QRS prolongation after premature stimulation is associated with polymorphic ventricular tachycardia in nonischemic cardiomyopathy: Results from the Leiden Nonischemic Cardiomyopathy Study

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BACKGROUND Progressive activation delay after premature stimulation has been associated with ventricular fibrillation in nonischemic cardiomyopathy (NICM).

OBJECTIVES The objectives of this study were (1) to investigate prolongation of the paced QRS duration (QRSd) after premature stimulation as a marker of activation delay in NICM, (2) to assess its relation to induced ventricular arrhythmias, and (3) to analyze its underlying substrate by late gadolinium enhancement cardiac magnetic resonance imaging (LGE-CMR) and endomyocardial biopsy.

METHODS Patients with NICM were prospectively enrolled in the Leiden Nonischemic Cardiomyopathy Study and underwent a comprehensive evaluation including LGE-CMR, electrophysiology study, and endomyocardial biopsy. Patients without structural heart disease served as controls for electrophysiology study.

RESULTS Forty patients with NICM were included (mean age 57 ± 14 years; 33 men [83%]; left ventricular ejection fraction 30% ± 13%). After the 400-ms drive train and progressively premature stimulation, the maximum increase in QRSd was larger in patients with NICM than in controls (35 ± 18 ms vs 23 ± 12 ms; $P = .005$) and the coupling interval window with QRSd prolongation was wider (47 ± 23 ms vs 31 ± 14 ms; $P = .005$). The maximum paced QRSd exceeded the ventricular effective refractory period, allowing for pacing before the offset of the QRS complex in 20 of 39 patients with NICM vs 1 of 20 controls ($P < .001$). In patients with NICM, QRSd prolongation was associated with the inducibility of polymorphic ventricular tachycardia (16 of 39 patients) and was related to long, thick strands of fibrosis in biopsies, but not to focal enhancement on LGE-CMR.

CONCLUSION QRSd is a simple parameter used to quantify activation delay after premature stimulation, and its prolongation is associated with the inducibility of polymorphic ventricular tachycardia and with the pattern of myocardial fibrosis in biopsies.

KEYWORDS Nonischemic cardiomyopathy; Activation delay; Electrophysiology study; Polymorphic ventricular tachycardia; Ventricular fibrillation

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Introduction

Implantable cardioverter-defibrillator (ICD) therapy can terminate, but does not prevent potentially life-threatening ventricular arrhythmias. Although progress has been made to treat monomorphic ventricular tachycardia (VT), therapies that prevent polymorphic VT and ventricular fibrillation (VF) in nonischemic cardiomyopathy (NICM) are lacking. Improved understanding and identification of the underlying substrate for these arrhythmias may allow personalized risk stratification and development of tailored treatment.

Activation delay after premature stimulation has been associated with a history of VF in patients with various noncoronary heart diseases on the basis of intracardiac recordings from the right ventricle (RV).[1] Total biventricular activation delay may be quantified by measuring paced QRS duration (QRSd) on surface electrocardiograms, which is

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easy to apply and may better reflect activation delay in left dominant myocardial disease.

The objectives of this study were (1) to investigate prolongation of the paced QRSd after decremental premature stimulation as a marker of activation delay in NICM, (2) to assess its relation to induced ventricular arrhythmias, and (3) to analyze its association with the extent and distribution of fibrosis in endomyocardial biopsy specimens and on late gadolinium enhancement cardiac magnetic resonance imaging (LGE-CMR).

Methods

Patients

The Leiden Nonischemic Cardiomyopathy Study is a single-center prospective cohort study designed to analyze the substrate and mechanisms of ventricular arrhythmias in NICM (ClinicalTrials.gov Identifier: NCT01940081). From October 2011, patients aged 18–80 years with idiopathic (dilated) cardiomyopathy and documented sustained ventricular arrhythmia, with suspected sustained ventricular arrhythmia (eg, because of out-of-hospital cardiac arrest, palpitations, or syncope), or considered to be at intermediate or high risk of ventricular arrhythmias were enrolled. A high risk of ventricular arrhythmias was defined as a left ventricular ejection fraction (LVEF) of $\leq$35%; an intermediate risk was defined as an LVEF of $\leq$50% and late enhancement on LGE-CMR. Exclusion criteria were inability to understand the nature and risks of the study procedures, pregnancy, inability to comply with the protocol owing to hemodynamic instability, and other cardiomyopathies (eg, tachycardiomyopathy, previous myocardial infarction, sarcoidosis, infiltrative cardiac disease such as amyloidosis, Chagas cardiomyopathy, arrhythmogenic RV cardiomyopathy, hypertrophic cardiomyopathy, noncompaction cardiomyopathy, and congenital heart disease).

Patients underwent a comprehensive evaluation including transthoracic echocardiography, LGE-CMR, 24-hour Holter monitoring, exercise testing, blood sampling for N-terminal pro-b-type natriuretic peptide (NT-proBNP) levels, invasive electrophysiology study, endomyocardial biopsy, iodine-123 metaiodobenzylguanidine scan, and genetic analysis of 55 cardiomyopathy-related genes. Targeted next generation sequencing of these 55 genes, a list of which is provided in the Online Supplemental Methods, was performed analyzing 151-bp paired-end reads on an Illumina MiSeq sequencer, as described previously.[2] The (likely) pathogenicity of single nucleotide polymorphisms and small insertion deletion mutations was based on the nature and location of the variant, conservation of the affected amino acid residue(s) and the surrounding region, frequency of the variant in (healthy) population databases, its predicted pathogenicity according to multiple available prediction programs, the scientific literature and/or variant databases.

The study protocol was approved by the local ethics committee and by the appropriate national ethics committee. All patients provided written informed consent. Patients who refused endomyocardial biopsy but agreed with all other study procedures were allowed to participate. Premature ventricular contraction or VT ablation and ICD implantation were performed if clinically indicated.

The control group for electrophysiology study measurements consisted of 20 patients referred for catheter ablation of atrioventricular nodal reentrant tachycardia or a concealed bypass tract in the absence of structural heart disease, who underwent routine electrophysiology study according to our institutional protocol. This protocol includes single, but not multiple, extrastimuli from the RV.

LGE-CMR acquisition and analysis

CMR imaging included cine and LGE images in long and short axes. Left ventricular (LV) and RV volumes were calculated and indexed to body surface area. Myocardial scar was considered to be present only if LGE was visible in 2 orthogonal views. LGE was defined by signal intensity $\geq$35% of the maximal myocardial signal intensity and subdivided into core ($\geq$50% of the maximal signal intensity) and border zone (35%–50% of the maximal signal intensity).[3] Details are provided in the Online Supplemental Methods.

Electrophysiology study

Antiarrhythmic drugs were discontinued for at least 5 half-lives, if possible. Programmed electrical stimulation consisted of 3 drive trains of 8 beats (S1) (cycle length 600, 500, and 400 ms) with 1–3 ventricular extrastimuli and burst pacing at twice diastolic threshold from the RV apex and the RV outflow tract using the distal electrode pair of a quadripolar catheter. The first extrastimulus (S2) was applied at a coupling interval (CI) identical to the cycle length of the drive train, followed by a CI of 350 ms, which was then progressively decreased by steps of 10 ms until the ventricular effective refractory period (VERP) was reached. Consecutive extrastimuli (S3 and S4) and burst pacing were applied from a CI of 350 ms down to 200 ms. Polymorphic VT was defined by $\geq$5 consecutive ventricular beats at $\geq$120 beats/min with continuously changing QRS morphology[4,5] and was regarded as sustained if lasting $>$30 seconds or requiring termination because of hemodynamic compromise. Sustained monomorphic VT was defined by similar beat-to-beat QRS morphology and duration $>$30 seconds or the requirement for termination because of hemodynamic compromise. If no sustained ventricular arrhythmia was induced, stimulation was repeated with isoprenaline (2–10 μg/min).

In the control group for electrophysiology study measurements, drive trains of 600 and 400 ms with a single extrastimulus were applied from the RV apex as part of the routine electrophysiology study protocol. All studies were recorded on an electrophysiology recording system for off-line analysis, with a sweeping speed of 200 mm/s and bipolar electrograms filtered at 30–500 Hz (Prucka CardioLab recording system, Houston, TX).

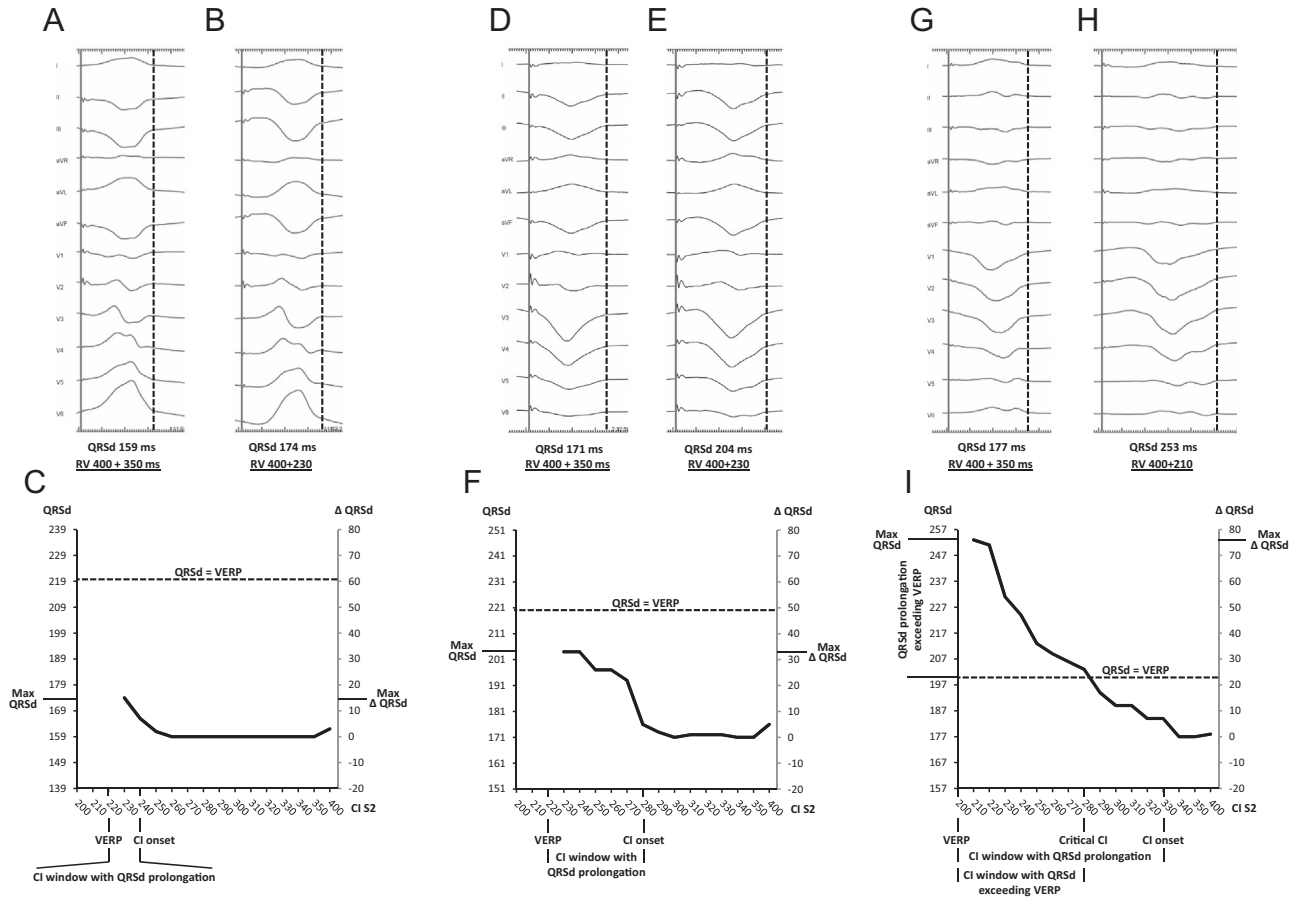

Figure 1 Examples of QRSd restitution curves and derived parameters. QRSd measurements and restitution curves in a control without structural heart disease after premature stimulation (**A–C**), in a patient with cardiomyopathy and more pronounced QRSd prolongation, but without exceeding the VERP (**D–F**), and in a patient with cardiomyopathy and marked QRSd prolongation exceeding the VERP (**G–I**). In this patient the maximal QRSd exceeds the VERP (indicated by the horizontal dashed line). CI = coupling interval; QRSd = QRSd duration; RV = right ventricular; VERP = ventricular effective refractory period.

Electrophysiology study analysis

The paced QRSd was measured from the pacing artifact to the latest offset of the QRS complex in any 12-lead electrocardiogram lead (examples in Figure 1) by using electronic calipers. For the 600- and 400-ms drive trains with single extrastimuli from the RV apex, QRSd restitution curves were constructed and the following parameters were derived:

1. CI at the onset of QRSd prolongation (CI_{onset}): the longest S1S2 CI causing $\geq$5 ms increase in QRSd compared to the previous S1S2, applied at a 10 ms longer CI.
2. CI window with QRSd prolongation: the interval between the CI_{onset} and the VERP.
3. Maximal ΔQRSd: maximum increase in QRSd after premature stimulation.
4. QRSd prolongation exceeding the VERP: QRSd prolongation exceeding the VERP after the last S1 of the drive train (calculated as QRSd-VERP).
5. CI window with QRSd exceeding the VERP: the CI window at which the paced QRSd exceeds the length of the VERP.

Examples of QRS restitution curves and calculations of the derived parameters are displayed in Figure 1.

Endomyocardial biopsy

After the electrophysiology study, endomyocardial biopsies were obtained; immediately thereafter, 2 were stored in liquid nitrogen and 1 specimen was fixed in 4% formaldehyde and embedded in paraffin. Cross sections were cut at 4 μm and stained with picrosirius red to evaluate collagen content and collagen distribution patterns. Mayer's hematoxylin was used to counterstain cell nuclei. High-resolution photomicrographs were taken using a fluorescent microscope equipped with a digital camera (Nikon Eclipse, Nikon Europe, Badhoevedorp, The Netherlands) and analyzed using dedicated software (ImageJ 1.49c, National Institutes of Health, Bethesda, MD). All staining and subsequent analyses were performed by an independent investigator blinded to all clinical and procedural data. Fibrosis content was quantified by calculating the collagen area percentage. Distribution of fibrosis was categorized according to the predefined patterns:

1. Interstitial: collagen strand thickness exceeds 10% of the width of adjacent cardiomyocytes without interrupting myocardial fiber continuity.
2. Short thin strands: dispersed presence of 2 or more fibrotic areas smaller than the width of 5 cardiomyocytes that disrupt myocardial fiber continuity.

Table 1 Baseline characteristics (N = 40)

Characteristic	Value
Age (y)	57 ± 14
Sex: male	33 (83)
BMI (kg/m²)	27 ± 4
Diabetes mellitus	6 (15)
Hypertension	10 (25)
NYHA functional class	
I	7 (18)
II	20 (50)
III or IV	13 (33)
History of AF/atrial flutter	16 (40)
ECG	
Heart rate (beats/min)	73 ± 13
PR interval (ms)	182 ± 32
QRS duration (ms)	129 ± 34
eGFR (mL/(min · 1.73 m^2))	86 ± 25
NT-proBNP level (pg/mL)	932 (499–1773)
Exercise test (n = 29)	
Peak oxygen consumption (mL/(kg · min))	20 ± 6
Peak oxygen consumption (% of predicted)	80 ± 22
AAD during electrophysiology study	
Class I	0 (0)
Sotalol	1 (3)
Amiodarone	2 (5)
Genetic screening (n = 39)	
(Likely) pathogenic mutation or VUS	19 (49)
Device at discharge	
CRT-defibrillator	20 (50)
Implantable cardioverter-defibrillator	13 (33)
None	7 (18)

Variables are expressed as mean ± SD, as n (%), or as median (interquartile range).

AAD = antiarrhythmic drug; AF = atrial fibrillation; BMI = body mass index; CRT = cardiac resynchronization therapy; ECG = electrocardiography; eGFR = estimated glomerular filtration rate; NT-proBNP = N-terminal pro-b-type natriuretic peptide; NYHA = New York Heart Association; VUS = variant of unknown significance.

3. Long, thick strands: 1 or more fibrotic areas exceeding the width of 5 cardiomyocytes that disrupt the myocardial fiber continuity.

Underlying substrate of QRS prolongation

The relation between QRSd restitution curve parameters and the following parameters was analyzed: (1) CMR-derived LV and RV volumes and ejection fraction, (2) LGE on CMR, (3) NT-proBNP levels, (4) collagen area percentages in biopsy specimens, (5) pattern of fibrosis in biopsy specimens, and (6) the presence of a (likely) pathogenic mutation or variant of unknown significance (VUS).

Statistical analysis

Categorical variables are expressed as number (percentage), and continuous variables are expressed as mean ± SD or median (interquartile range [IQR]). Categorical variables were compared using the χ^2 test or the Fisher exact test. Continuous variables were compared using the Mann-Whitney *U* test because of the small sample size. For paired variables, the Wilcoxon signed rank test was used. The association between QRS restitution curve parameters and patient characteristics was analyzed by univariate linear regression analyses. For this purpose, NT-proBNP levels were log-transformed because of the skewed distribution. All analyses were performed with SPSS version 20.0 (IBM, Somers, NY). All tests were 2-sided, and probability values $<.05$ were considered statistically significant.

Results

Patients

Between October 2011 and August 2013, 40 patients (age 57 ± 14 years; 83% men) were enrolled in the Leiden Nonischemic Cardiomyopathy Study. Baseline characteristics are summarized in Table 1. Eight patients (20%) had documented sustained ventricular arrhythmia, 6 (15%) had suspected sustained ventricular arrhythmia, and 26 (65%) were considered to be at intermediate or high risk of ventricular arrhythmia. Cine CMR was performed in 36 patients (90%), while the remaining 4 (10%) had an implanted device and underwent echocardiography. Based on combined CMR and echocardiography data, the LVEF was considered reduced in all patients and the LV end-diastolic volume index was found to be increased in 34 patients (85%) compared with published reference ranges normalized for sex, age, and body surface area.[6] LGE was observed in 26 of 34 patients with LGE images (77%), and the median LGE mass was 8.8 g (IQR 1.1–19.3 g). CMR and biopsy data are summarized in Table 2. Genetic analysis of 55 cardiomyopathy-related genes

Table 2 CMR and biopsy data

Characteristic	Value
Cine CMR images (n = 36)	
LVEDV (mL)	303 ± 90
LVEDV index (mL/m^2)	147 ± 42
LVESV (mL)	221 ± 93
LVESV index (mL/m^2)	107 ± 44
LVEF (%)	30 ± 13
LV mass (g)	143 ± 34
LV mass index (g/m^2)	70 ± 16
RVEDV (mL)	189 ± 45
RVEDV index (mL/m^2)	92 ± 19
RVESV (mL)	105 ± 39
RVESV index (mL/m^2)	51 ± 18
RVEF (%)	46 ± 11
LGE CMR images (n = 34)	
LGE presence	26/34 (77)
LGE total (g)	8.8 (1.1–19.3)
Core mass (g)	3.3 (0.4–8.7)
Border zone mass (g)	4.9 (0.6–13.4)
Endomyocardial biopsy (n = 23)	
Collagen area percentage (%)	33 (19–42)
Collagen distribution type	
Interstitial	8/23 (35)
Short thin strands	7/23 (30)
Long, thick strands	8/23 (35)

Variables are expressed as mean ± SD, as n (%), or as median (interquartile range).

LGE = late gadolinium enhancement; LV = left ventricular; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume; RVEDV = right ventricular end-diastolic volume; RVEF = right ventricular ejection fraction; RVESV = right ventricular end-systolic volume.

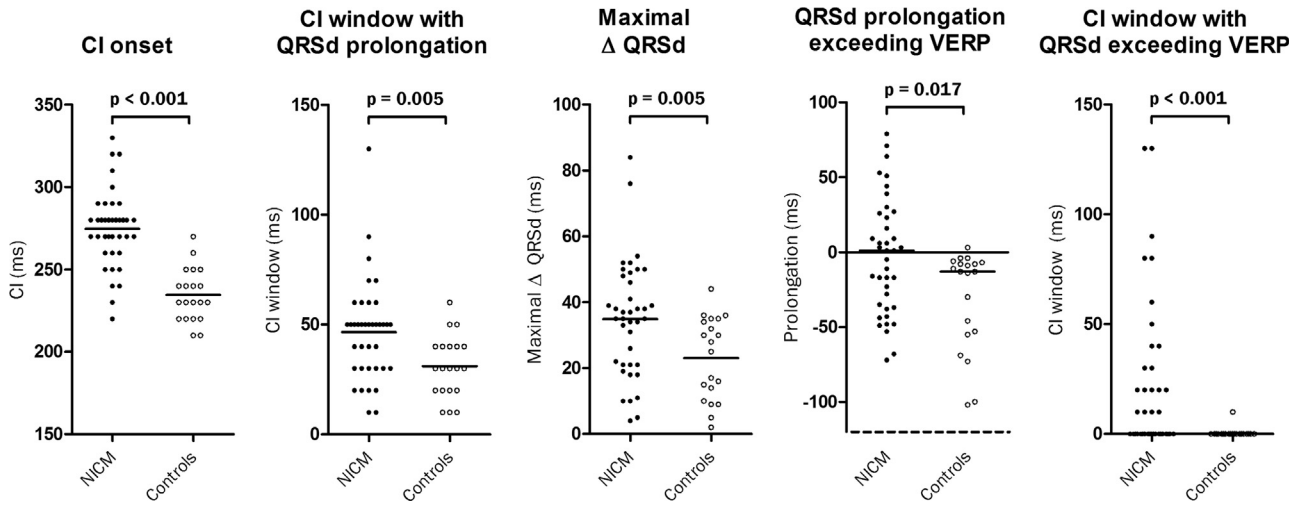

Figure 2 QRSd prolongation in patients with NICM and controls. The QRSd restitution curve parameters based on 400-ms drive trains with single extrastimuli differed significantly between patients with NICM and controls. CI = coupling interval; NICM = nonischemic cardiomyopathy; QRSd = QRS duration; VERP = ventricular effective refractory period.

was performed in all but one patient, and it revealed a (likely) pathogenic mutation or VUS in 19 (49%) (details are listed in Online Supplemental Table 1).

QRSd prolongation in patients with NICM and controls

Both in patients with NICM and in controls, QRSd increased after premature stimulation (examples in Figures 1A–1I). Differences between patients with NICM and controls were more pronounced after the 400-ms drive train than after the 600-ms drive train (Online Supplemental Figure 1 and Online Supplemental Table 2), and therefore, the 400-ms drive train was used for further analyses. After the 400-ms drive train, the onset of QRSd prolongation occurred at longer CI (CI_{onset}) in patients with NICM than in controls (275 ± 23 ms vs 235 ± 16 ms; $P < .001$) (Figure 2), the CI window with QRSd prolongation was wider (47 ± 23 ms vs 31 ± 14 ms; $P = .005$), and the maximal ΔQRSd was larger (35 ± 18 ms vs 23 ± 12 ms; $P = .005$). QRSd prolongation exceeding the VERP, which allowed to apply an additional extrastimulus S3 within the paced QRS complex, was observed in 20 of 39 patients with NICM (51%) compared with only 1 of 20 controls (5%) ($P < .001$) (Figures 1G–1I, Figure 2, and example in Figure 3). The CI window with QRSd exceeding the VERP ranged from 10 to 130 ms in patients with NICM as compared with only 1 case with a CI window of 10 ms in controls ($P < .001$) (Figure 2). The results were similar when 3 patients with cardiomyopathy who were on class III antiarrhythmic drugs were excluded.

QRSd prolongation and inducibility of ventricular arrhythmia

The programmed electrical stimulation protocol could not be completed in 1 patient because of incessant VT. Sustained monomorphic VT was induced in 11 of the remaining 39 patients with NICM (28%) and polymorphic VT ≥5 beats in 16 of 39 (41%) (Figure 3). The longest polymorphic VT was induced with triple extrastimuli in all 16 patients, and it was induced during isoprenaline infusion in only 1 patient. None of the baseline characteristics were associated with the inducibility of polymorphic VT (Table 3).

In contrast, 3 of 5 QRSd restitution curve parameters were associated with the inducibility of polymorphic VT (Figure 4 and Online Supplemental Table 3). The CI_{onset} was similar in patients with vs without inducible polymorphic VT (276 ± 20 ms vs 274 ± 26 ms; $P = .92$), but the CI window with QRSd prolongation was wider (57 ± 25 ms vs 39 ± 18 ms; $P = .015$) and the maximal ΔQRSd was larger (46 ± 17 ms vs 27 ± 14 ms; $P = .001$). QRSd prolongation exceeding the VERP was significantly longer, allowing to pace further within the QRS complex (15 ± 37 ms vs −15 ± 36 ms; $P = .017$). The CI window with QRSd exceeding the VERP was not statistically different between patients with and without inducible polymorphic VT (median 20 ms [IQR 0–75 ms] vs median 0 ms [IQR 0–20 ms]; $P = .11$).

The CI window with QRSd prolongation and the maximal ΔQRSd did not differ between patients with and without inducible sustained monomorphic VT (Online Supplemental Table 4). However, QRSd prolongation exceeding the VERP was significantly longer in patients with vs without inducible sustained monomorphic VT (24 ± 39 ms vs −13 ± 34 ms; $P = .009$) and the CI window with QRSd exceeding the VERP tended to be wider (median 50 ms [IQR 0–80 ms] vs median 0 ms [IQR 0–20 ms]; $P = .063$).

CMR-derived data, NT-proBNP levels, and genetics: Relation to QRSd prolongation

Larger end-diastolic LV volume indexes were associated with a wider CI window with QRSd prolongation, and larger end-systolic LV volume indexes and lower LVEF were associated with a smaller maximal ΔQRSd, but neither was associated with any of the other QRSd restitution curve parameters (Online Supplemental Table 5). The extent of LGE (total LGE, core, and border zone), NT-proBNP levels,

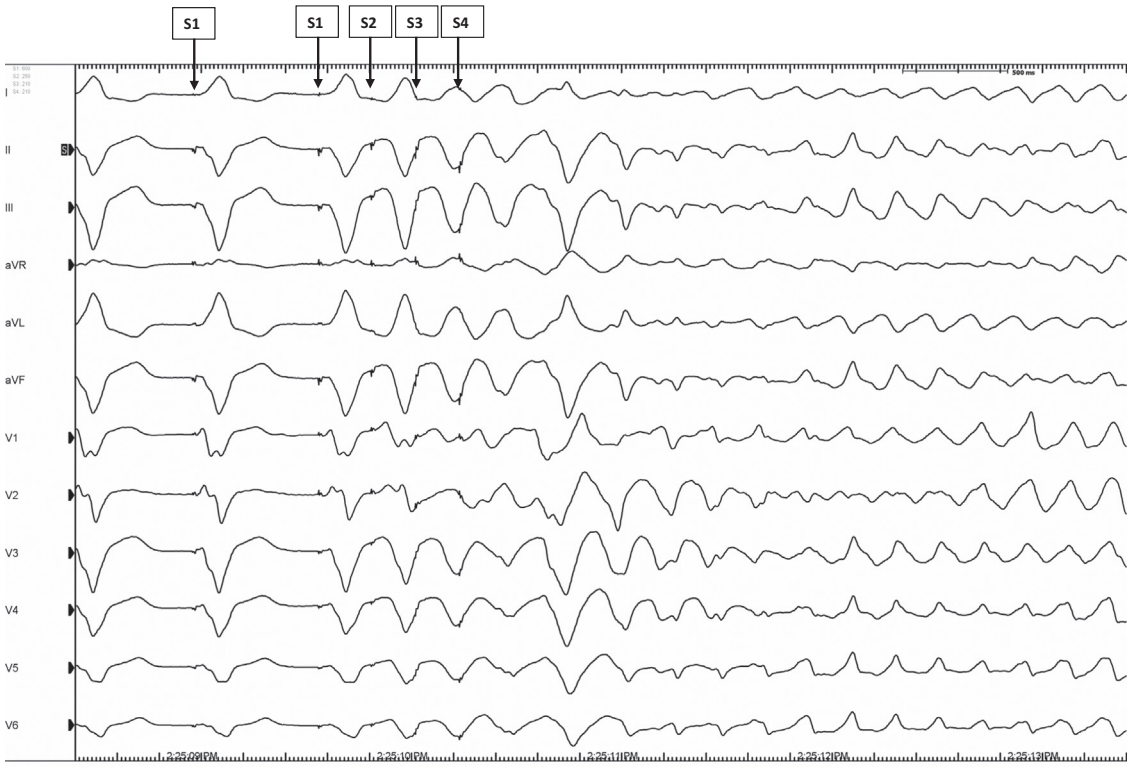

Figure 3 Example of pacing within the QRS complex and induction of polymorphic ventricular tachycardia. During application of triple extrastimuli after a 400-ms drive train, the QRS complex prolongs after premature stimulation. S3 and S4 are applied within the paced preceding QRS complexes, resulting in the induction of polymorphic ventricular tachycardia.

and the presence of a (likely) pathogenic mutation or VUS were not associated with any QRSd restitution curve parameter ($P > .05$ for all).

Extent and distribution of fibrosis in biopsy specimens and association with QRSd prolongation

Endomyocardial biopsy was performed in 24 patients (60%; LV in 23 patients and RV side of septum in 1 patient); no biopsies were obtained in the remaining 16 patients (40%) because of a potential risk of complications or patient preference. One set of biopsies was excluded because of poor quality. In the remaining 23 patients, the median collagen area percentage was 33% (IQR 19%–42%) and the collagen distribution type was interstitial in 8 (35%), short thin strands in 7 (30%), and long, thick strands in 8 (35%) (examples in Figure 5). The density of fibrosis quantified as area percentages was weakly associated with a smaller maximal increase in QRSd but not with any other parameter of QRSd prolongation (Online Supplemental Table 5). However, long, thick strands of fibrosis were associated with a wider CI window with QRSd prolongation and with a wider CI window with QRSd exceeding the VERP and, therefore, the ability to pace within the QRS complex after S2.

Discussion

The key findings of this study are as follows: (1) both in patients with NICM and in controls, the paced QRSd increased after premature stimulation; (2) in patients with NICM, QRSd started to increase at longer CI than in controls, the maximum increase in QRSd was larger, and the paced QRSd frequently exceeded the VERP, which allowed to apply an extrastimulus within the paced QRS complex; (3) the CI window with QRSd prolongation, the maximal ΔQRSd, and QRSd prolongation exceeding the VERP were associated with the inducibility of polymorphic VT in NICM; (4) not the density of fibrosis but the presence of long, thick strands of fibrosis was associated with a wider CI window of progressive QRSd prolongation and a wider CI window at which QRSd exceeds the VERP, which may indicate a substrate for polymorphic VT or VF; and (5) none of the QRSd restitution curve parameters was related to the presence or extent of LGE on CMR, NT-proBNP levels, and the presence of (likely) pathogenic mutations or VUS.

Quantification of activation delay after premature stimulation

Activation delay after premature stimulation has previously been quantified and related to VF in humans with various cardiac diseases, in particular hypertrophic cardiomyopathy, by applying stimulation protocols sequentially from 4 RV catheters.[1] LV activation could not be assessed, as no catheters were placed in the LV. Of interest, both normal and abnormal electrogram responses were frequently observed at the different positions of the 4 RV catheters, suggesting that conduction delay may not be uniform, and therefore, multiple recording sites may be required. In

Table 3 Baseline characteristics and inducible PVT

Characteristic	PVT (n = 16)	No PVT (n = 23)	*P*
Age (y)	60 ± 13	56 ± 13	.40
Sex: male	13 (81)	19 (83)	1.00
BMI (kg/m²)	27 ± 4	28 ± 4	.47
Diabetes mellitus	9 (56)	7 (30)	.11
Hypertension	4 (25)	6 (26)	1.00
NYHA functional class III or IV	4 (25)	9 (39)	.36
History of AF/atrial flutter	2 (13)	4 (17)	1.00
eGFR (mL/(min · 1.73 m^2))	87 ± 24	86 ± 27	1.00
NT-proBNP level (pg/mL)	1058 (487–2232)	872 (498–1646)	.64
ECG			
Heart rate (beats/min)	72 ± 15	73 ± 12	.77
PR interval (ms)	188 ± 33	181 ± 31	.49
QRS duration (ms)	126 ± 32	133 ± 36	.69
Exercise test			
Peak oxygen consumption (mL/(kg · min))	19 ± 7	20 ± 5	.65
Peak oxygen consumption (% of predicted)	81 ± 28	80 ± 16	.91
Genetic screening			
(Likely) pathogenic mutationor VUS	8/16 (50)	10/22 (46)	.78
Cine MRI images			
LVEDV index (mL/m^2)	136 ± 44	153 ± 41	.27
LVEF (%)	35 ± 15	27 ± 12	.19
LV mass index (g/m^2)	67 ± 7	71 ± 19	.51
RVEDV index (mL/m^2)	96 ± 22	89 ± 18	.63
RVEF (%)	41 ± 13	49 ± 10	.057
LGE MRI images			
LGE presence	11/13 (85)	15/20 (75)	.68
LGE total (g)	15.0 (4.3–22.7)	8.2 (0.4–22.6)	.32
LGE core (g)	5.5 (1.9–11.1)	2.9 (0.1–8.4)	.41
LGE border zone (g)	7.7 (2.4–14.0)	4.3 (0.2–14.0)	.39
Endomyocardial biopsy specimens			
Collagen area percentage	22 (10–39)	38 (23–51)	.093
Interstitial fibrosis	3/10 (30)	4/12 (33)	
Short thin strands	2/10 (20)	5/12 (42)	.42
Long, thick strands	5/10 (50)	3/12 (25)	

Variables are expressed as mean ± SD, as n (%), or as median (interquartile range).MRI = magnetic resonance imaging; PVT = polymorphic ventricular tachycardia. Other abbreviations as in Tables 1 and 2.

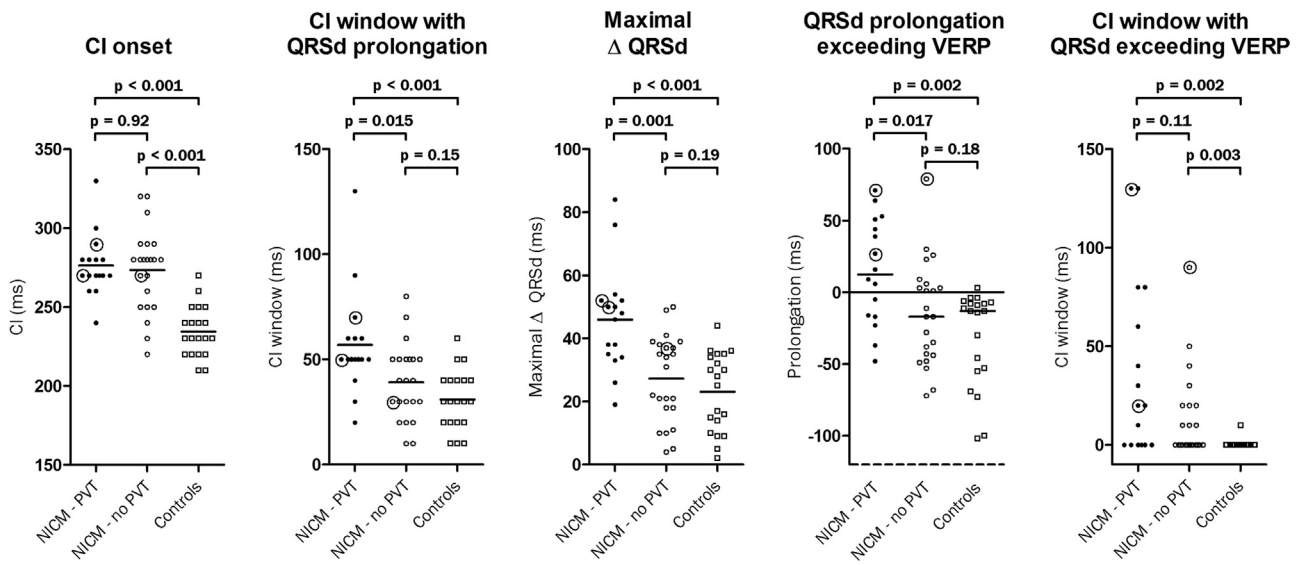

Figure 4 QRSd prolongation and inducibility of PVT. Three QRSd restitution curve parameters were associated with the inducibility of PVT in patients with NICM. The 3 patients on antiarrhythmic drugs are marked with circles. CI = coupling interval; NICM = nonischemic cardiomyopathy; PVT = polymorphic ventricular tachycardia; QRSd = QRS duration; VERP = ventricular effective refractory period.

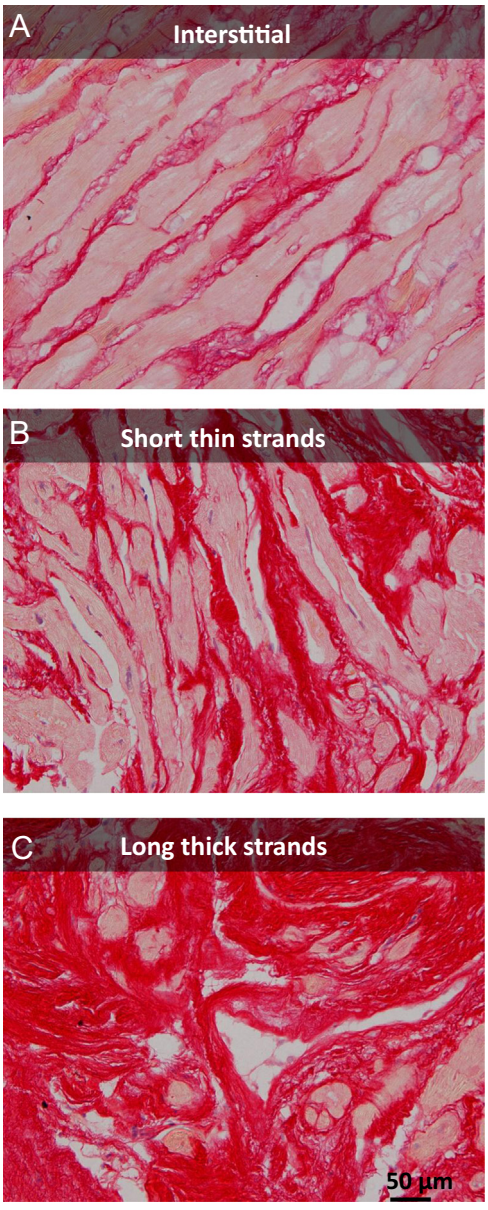

Figure 5 Examples of biopsy specimens. Typical photomicrographic examples demonstrating different patterns of fibrosis in endomyocardial biopsies as visualized by sirius red staining. **A:** Typical example from a patient showing an interstitial fibrosis pattern. **B:** Typical example from a patient with a fibrosis pattern that primarily consists of short thin fibrotic strands. **C:** Typical example from a patient with a fibrosis pattern predominantly containing long, thick strands of fibrosis.

contrast, the paced QRSd is easily available, can be measured during a standard programmed electrical stimulation protocol, does not require multiple intracardiac catheters, and reflects both RV and LV activation.

Activation delay and ventricular arrhythmia

In the present study, we could demonstrate that the CI window with QRSd prolongation, the maximal ΔQRSd, and QRSd prolongation exceeding the VERP were associated with the inducibility of polymorphic VT. In contrast, none of the baseline characteristics, including CMR-derived volumes, ejection fraction and LGE, NT-proBNP levels, and the presence of a (likely) pathogenic mutation or VUS were associated with the inducibility of polymorphic VT.

A larger CI window with QRSd prolongation may increase the likelihood that a spontaneous premature ventricular contraction is discontinuously propagated, resulting in progressive activation delay, wave break, and increased dispersion of repolarization. The maximal ΔQRSd may be considered as a marker of the cumulative activation delay throughout the heart after the shortest-coupled (VERP + 10 ms) premature stimulus. Prolonged and pronounced activation delay may reflect nonuniform anisotropic conduction, which, together with dispersion of repolarization, may predispose for polymorphic VT and VF.[7]

These findings are consistent with a previous study using extensive stimulation protocols and multiple RV catheters in patients with various noncoronary heart diseases (but only 5 patients with dilated cardiomyopathy), which demonstrated that the maximum increase in RV intracardiac electrogram duration was larger, as well as that the S1S2 CIs at which the electrograms started to increase were longer in patients with a history of VF.[1] In a retrospective study in consecutive patients with and without structural heart disease who underwent electrophysiology study for various reasons, activation delay, measured from the distal stimulation electrodes to the proximal electrodes located at a distance of 35 mm, was substantially longer in patients with vs without inducible VF.[8] Finally, in a retrospective study conducted in patients with borderline NICM with LVEF $>50\%$ and LV end-diastolic dimension <65 mm, 15 patients resuscitated from polymorphic VT/VF had longer paced QRSd after S3 (at CI 10 ms above the VERP) than did patients without documented ventricular arrhythmias.[9]

Of note, the amount of QRSd exceeding the VERP was also associated with sustained monomorphic VT inducibility, as was the local VERP. The inducibility of sustained monomorphic VT may depend not only on the presence of a slow-conducting pathway through a myocardial scar but also on functional components such as (rate-dependent) inhomogeneous activation delay within the myocardium between the pacing site and the scar.

Substrate and mechanisms of activation delay and polymorphic VT

The QRSd restitution curve parameters that were associated with the inducibility of polymorphic VT did not correlate with CMR-derived volumes, ejection fraction, or LGE and were not related to larger fibrosis area percentages on biopsy, but tended to be related to long, thick strands of fibrosis in biopsy. This is in line with 2 studies in explanted hearts from patients with end-stage heart failure, which suggested that activation delay after premature stimulation may be related to the presence of long fibrotic strands.[10,11] In one of these studies, even large amounts of diffusely distributed fibrosis with short strands only marginally affected conduction velocity, whereas large fibrotic strands appeared to serve as

inexcitable barriers with consecutive conduction delay, supporting that the geometry rather than the total amount of fibrosis creates a potential substrate for arrhythmias.[11] The authors elegantly demonstrated how such barriers may contribute to activation delay, namely, by increased path length, transverse instead of longitudinal conduction, wavefront curvature, and reduced conduction velocity.[11] In addition, activation delay caused by long strands of fibrosis may promote wave break and thereby predispose for polymorphic VT/VF.[12] Of interest, treatment with eplerenone, losartan, or both could reduce the development of age-related interstitial and patchy fibrosis in adult mice.[13] Importantly, only the reduction of patchy fibrosis correlated with the reduced inducibility of ventricular arrhythmias based on anisotropic reentry, further supporting the link between type of fibrosis and arrhythmogenicity.

Other potential mechanisms of activation delay after premature stimulation have been described in patients with various structural heart diseases.[14] In particular, extrastimuli that capture at an earlier and less repolarized level of the preceding beat correlated with delayed propagation. Mechanisms are not necessarily mutually exclusive, and the observed QRS prolongation may be dependent on the combination of different underlying structural and functional changes, resulting in an increased vulnerability for polymorphic VT.

Clinical implications

Activation delay after premature stimulation, which was associated with ventricular arrhythmias in the present and in previous studies, can be easily quantified by any clinical electrophysiologist by measuring QRSd after 400-ms drive trains with a single extrastimulus. The protocol and calculation of the suggested parameters can be performed in a couple of minutes and can be obtained noninvasively in patients with pacemakers or ICDs. Whether QRSd restitution curve parameters predict spontaneous ventricular arrhythmias requires further studies with larger sample sizes and long follow-up duration.

Study limitations

The number of subjects is small. As this study focused on identifying electrophysiological parameters associated with a predisposition toward polymorphic VT, several factors such as dispersion of repolarization after premature stimulation not caused by activation delay,[7] connexin 43 expression, distribution, and colocalization with N-cadherin[10] were not analyzed. Local conduction latency was not measured; however, activation delay was consistent with previous studies that have excluded local conduction latency as a cause of activation delay.[1,11,12] The VERP was assessed only for the RV apex after applying an extrastimulus after a 400-ms drive train. Regional differences in VERP and the potentially shorter VERP after S2 were not taken into account. The present study did not analyze the association between activation delay and spontaneous polymorphic VT/VF as such analyses require larger patient cohorts and long follow-up duration.

Conclusion

Premature stimulation in patients with NICM causes progressive activation delay, which can be easily quantified by measuring QRSd after a 400-ms drive train with single extrastimuli. The CI window with QRSd prolongation was wider in patients with NICM than in controls, the maximum increase in QRSd was larger, and a pronounced QRSd prolongation exceeding the VERP was demonstrated, allowing for extrastimuli being applied within the QRS complex. These 3 phenomena, which are easily assessed during a 3-minute stimulation protocol, were associated with the inducibility of polymorphic VT. QRSd prolongation after premature stimulation tended to be related to long, thick strands of fibrosis, but not to overall fibrosis area percentages in biopsy specimens, supporting the link between the geometry of fibrosis and arrhythmogenicity. Measuring QRSd may be a simple tool to quantify progressive activation delay after premature stimulation as a risk factor for polymorphic VT/VF.

Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.hrthm.2015.12.021.

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CLINICAL PERSPECTIVES

Polymorphic ventricular tachycardia (VT) and ventricular fibrillation in patients with nonischemic cardiomyopathy (NICM) are poorly understood, and therapies that effectively prevent their occurrence are still lacking. Activation delay after premature stimulation, measured using multiple intracardiac catheters and an extensive pacing protocol, has previously been associated with ventricular fibrillation in various nonischemic heart diseases. The present study investigated QRS duration (QRSd) prolongation after premature stimulation as a simple marker of biventricular activation delay in NICM. It is demonstrated that the coupling interval window with QRSd prolongation was wider in patients with NICM than in controls, the maximum increase in QRSd was larger, and the prolonged QRSd frequently exceeded the ventricular effective refractory period, allowing for extrastimuli being applied within the QRS complex. Importantly, these newly proposed parameters, but not clinical or imaging data, were associated with the inducibility of polymorphic VT in patients with NICM. The activation delay parameters were not consistently associated with N-terminal pro-b-type natriuretic peptide levels, left ventricular or right ventricular volumes/function, or late gadolinium enhancement cardiac magnetic resonance imaging, but tended to be related to long, thick strands of fibrosis in endomyocardial biopsy specimens, supporting a previously suggested link between activation delay and the geometry of fibrosis. QRSd may be a simple parameter to quantify activation delay after premature stimulation as a risk factor for polymorphic VT in NICM. The parameters can be easily measured after a 400-ms drive train with single extrastimuli and may even be obtained noninvasively in patients with pacemakers or implantable cardioverter-defibrillators. Further studies are required to analyze the predictive value of QRSd-quantified activation delay for spontaneous ventricular arrhythmias during follow-up.