventricular ectopy	BrS relative	P value
	(n=14)	(n=10)	(n=11)	(n=11)	
Proportion men	0.79±0.4	0.9±0.3	0.46±0.5	0.73±0.5	0.11
Age, y	36.7±8	58.3±8.5	42.5±14.8	45.5±11.6	0.002*
Peak phase heart rate, bpm	150.7±24.1	119.0±28.5	132.5±26.4	136.2±14.1	0.04*
Recovery phase heart rate, bpm	93.4±10.8	81.5±11.3	92.2±23.6	97.5±9.9	0.01*
Peak phase, % pMHR	79.9±11	73.3±16	75.0±15.3	78.3±8.9	0.79
Recovery phase, % pMHR	51.3±4.2	50.4±6	52.2±13.1	56.1±7.4	0.21
Peak QRS duration, ms	97.7±15.8	92.6±18.3	91.2±12.8	90.9±11.9	0.75
Recovery QRS duration, ms	110.4±20.6	100.4±17.5	106.8±11.7	102.8±11.4	0.57

Data are given as mean±SD. Statistical significance judged by the Kruskal-Wallis test. Bpm indicates beats per minute; BrS, Brugada syndrome; pMHR, predicted maximum heart rate; and VF, ventricular fibrillation.

* $P < 0.05$.

considered sufficiently artifact free for analysis were selected following each of these bookmarks. Effort was made to adhere to bookmark timing as closely as possible, and heart rates were recorded for each of the sampled segments to determine if differences between the test groups existed. Reconstruction of the selected time strip was performed, producing 1000 to 2000 continuous epicardial EGM traces containing 10 cardiac cycles (similar to Figure 1D–1F).

Using a modified version of the original V-CoS software, the 10 QRS complexes were automatically selected by finding largest 10 peaks in the squared mean electrogram trace. QRS regions of interest could be manually altered by the user in case of poor automatic selection. Local activation times were calculated for each epicardial location of the 10 cardiac cycles from exercise and the 10 from recovery (similar to Figure 1H) but with fully automatic deselection by our software based on compared electrogram morphology.

V-CoS scores were calculated for each exercise-recovery QRS pairing (similar to Figure 1I). The V-CoS matrix allows the comparison of every combination of exercise QRS and recovery QRS (Figure 2B). For example, the first cell in the first row of the matrix is the V-CoS comparison of the first exercise beat and first recovery beat activation times. To minimize the effect of outlier measurements compared with the single lowest value method, the mean score from each matrix was used as the representative score for each patient. Individual cells in a matrix are color coded to allow rapid visual estimation of score magnitude and variability for a given patient.

When selecting body surface signals from immediately postexercise and 10 minutes of recovery, care was made to ensure the same array of electrodes were used in cardiac cycles compared with each other. In effect, if any surface electrode recorded too much

artifact to be analyzed in either exercise or recovery, it was excluded from analysis altogether.

By automating several steps in the process and ensuring consistent electrogram deselection between cardiac cycles, the V-CoS matrix method aims to reduce the potential variation between cardiac cycles from nonuniform user choices. This leaves the influence of (1) true differences in epicardial electrophysiology and (2) bioelectric noise from the body-electrode interface. To separate these, we opted for a simulation-based approach, described in Data S1 ("Periodic noise simulations").

Analysis of Surface ECG Markers

To examine for body surface recording signs of conduction pathology, QRS durations were measured for the peak exercise and recovery data sets. The output of the same vest supplying data to the ECGi reconstruction was used over conventional 12-lead method to avoid possible timing issues with the exercise machine output. The positional equivalent of 12-lead ECG V2 was used (electrodes 71–76 on the CardiInsight vest), with the first beat from the sample as the representative measurement.

Statistical Analysis

Comparisons between >2 groups were performed using the Kruskal-Wallis test. When pairwise comparisons were required within >2 groups, the Dunn test was used with Benjamini-Hochberg correction to control the family-wise error rate. Comparisons between 2 groups were made with the Wilcoxon rank-sum test. Repeated statistical analysis was performed using the subgroup of patients with idiopathic VF with a history of multiple arrhythmic events. Summary statistics are presented as mean±SD where normally distributed and median±interquartile range where not normally

distributed. Calculations and graphical representations were performed in R 4.0.2 and the ggplot2 library, respectively.

RESULTS

Patient Characteristics

A total of 47 patients were recruited; details are given in the [Table](#). Further clinical information about other investigations is provided in [Tables S1 through S4](#). Age was significantly different between groups. The idiopathic VF group was the youngest, the ischemic heart disease (IHD) VF group was the eldest, with the patients with VE and Brugada syndrome (BrS) relatives in the middle (means, 36.8, 58.3, 42.5, and 45.4 years, respectively; $P=0.002$). Heart rate was lower in IHD VF patients than the other groups, both during the peak exercise measurements and during recovery. This turned out to be a manifestation of their greater age, because when heart rate was expressed as a percentage of predicted ($220 - \text{Age}$), there was no longer a difference between groups.

QRS durations were not significantly different across the groups during either phase of data collection. Sex balance was not significantly different between groups.

Comparisons of V-CoS Between Groups

All patients successfully completed the testing protocol, reaching at least 85% of maximum predicted heart rate for age on the treadmill without complications.

Idiopathic VF survivors had significantly lower mean V-CoS matrix scores than pooled controls (median $\pm$ interquartile range V-CoS versus control, $96.9\pm 2.3\%$ versus $98.8\pm 1.6\%$; $P=0.0014$; [Figure 3A](#)). Median $\pm$ interquartile range V-CoS was $96.9\pm 2.3\%$ for idiopathic VF survivors, $97.8\pm 1.5\%$ for IHD VF, $98.8\pm 1.2\%$ for patients with VE, and $99.2\pm 0.9\%$ for BrS relatives. In pairwise comparisons, idiopathic VF survivors had significantly lower mean V-CoS matrix scores than patients with VE and Brugada relatives ($P=0.039$ and $P=0.0096$, respectively; [Figure 3B](#)). The IHD VF survivors could not be differentiated in corrected comparisons with idiopathic VF, VE, or Brugada relatives. Brugada relatives and patients with benign VE had comparable V-CoS scores. For the numeric P values of all pairwise comparisons, please see [Table S5](#).

Two patients in the idiopathic VF group appeared to form a particularly low V-CoS group. To ascertain whether they might have features suggestive of a distinct structural phenotype not appreciated by imaging, we examined electrogram fractionation and voltage. Fractionation was defined as the number of steep negative deflections ($>70\%$ maximum gradient and $>10\%$ maximum voltage of the parent EGM).¹¹

Unipolar but not bipolar EGMs are available for voltage measurement in CardiInsight. The 2 lowest patients ranked second and ninth of 14 idiopathic VF survivors for fractionation (mean, 1.05 and 1.08 steep negative deflections per EGM across the whole heart; cohort range, 1.04–1.20). For voltage, they ranked second and sixth of 14 (mean, 2.85 and 3.94 mV across the whole heart; cohort range, 2.85–4.63 mV). This suggests they are not outliers for the idiopathic VF group and unlikely to have a structural substrate distinct from the others.

To determine whether a larger effect size could be seen in patients with idiopathic VF with multiple arrhythmic events, patients with >1 clinical episode of VF or appropriate implantable cardioverter-defibrillator therapy were analyzed against those with a single such episode and compared with controls.

Subgroups of patients with multiple ($n=5$) or single ($n=9$) episodes of ventricular arrhythmia had significantly lower V-CoS scores than controls ($96.7\pm 3.9\%$ and $97.0\pm 2.4\%$ versus $98.8\pm 1.6\%$; $P=0.028$ and $P=0.036$, respectively; [Figure 3C](#)). V-CoS scores trended nonsignificantly lower in the multiple episode group. For the numeric P values of all pairwise comparisons, please see [Table S6](#).

Variability of V-CoS

V-CoS matrices were graphically expressed with consecutive peak exercise beats in the horizontal axis and consecutive recovery beats in the vertical axis. Color scaling was standardized across all patients; V-CoS scores were shaded yellow if 100%, with increasing red intensity to a lower bound of 85%.

[Figure 4](#) shows 8 examples. Idiopathic VF matrices are more intensely red than the IHD VF matrices, which are, in turn, more intensely red than the VE or BrS relative matrices, reflecting the differing means between the groups. All groups demonstrate variability, with the magnitude varying between groups. Variability follows a periodic pattern, with certain beats interacting to produce lower V-CoS scores than others. To quantify, we calculated the range in a single matrix (maximum V-CoS minus minimum V-CoS).

Median $\pm$ interquartile range V-CoS ranges were $7.6\pm 4.1\%$ for idiopathic VF survivors, $5.9\pm 4.8\%$ for IHD VF survivors, $2.9\pm 2.9\%$ for patients with VE, and $2.8\pm 1.2\%$ for BrS relatives. The range of individual beat V-CoS comparisons was significantly larger in the idiopathic VF group than either patients with VE or BrS relatives ($P=0.04$ and $P=0.01$, respectively; Dunn test). Patients with IHD VF also had significantly larger V-CoS range than BrS relatives ($P=0.01$). There was no significant difference between the ranges of idiopathic VF and IHD VF groups ($P=0.31$), and the VE and BrS

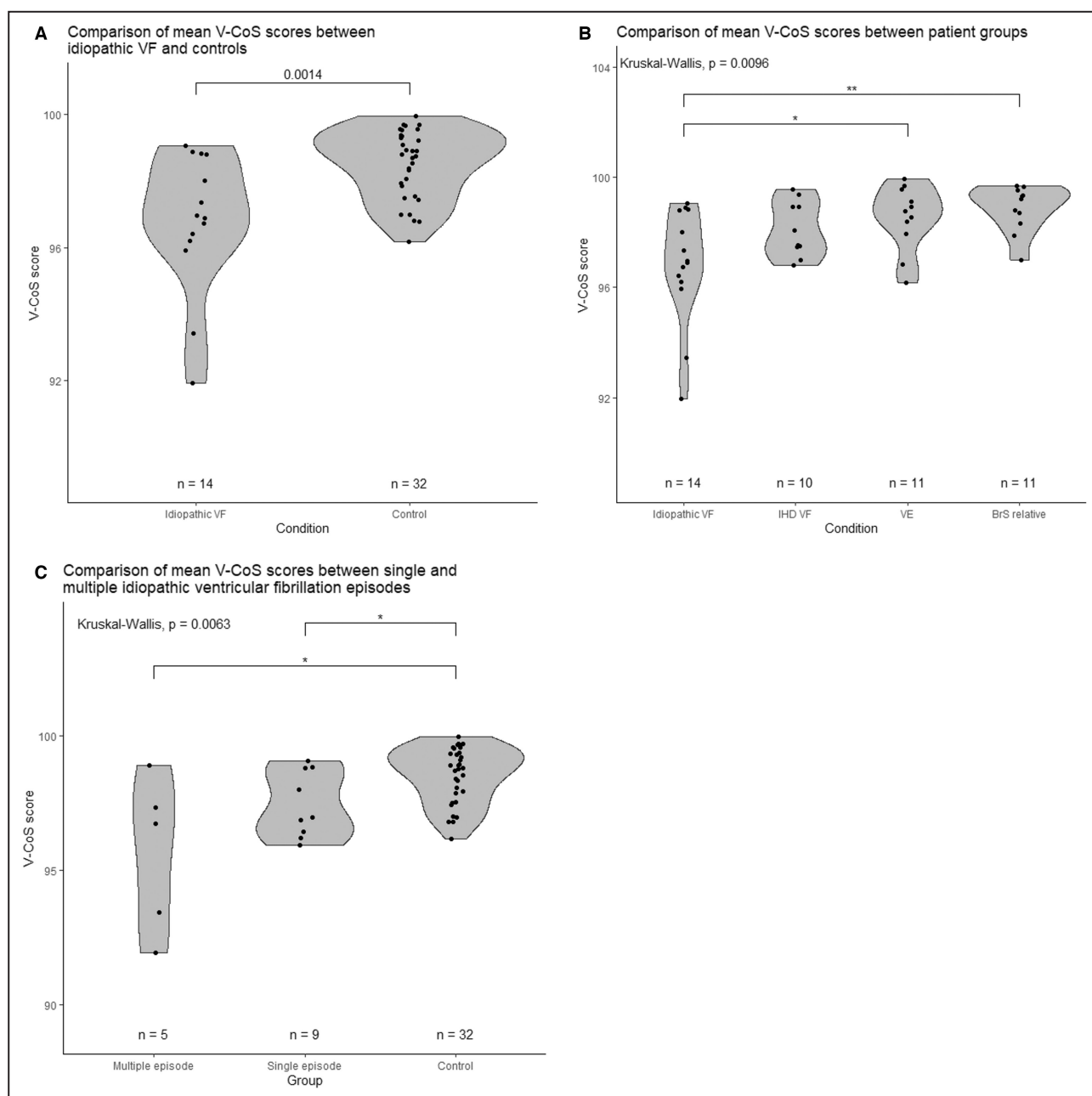


Figure 3. Mean V-CoS scores compared between groups.

A, Comparison between survivors of idiopathic VF with a pooled control set of patients with ischemic VF with full recovery of left ventricular function and full revascularization, patients with benign ventricular ectopy, and unaffected BrS relatives. **B**, Comparison between survivors of idiopathic VF with control groups separated. **C**, Comparison between survivors of idiopathic VF experiencing either multiple or single episodes of ventricular arrhythmia and controls. Group comparisons were determined using the Kruskal-Wallis test, and pairwise comparisons were determined using Dunn test with Benjamini-Hochberg correction. For the numeric *P* values of all comparisons, please see Data S1. BrS indicates Brugada syndrome; IHD, ischemic heart disease; V-CoS, ventricular conduction stability; VF, ventricular fibrillation; and VE, ventricular ectopy.

relative groups could not be differentiated ($P=0.53$). The IHD VF group could not be differentiated from any group (versus idiopathic VF, $P=0.2$; versus VE, $P=0.53$; and versus BrS relative, $P=0.27$).

Figure 5 describes the underlying electrograms and V-CoS maps that form the low scores in a matrix.

DISCUSSION

V-CoS revealed evidence of loss of normal rate adaptation in patients surviving idiopathic VF compared with a control group that included patients with a reversible ischemic cause for VF, patients with benign

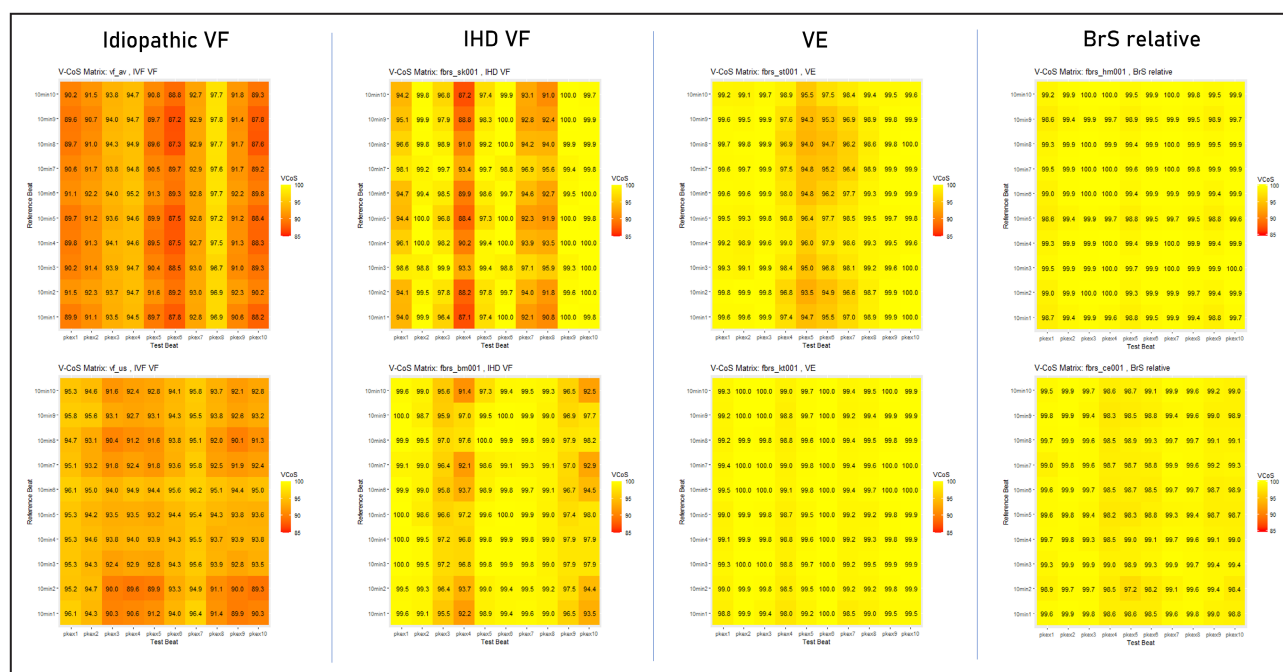


Figure 4. Eight example V-CoS matrices: 2 from each condition group.

All patients demonstrate some variability in V-CoS scores, but this seems to be greater in patients with idiopathic VF than those with recovered ischemic VF or the other controls. Beats immediately after peak exercise (consecutive beat x), pkex(x); beats from full recovery (consecutive beat x), 10minutes (x). BrS indicates Brugada syndrome; IHD, ischemic heart disease; V-CoS, ventricular conduction stability; VE, ventricular ectopy; and VF, ventricular fibrillation.

ventricular ectopy, and asymptomatic relatives with normal cardiac investigations. Interestingly, this loss of normal rate adaptation appeared to occur in a periodic cycle and was accentuated in those susceptible to VF.

Activation Patterns Are Preserved in Normal Subjects Undertaking Exercise

Epicardial activation patterns in normal subjects undergoing exercise were previously unknown.¹² Surface ECG studies demonstrate either insignificant change or reduction in QRS duration in response to exercise.^{15–17} We previously demonstrated the feasibility of using exercise ECGi to study epicardial activation at physiologically elevated heart rates but only included patients with underlying cardiac conditions.⁸ In the current study, we present normal subjects following echocardiography, resting ECG, and negative Ajmaline challenge test undergoing exercise ECGi. Following exercise, these normal subjects had a median V-CoS of 99%, indicating that the activation pattern was almost entirely preserved between rest and following exercise. This contrasts with invasive and ex vivo studies showing poor preservation of activation pattern in response to rate or stressor in proarrhythmic substrates.^{2,4,5,18}

Arrhythmic Substrate for Idiopathic VF

Purkinje system ectopic beats can trigger VF, and radiofrequency ablation of these sites can prevent recurrences.⁹ It was not known if an abnormal substrate was also necessary for these ectopics to initiate sustained arrhythmia. In the current study, the difference in V-CoS scores between patients with multiple VF events and controls was greater than for those with single VF events, suggesting the presence of a predisposing proarrhythmic substrate.

In the current study, we only investigated the preservation of activation patterns to exercise; some patients had V-CoS scores of ~99%, suggesting normal rate adaptation. However, we previously demonstrated changes in activation recovery intervals during treadmill testing seen on ECGi in idiopathic VF but not on the surface ECG.^{8,19} Others have demonstrated steep repolarization time gradients and flattened T waves in the patients with idiopathic VF not seen in their single control patient.²⁰ These varying observations in the idiopathic VF myocardium may reflect a spectrum of the same pathology or possibly different conditions. “Idiopathic” VF survivors with lower V-CoS scores could be subgrouped; future study could focus on factors connecting these patients to identify new, distinct pathological features. Clinical tools may need to combine multiple physiological parameters rather than relying on single

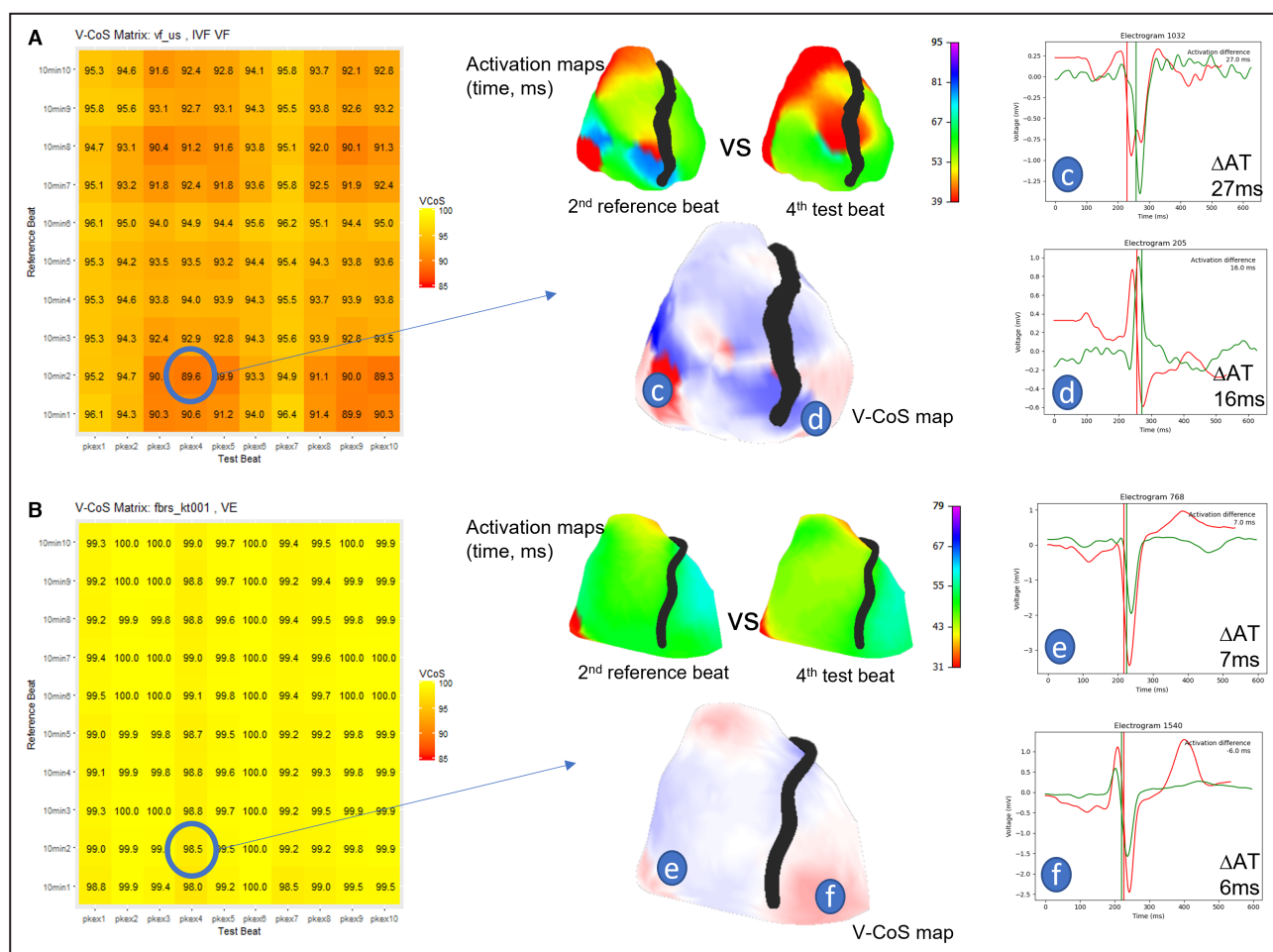


Figure 5. Comparison of V-CoS maps and edvlectrograms used to build the matrices.

Low V-CoS scores from 2 matrices are selected, and the source epicardial activation maps are displayed. V-CoS is the percentage of epicardial points with <10-ms discordance in activation time. This discordance can be mapped: blue as advancement and red as delay of the test phase, with increasing color intensity representing the magnitude. **A**, Idiopathic VF survivor has clear differences between activation maps, resulting in greater discordance on the V-CoS map and a low V-CoS score. Example electrograms from regions “c” and “d” show that despite baseline noise, the software accurately finds the minimum dV/dt to define activation. The difference in activation times is stated as ΔAT . **B**, Control patient has much less discordance, and example electrograms from the most intensely colored regions “e” and “f” reveal the lower levels of discordance compared with the idiopathic VF survivor’s map. dV/dt, change in voltage divided by change in time; V-CoS indicates ventricular conduction stability; VE, ventricular ectopy; and VF, ventricular fibrillation.

factors, which may not encompass the spectrum of disease.

VF is inducible with aggressive programmed stimulation in normal hearts but often self-terminates, indicating the need for a substrate.²¹ Extreme stimuli, such as commotio cordis and electrocution, can also initiate VF in the normal heart.^{22,23} Our study suggests that for some patients with idiopathic VF and no further events during the follow up period, their substrate assessment (at least by standard methods and V-CoS) is within the normal range. It is possible that these patients have no underlying myocardial abnormalities and were the victims of a rare external initiating stimulus.

Arrhythmic Substrate of Ischemic VF Survivors

VF is a common cause of death related to myocardial infarction, but arrhythmia susceptibility in patients with acute myocardial infarction is not uniform. Familial sudden death has been shown to be a risk factor in 2 case-control studies of patients presenting with ST-segment-elevation myocardial infarction.^{24,25} Genome-wide study of 1 cohort revealed an association at 21q21 with an odds ratio of 1.78, colocated with the Coxackie-adenovirus receptor gene (*CXADR*), also implicated in myocarditis and dilated cardiomyopathy.²⁶ Another study found mutations in sodium

voltage-gated channel alpha subunit 5 (SCN5A) with acute myocardial infarction complicated by VF, but only in a small proportion of cases.²⁷

The patients with IHD VF in our control group had an intermediate range of V-CoS that was not significantly different from either the idiopathic VF cases or those controls without a history of VF. Although this group are considered to be at low risk of future arrhythmia, our findings suggest either the presence of an underlying proarrhythmic substrate or that the historic VF episode may have caused myocardial injury that is being detected by the V-CoS method.

Periodic Variation in Activation Pattern

Periodic drops in V-CoS were found across all the groups studied and have not been described previously. Small fluctuations in V-CoS were seen in controls, becoming more obvious and marked in idiopathic VF survivors. Detailed in Data S1 and Figures S1 and S2, we investigated the possibility that this was artifact of the experimental method and simulated the effect of noise at the 0.3- to 1-Hz frequency characteristic of V-CoS periodicity. As noise-simulation scores were no different from recovery, and significantly higher than the true exercise scores, we concluded that the periodic drops in V-CoS were not attributable to exercise-induced electrical artifact at this frequency. Periodicity has been described in mechanical function as well as electrical parameters, such as repolarization and T-wave alternans.^{28–30} These have been proposed as being proarrhythmic risk markers. As we detected periodicity in all groups, it may be a physiological phenomenon that is more marked in those further along the spectrum of arrhythmia vulnerability.

Risk stratification for idiopathic VF is uniquely challenging as patients do not have a “cardiac diagnosis” until after the event. The primary purpose of any risk stratification tool at present would be for screening family members of an index case of unknown cause. In the cases where the index case has a clearly abnormal V-CoS test, then it may be reasonable to consider using it as a diagnostic tool. Health screening programs often perform a panel of tests, including 12-lead ECG, which may reveal minor abnormalities. These findings are usually nondiagnostic and nonprognostic in asymptomatic individuals, but present clinicians with a dilemma of whether to perform further investigations. The V-CoS test may potentially be helpful in those cases where exercise-related risk needs further assessment.

Limitations

This was a post hoc study, and it is uncertain whether these changes existed before the VF event. We used exercise as the physiological stressor, and other potential triggers need investigation. The number of patients

included was too small to extend this to a wider risk stratification system.

Age- and sex-matched healthy controls were not used in this study because of ethical concerns about radiation exposure during experimental CT scan, but these volunteers would be a useful comparator to our groups with various pathological conditions.

The accuracy of noninvasive ECG imaging is also a limitation. It is difficult to assess against contact mapping as the former estimates a panoramic map in a single beat, whereas the latter measures a map over many cardiac cycles and is thereby unable to assess beat-to-beat variability in the same way. Validation studies in open-chest or ex vivo preparations may not be wholly representative of hearts in live, intact patients. It is therefore possible that the proposed changes in activation patterns are mechanistically different from our interpretation here, which appears periodic through the lens of ECG imaging. Further study might investigate whether ex vivo preparations can be stimulated to produce these changes.

CONCLUSIONS

We used the V-CoS test to show that patients with a history of VF demonstrated periodic loss of uniform cardiac conduction following exercise. This contrasted with normal subjects whose epicardial activation patterns remained the same following exercise. The V-CoS test has the potential to enhance diagnostic yield for investigations of proarrhythmic substrate.

ARTICLE INFORMATION

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Disclosures

Imperial Innovations holds the patent for the intellectual property of the algorithm described here on behalf of the authors (Drs Shun-Shin, Leong, Ng, Varnava, Francis, and Kanagaratnam). The remaining authors have no disclosures to report.

Supplemental Material

Data S1
Tables S1–S6
Figures S1–S2

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Periodic noise simulations

Preliminary inspection of V-CoS matrices in Figure 4 suggested periodic drops in V-CoS score every 3-5 beats, or a frequency of 0.3-1Hz at 120-150 beats per minute. This is close to the frequency of baseline wander and electrode motion artefacts, both of which may be exaggerated immediately following exercise. This could be seen in our peak phase electrograms at a comparable frequency to drops in V-CoS score (Figure S1, panel A and B).

To test the hypothesis that this low frequency periodic noise was the cause of our observations, a simulation experiment was conducted.

10 consecutive cardiac cycles immediately following peak exercise and 10 consecutive cardiac cycles following 10 minutes of recovery were collected for analysis. V-CoS matrices were calculated for the exercise vs recovery beats, producing 100 exercise-induced V-CoS scores. The recovery beats were then compared to themselves, producing 100 recovery-baseline V-CoS scores.

Noise was extracted from the exercise traces using a low-pass finite impulse response filter, frequency 1Hz, order 600 and sampling rate 1kHz (Figure S1, panel C). ECGi datasets are formed of one electrogram $\frac{\text{Voltage}}{\text{Time}}$ graph per reconstructed point in the epicardial mesh. For a numeric array of m electrogram traces by n samples, the filter resulted in an identically sized numeric array of m noise traces by n samples.

One-dimensional linear interpolation was used to ensure noise trace arrays had identical length to the recovery trace. Then, each individual noise trace was added onto its counterpart recovery trace (Figure S1, panel E and F). Each synthetic electrogram had the QRS properties of recovery but noise characteristics of exercise. These synthetic, noise-augmented traces were then compared to the unmodified recovery traces to calculate a final, simulated noise V-CoS matrix.

Adding synthetic noise to true recovery matrices could not induce periodic drops in V-CoS ($p = 0.46$). True exercise V-CoS was lower than both simulated and recovery V-CoS ($p < 0.0001$), mean $\pm$ SD: 97.4 $\pm$ 3.0% for true exercise, 99.9 $\pm$ 0.08% for noise-augmented synthetic and 99.9 $\pm$ 0.2% for true recovery). As noise-simulation scores were no different from recovery, and significantly higher than the true exercise scores, we can conclude that the periodic drops in V-CoS seen in our groups are not a side-effect of exercise-induced electrical artefact at this frequency.

To understand whether QRS frequency spectrum noise could in some way be causing the periodic drops in V-CoS we needed to take a generated simulation approach as a band pass method for obtaining QRS frequency noise would not be able to distinguish the QRS from the desired noise, resulting in the QRS complexes themselves being retained.

Using Fourier analysis we measured the frequency spectrum of the EGM QRS complexes in our study. Most frequencies were between 1-60Hz. To cover high frequency deflections that might also influence the measurement, we doubled this to define a 1-120Hz band pass to filter an inverse discrete Fourier transform of a random array scaled to visually obliterate the ST-T waves (Figure S1, panels H-K).

For noise in the QRS spectrum, the true exercise V-CoS was also lower than both simulated and recovery V-CoS ($p < 0.0001$), mean $\pm$ SD: 97.4 $\pm$ 3.0% for true exercise, 99.4 $\pm$ 0.5% for noise-augmented synthetic and 99.9 $\pm$ 0.2% for true recovery). However, this time the simulated noise V-CoS were differentiable from the recovery V-CoS ($p < 0.0001$), albeit with a negligible effect size (0.5% V-CoS). Significant periodic drops were not seen in the idiopathic VF survivors and therefore our technique appears robust to generated noise in the QRS spectrum.

Example matrices are displayed in Figure S2. The noise augmented recovery trace matrices (middle row) are visually far more like the true recovery matrices (bottom row) than the true exercise matrices (top row).

Table S1: Ischemic VF

Count	10
Age (years)	58.3 ±8.0
Sex M:F	9:1
VF during presentation	10/10
Anginal symptoms in year prior to testing	0/10
ECG at testing	3 normal sinus rhythm 3 residual ST elevation (<1mm) 1 anterior early repolarization 3 residual T wave inversion
MRI	Performed in 1, late Gadolinium enhancement of papillary muscle but no regional motion abnormality
Normal LV function, no regional wall motion abnormalities on echocardiogram	10/10
Infarct location/revascularization	2 LAD 2 Circumflex/OM 2 RCA/PDA 4 triple vessel disease
Mean peak troponin I (ng/L)	15387 ±20919

Table S2: Brugada relatives

Count	11
Age (years)	45.5 ±11.0
Sex M:F	8:3
ECG at testing	8 normal sinus rhythm 1 RSR' in V1-2 1 early repolarization in V1-2 1 W pattern in V1
Echocardiogram	10 normal 1 bicuspid aortic valve, otherwise normal
MRI	Performed in 1, normal
Coronary assessment	10 normal ETT 1 normal ETT and DSE
Negative Ajmaline challenge	11/11
Family history	4 (aborted) sudden death 4 spontaneous Type 1 ECG 3 concealed Type 1 ECG

Table S3: Idiopathic VF survivors

Count	14
Age (years)	36.9 ±7.5
Sex M:F	11:3
Presentation rhythm	14 VF
Lifetime events	5 multiple events 9 single event
ICD implanted at time of test	14/14
Normal echocardiography (including LV function)	14/14
Normal ETT/adrenaline challenge	14/14
Normal coronary angiogram/CT CA	14/14
MRI	10 normal 1 mild LV volume increase in context of multiple ectopy 1 subepicardial late Gadolinium enhancement 1 too claustrophobic 1 transient apical hypokinesia post arrest with full resolution on subsequent echo
Monomorphic ectopy identified	5/14
Ajmaline	14/14 negative
ECG	5 normal sinus rhythm 3 T wave abnormalities 3 Early repolarization 2 RBBB 1 frequent ectopy
Index arrhythmia context	3 walking 2 intense exercise 3 at rest but awake 3 during sleep 1 after alcohol 1 practicing yoga 1 not identified

Genetics	5 gene negative 4 with VUS (AKAP9; KCNH2; TMEM43, MYBPC3) 5 not submitted for testing
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Table S4: Ventricular ectopy ablation

Count	11
Age (years)	44.5 ±14.3
Sex M:F	6:5
ECG (excluding ectopy)	9 normal sinus rhythm 1 RBBB 1 T wave inversion V1-4
Echocardiogram	10 normal 1 mitral valve prolapse, otherwise normal
MRI	3 normal 2 mild LV dysfunction in the context of ectopy without evidence of fibrosis 6 not performed
Ectopy location	8 RVOT 1 LVOT 1 Basal septal LV 1 inferior LV
Ectopy burden	17.2 ±10.2%

Multiple corrected comparisons results

Where multiple groups were compared, Dunn's test was used with Benjamini-Hochberg correction to control the family-wise error rate. The following tables summarize the corrected and uncorrected p-values for intergroup comparisons from Figure 3.

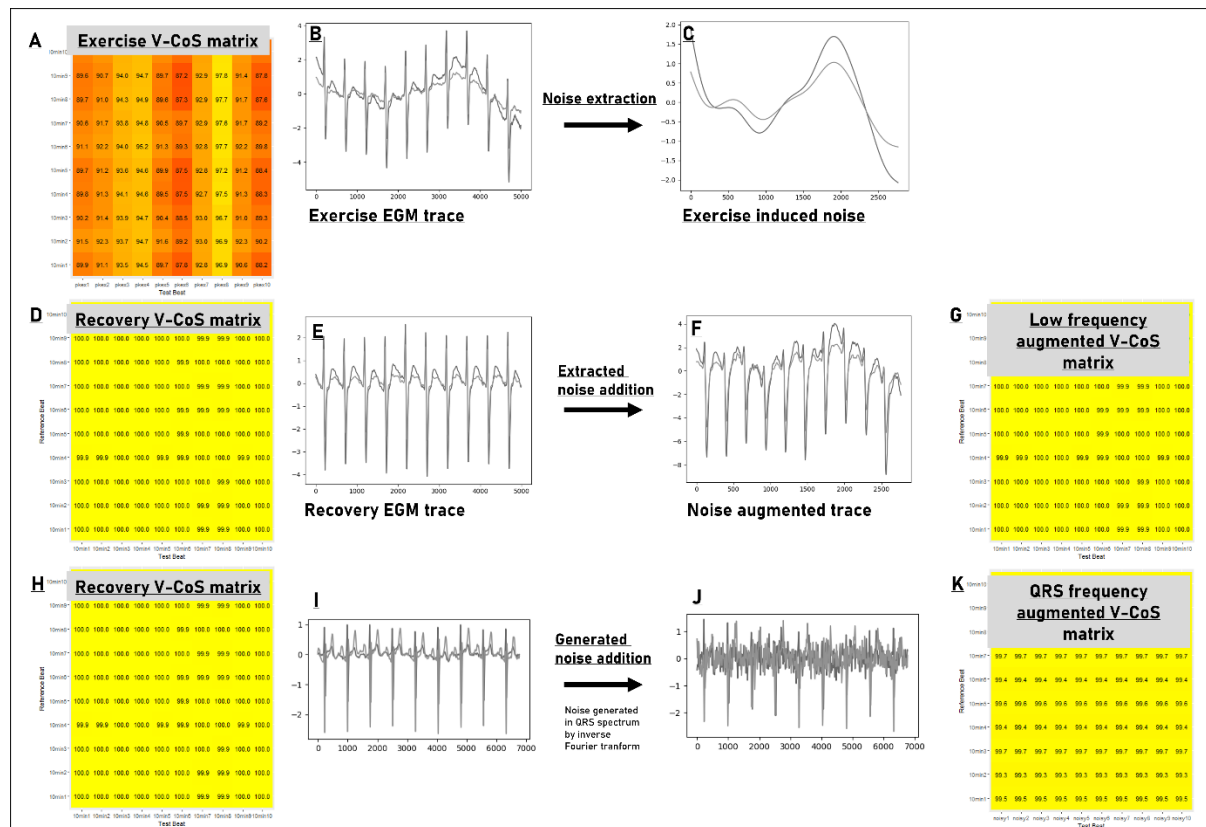
Table S5: Significance of mean V-CoS scores comparisons between survivors of idiopathic ventricular fibrillation, ischaemic ventricular fibrillation patients with full recovery of left ventricular function and full revascularization, patients with benign ventricular ectopy and unaffected Brugada relatives. Ventricular fibrillation, VF; Ischaemic heart disease, IHD; Ventricular ectopy, VE; Brugada, BrS.

Group 1	Group 2	p-value	Adjusted p-value
Idiopathic VF	IHD VF	0.098	0.2
Idiopathic VF	VE	0.013	0.039
Idiopathic VF	BrS relative	0.0016	0.0097
IHD VF	VE	0.47	0.53
IHD VF	BrS relative	0.18	0.27
VE	BrS relative	0.53	0.53

Table S6: Significance of mean V-CoS scores comparisons between survivors of idiopathic ventricular fibrillation experiencing either multiple or single episodes of ventricular arrhythmia, and pooled controls.

Group 1	Group 2	p-value	Adjusted p-value
Multiple episode	Single episode	0.48	0.48
Multiple episode	Control	0.0094	0.028
Single episode	Control	0.024	0.036

Figure S1: Exercise V-CoS matrices show periodic changes (A) at a frequency comparable to the baseline noise on an EGM trace (B).



This baseline noise is made up of extracardiac signals, including movement of the chest wall in respiration – exaggerated following exertion. QRS complexes represent cardiac activation. Two EGM traces are shown per graph to illustrate the example and demonstrate that each trace has a unique noise signature, but the V-CoS calculation includes ~1500 epicardial EGM traces per patient. It is unclear whether this baseline noise is the cause of the periodic change in V-CoS score. The baseline noise can be extracted from the EGM traces by a low-pass filter (C). Recovery V-CoS matrices do not show such large periodic changes (D). The EGM can also be noted to have less baseline noise (E). The EGM can be artificially augmented with the baseline noise extracted from the exercise traces (C) to make a synthetic noise-augmented recovery trace (F). If extracardiac noise (as opposed to true changes in QRS, i.e. cardiac activation patterns) is responsible for the 0.3-1Hz periodic changes in V-CoS (A), the noise-augmented recovery traces would be expected to produce similar magnitude changes in V-CoS to the exercise matrix (G). To understand whether our technique was robust to QRS spectrum noise, we measured the QRS spectrum at mostly ranging from 1-60Hz. The range of generated noise was doubled to ensure high frequency components were accounted for and scaled to mask the T waves in recovery EGMs (I, J). These higher frequency augmented EGMs could also not replicate the low exercise V-CoS scores (K), suggesting that noise is not the reason for our results.

Figure S2: Four V-CoS matrices for each of 3 volunteers: one survivor of idiopathic ventricular fibrillation, one ischaemic ventricular fibrillation survivor, one patient with benign ventricular ectopy.



Each volunteer occupies a column; the first row demonstrates V-CoS scores when exercise and recovery beats are compared; the second when a synthetic combination of recovery beats and extracted bio-electrical noise are compared to un-augmented recovery beats; the third row compares QRS spectrum noise augmented recovery beats and un-augmented recovery beats; the fourth row comparing unaugmented recovery beats to each other. The exercise V-CoS matrices demonstrate lower scores with periodic loss of uniform conduction, absent in recovery matrices. The synthetic matrices cannot replicate the exercise scores, suggesting that periodic noise is unlikely to be the cause of periodic low V-CoS scores.