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Endocardial Approach for Substrate Ablation in Brugada Syndrome: Epicardial, Endocardial or Transmural Substrate?

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Abstract

Background: Radiofrequency ablation (RFA) in Brugada syndrome (BrS) has been performed both endocardially and epicardially. The substrate in BrS is thus unclear.

Objectives: To investigate the functional endocardial substrate and its correlation with clinical, electrophysiological and ECG findings in order to guide an endocardial ablation.

Methods: Thirteen patients (38.7±12.3 years old) with spontaneous type 1 ECG BrS pattern, inducible VF with programmed ventricular stimulation (PVS) and syncope without prodromes were enrolled. Before to endocardial mapping the patients underwent flecainide testing with the purpose of measuring the greatest ST-segment elevation for to be correlated with the size and location of substrate in the electro-anatomic map. Patients underwent endocardial bipolar and electro-anatomic mapping with the purpose of identify areas of abnormal electrograms (EGMs) as target for RFA and determine the location and size of the substrate.

Results: When the greatest ST-segment elevation was in the 3rd intercostal space (ICS), the substrate was located upper in the longitudinal plane of the right ventricular outflow tract (RVOT) and a greatest ST-segment elevation in 4th ICS correspond with a location of substrate in lower region of longitudinal plane of RVOT. A QRS complex widening on its initial and final part, with prolonged transmural and regional depolarization time of RVOT corresponded to the substrate locateded in the anterior-lateral region of RVOT. A QRS complex widening rightwards and only prolonged transmural depolarization time corresponded with a substrate located in the anterior, anterior-septal or septal region of RVOT. RFA of endocardial substrate suppressed the inducibility and ECG BrS pattern during 34.7±15.5 months. After RFA, flecainide testing confirmed elimination of the ECG BrS pattern. Endocardial biopsy showed a correlation between functional and ultrastructural alterations in two patients.

Conclusion: Endocardial RFA can eliminate the BrS phenotype and inducibility during PVS.

Keywords: Brugada syndrome; Radiofrequency catheter ablation; Electrocardiography; Mapping; Biopsy.

List of Abbreviations: AP: Action potential; BRS: Brugada syndrome; ECG: Electrocardiogram; EGMs: Electrograms; EPS: Electrorophysiology study; f-QRS: Fragmented QRS; ICD: Implantable cardioverter-defibrillator; LPs: Late potentials; PVC: Premature ventricular contractions; PVS: Programmed ventricular stimulation; RF: Radiofrequency; RP: Refractory period; RV: Right ventricle; RVIT: Right ventricular inflow tract; RVOT: Right ventricular outflow tract; SCD: Sudden cardiac death; TEM: Transmission electron microscopy; VF: Ventricular fibrillation; VRP: Ventricular refractory period ; VT: Ventricular tachycardia

Introduction

Knowledge about the substrate of arrhythmias has allowed its rational treatment in the era of ablation. Brugada Syndrome (BrS) is characterized by an elevated ST segment in the right precordial leads (V1-3) on the electrocardiogram (ECG) and risk of ventricular tachycardia/ ventricular fibrillation (VT/VF) episodes and sudden cardiac death (SCD) [1,2]. The incidence of SCD in subjects with Brugada type 1 ECG pattern and no previous cardiac arrest is 2 per 1000 patients per year [3,4]. Since the original publication in 1992, many researchers have tried to explain the mechanisms and substrate that causes an abnormal ECG pattern and ventricular arrhythmias and few therapeutic options have been found. Initially three hypotheses were proposed for explain the mechanism and arrhythmias in BrS, the abnormal repolarization theory, the abnormal depolarization theory and the abnormal expression of neural crest cells during cardiac development [1,5-7]. At present, there are just two therapeutic strategies, which include implantable cardioverter-defibrillator (ICD) and/or chronic quinidine therapy [3,4]. However, quinidine is not effective in many patients and its use is frequently associated with intolerable adverse effects. ICD implantation may be effective in preventing sudden cardiac deaths, and is currently recommended as a class I indication for symptomatic patients with type 1 Brugada ECG pattern. Unfortunately, ICD therapy in many patients is associated with inappropriate shocks (overall ICD complication rate is 9.1% and inappropriate shocks in BrS occur in 13.7%), lead fractures/failure, device infections and frequent ICD discharges or electric storms [4,8,9]. Additionally, high-risk patients with BrS have recurrent VF episodes, which cause frequent ICD discharges or storms. As an autosomal dominant disease with incomplete penetrance, BrS was initially linked to mutations in the SCN5A gene. Currently, more than 450 pathogenic variants have been identified in 24 genes encoding sodium, potassium, and calcium channels or associated proteins [10,11]. Known BrS-susceptibility genes can only partially explain the clinically diagnosed cases; therefore, many patients (65-70%) remain "genetically unresolved" [8,9]. For many years, BrS has been considered a purely electric disease even if, more recently, some authors have suggested the presence of morphological and functional abnormalities (regional conduction slow in the endocardium and epicardium), predominantly located in the right ventricle outflow tract (RVOT) [12-14].

Nevertheless, in two previous reports endocardial radiofrequency ablation (RFA) was effective in preventing VF in BrS [15,16]. Two studies have recently shown fractionated systolic electrograms (EGMs) in epicardium of RVOT and RFA normalized the ECG pattern and prevented VT/VF occurrence in a short follow-up [17,18].

The aim of the present study was to investigate the functional endocardial substrate and its correlation with clinical, electrophysiological and ECG findings, in symptomatic and inducible patients with spontaneous type 1ECG BrS pattern in order to guide an endocardial ablation. The end points were non inducibility, disappearance of ECG pattern and long-term survival. In addition, we investigated the correlation between the functional and ultrastructural substrate, in endomyocardial biopsy of two patients.

Methods

Patient Characteristics

A prospective single-center study in thirteen caucasian patients between 2011 and 2016 was conducted. Patients referred to the arrhythmology department were consecutively included when presented all three high-risk criteria: 1) documented spontaneous type 1 BrS ECG pattern, 2) syncope of probable arrhythmic cause (syncope was defined as a nontraumatic and reversible loss of consciousness, and was considered of arrhythmic origin in the absence of a prodrome or triggering circumstances), 3) inducible VF with programed ventricular stimulation (PVS). These were associated with at least one of the following conditions: family history of SCD at age <45 years, type 1 BrS ECG pattern in family members, early repolarization pattern, and/or nocturnal agonal respiration [3,9]. The informed consent was signed by the patients.

Structural heart disease, systemic diseases and phenocopies was ruled out in each case on the basis of clinical history and extensive evaluation with 2D Echocardiography, tilt test, brain computed tomography, 24-hour ambulatory ECG monitoring, HIV test, coxsackie and parvovirus B19 test, Chagas disease test, myocardial perfusion and cardiac nuclear magnetic resonance. All patients had 5 points according to the risk score model currently proposed by Sieira *et al.* (spontaneous type 1 ECG pattern =1 point, inducible VF =2 points and syncope =2 points) [19]. None had a history of SCD or documented spontaneous VT/VF and did not receive antiarrhythmic drugs. Patients were submitted to endocardial bipolar and electroanatomic mapping. One month before of mapping and RFA ten patients accepted the implant of an ICD with class IIa indication [9]. Local Ethics Committee approved the study.

Electrocardiographic analysis

QRS complex duration, R wave amplitude in aVR lead, presence of fragmented QRS (f-QRS) and end-QRS slur or notch in DI, aVL, DII, DIII and aVF leads with J point peak ≥0.2 mv with descending ST segment, corresponding to an early repolarization or "J wave" were analyzed.

Before of endocardial mapping the patients were underwent flecainide testing (400 mg, orally) with the purpose of measuring the greatest ST-segment elevation. The partial greatest ST-segment elevation in millimeters (ST-segment elevation in V1 + V2 leads in

the 3rd ICS and ST-segment elevation in V1+V2+V3 leads in 4th ICS), and total sum of greatest ST-segment elevation (ST-segment elevation in V1+V2 leads in the 3rd ICS plus ST-segment elevation in V1+V2+V3 leads in 4th ICS) were measured (Supplementary Figure 1). Correlation between size and location of substrate in the electro-anatomic map and the ST-segment elevation were analyzed.

Electrophysiological study, mapping and biopsy

Before of RFA, bipolar endocardial mapping of right ventricle and RVOT with 4 mm tip deflectable catheter was performed. AH and HV interval to DI and V2 lead and ventricular refractory period (VRP) were measured. Bipolar EGMs were filtered from 10 to 400 Hz and displayed at 100 to 200 mm/s speeds. Programmed ventricular stimulation (PVS) of RVOT with 3 cycle lengths (600, 500 and 400 ms) and up to two premature extrastimuli was performed. Premature extrastimuli was decreased in 20 ms step until a coupling interval of 200ms or the VRP was reached or VF lasting>10 seconds was induced. Regional depolarization time (RDT) from the endocardial EGM of right ventricular inflow tract (RVIT) recorder by the catheter located at the site of His, until the beginning of endocardial EGM of RVOT, recorded by the catheter located in the RVOT was measured. A value from 0 to 10 ms was considered normal. Moreover, the trans-mural depolarization time (TDT) of RVOT from the beginning of endocardial EGM of RVOT until the end of QRS complex in V2 lead was measured. The TDT measured at DI was considered as normal value.

High-density detailed endocardial electroanatomical and bipolar voltage mapping was performed using 3-dimensional (3D) mapping system En Site NavX™ under local anesthesia and sedation, during stable sinus rhythm. Systolic low voltage areas were identified using standard voltage cut-off values for dense scar (<0.5 mV) and border zone (<1.5 mV). Three zones of substrate of according to amplitude of the systolic EGMs were defined: central very low voltage zone <0.5mV, peripheral low voltage zone of 0.5 to 1.5mV (border) and normal voltage zone >1.5mV. The central zone of substrate using filling scaling was measured in mm^2 and located in the RVOT. Taking the pulmonary valve as upper limit and the supraventricular crest as lower limit in the longitudinal plane the RVOT was divided in top and bottom. In the transverse plane, anterior, lateral, posterior and septal areas were identified. Before RFA and after right internal jugular venous access through a steerable sheath 7.2 Fr, length 45 cm (Deflectable catheter delivery system ATTAI R. Medtronic Inc., USA), a bioptome was advanced to RVOT (Jawz TM Endomyocardical Biopsy Forceps Argon Medical, 6Fr, length 50 cm) and connected to 3-dimensional (3D) mapping system. Guiding by electroanatomic and voltage map two samples of endomyocardial biopsies of the three previously defined zones of substrate was obtained. Samples were fixed in 4% glutaraldehyde and 0.1% sodium phosphate (pH 7.4) for TEM study as was described previously [20].

Definition of Abnormal EGMs and ablation

Systolic EGMs with an amplitude ≤1.5 Mv, split or fractionated whit a duration >80 ms and delayed components extending beyond the end of QRS complex and accompanied by late potentials (LPs) were defined as abnormal. The EGMs found in the diastole were referred as "diastolic electrical activity". The number of diastolic EGMs (separated by isoelectric line) in two successive sinus cycles was counted. After the mapping, the 4mm tip catheter was replaced by an 8mm tip ablation catheter, looking to provoke a deeper and more extensive lesion from the endocardium in the thin wall of the RVOT. Abnormal EGMs were target for ARF. Radiofrequency was delivered for abnormal areas with 55 °C temperature control and 60 W power limit. Each radiofrequency application lasted 30 to 60 s, depending on complete elimination of the targeted EGMs. Abolition or persistence of abnormal EGMs was checked with the ablation catheter after each one of the applications. Immediate procedural end point was the elimination of all the abnormal EGMs identified inside the low-voltage areas during sinus rhythm as replaced by low-voltage (<0.5 mV) or until their disappearance in the ablation catheter and loss of activity (Suplementary Figure 3). Finally, the same PVS protocol to confirm loss of inducibility was applied.

Follow-up

Patients were monitored for at least 3 days after the procedure. Before hospital discharge, echocardiography and 12-lead ECG were performed and the patients were followed monthly. After discharge, a 12-lead ECG, a 24-hour ambulatory ECG monitoring and ICD interrogation were scheduled at each follow-up visits. In addition, 30 days after ablation a flecainide testing (400 mg, orally) was performed.

Statistical Analysis

Continuous variables are shown as mean±SD, and categorical variables are presented as number and percentage. Comparison of continuous variables was done by using the Student t test for normally distributed variables and the Mann-Whitney U test for no normally distributed variables. The *P* value≤0.05 was considered of statistical significance. A ROC curve was constructed to determine the sensitivity and specificity of the method used.

Results

Study population and risk factors

Table 1 shows the clinical characteristics of the study patients. Thirteen patients with spontaneous type 1 ECG BrS pattern,

symptomatic by syncope without prodromes, and VF induced during PVS were enrolled and completed the study protocol. Five males (38.5%) and eight females (61.5%), with an average age of 38.7±12.3 years (range 19 to 58 years) were enrolled. Most patients (54%) had a family history of SCD and all patients experienced previous syncopal episodes without prodromes. In four patients (31%) nocturnal agonal respiration and family historie of ECG 1 BrS pattern were evident.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	Total	%	Mean	SD
Age	41	33	57	55	58	27	46	26	24	19	38	38	41			38.7	12.3
Sex	M	F	F	M	F	M	M	F	F	M	F	F	F	F=8 M=5	F=61.5%-M=38.5%		
Spontaneous Type 1 ECG Pattern	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	13	100%		
Syncope Without Prodromes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	13	100 %		
Nocturnal Agonal Respiration	No	No	No	No	Yes	No	Yes	No	Yes	No	No	Yes	No	4	31%		
Palpitations	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	13	100%		
Family History Scd	No	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	7	54%		
Family History Brs	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No	4	31%		
VRP ≤ 200ms	180	180	160	200	160	180	200	180	160	180	180	200	180	13	100%	180ms	13.6
QRS Duration in V2 lead (> 120 ms)	160	105	120	180	120	120	140	100	90	140	170	140	100	6	46%	129.6ms	27
R Wave in aVR lead (≥ 3 mm) (#)	3	4.5	2	2.5	1.5	1	4	4	1.5	6	3	4	1	7	54%	3mm	1.4
QRS Fragmentation	No	Yes	No	No	No	No	No	Yes	No	No	No	No	Yes	3	23%		
J WAVE	No	No	No	No	No	No	Yes	No	No	No	Yes	No	No	2	15.4%		
HV Interval to DI lead (>55ms)	60	57	40	120	57	40	46	35	45	45	55	35	59	5	38.5%	53.4ms	21
HV Interval to V2 lead (>55ms)	40	40	35	80	45	40	46	35	40	45	55	35	46	1	7.7%	44.8ms	11.5
Follow-Up (months)	66	63	56	53	51	54	45	46	45	43	28	12	19	13	100%	44.7	15.5
CDI Implant	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	10	77%		
BIOPSY	No	No	No	No	No	No	No	No	No	No	Yes	No	Yes	2	15%		
Very Low-Voltage Area(≤ 0.5mV) in (mm2)	23	8	19	42	5	4	8	10	19	10	8	25	4	13	100%	14.2 mm^2	10.5
Nº Endocardial Diastolic Egms In 2 Succesive Cycles Sinus (≥4 EGMs)	8	8	5	7	7	9	6	6	9	6	6	7	4	13	100%	6.7	1.4
Procedure Time (min)	120	110	100	90	120	95	105	95	100	100	160	90	170			112	24.5
Fluoroscopy Time (min)	15	10	10	10	15	10	12	13	12	10	27	10	25			13.7	5.5

Table 1: Basal Characteristic and Risk Factors
M=Male , **F**=Female, **SD**=Standard deviation (#)=during flecainide test, **VRP**=Ventricular refractory period

All patients had a VRP≤ 200 m (180±13.6ms). A QRS complex duration > 120 ms in V1 or V2 leads (129.6±27ms) in 6 patients (46%) and a R wave with an amplitude ≥3 mm in aVR lead during flecainide testing (3±1.4mm) in 7 patients (54%) was found. In five patients (38.5%) a HV interval to DI lead >55 ms (53.4±21ms), a QRS fragmentation (23%) in 3 patients and a J wave (15.4%) in 2 patients were present. Interestingly, during bipolar mapping after PVCs, alternating T and J-wave and changes of the ST segment elevation were found (Supplementary Figure 2).

Identification of functional Substrate

Baseline endocardial voltage mapping was successfully acquired during sinus rhythm in all patients. Areas showing low-amplitude signals were mapped with greater point density to delineate the extent and borders of endocardial electroanatomic substrate. Abnormal endocardial electroanatomic voltage maps, characterized by very low-voltage EGMs with clean diastoles in the central

area of substrate were found. Only three patients (23%) during bipolar mapping showed fragmented systolic EGMs of low-voltage (≤1.5 mV) with duration greater of 80ms between central and peripheral zone (border zone) of voltage mapping (Figure 1). As shown in Figure 1, only in the peripheral zone of substrate the endocardial diastolic EGMs (mean 6.7±1.4 EGMs in two successive sinus cycles) were present. Overall, the median baseline very low voltage or central area of substrate (≤ 0.5mV) was 14.2 mm2 (SD=10.5) (Table I).

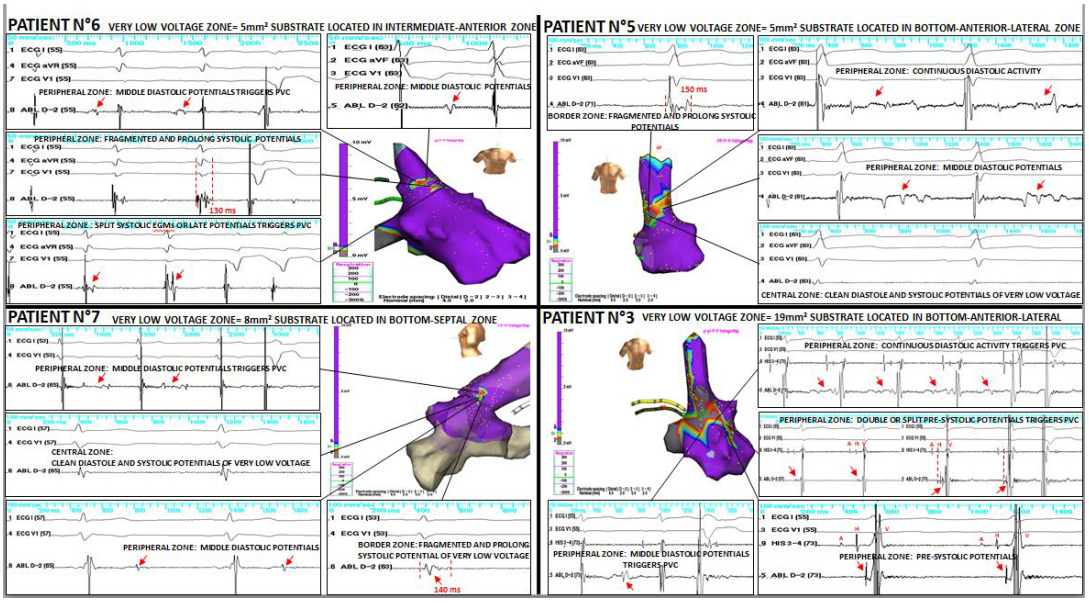

Figure 1: Endocardial electroanatomic maps and location of abnormal EGMs. Double systolic EGMs, late potentials, middle diastolic potentials and continuous diastolic activity in peripheral zone that triggers PVCs is displays. Prolonged and fragmented systolic EGMs of low voltage in border zone in three patients can be observed. In the central zone of substrate observe clean diastole with very low-voltage systolic potentials. The N°3 patient displays single and split pre-systolic potentials in peripheral zone of substrate which triggers PVCs

Size of substrate and ST-segment elevation

As shown in Figure 2 and Table 2, with a cut-off ≥13 mm in the total sum of ST segment elevation two variants were found: 1) A total sum of ST-segment elevation <13 mm (n=8, 61.5%, mean 9.6±1,3mm) corresponded to a central area of substrate of 7.7±1.8mm^2; 2) A total sum of ST segment elevation ≥13 mm (n=5, 38.5%, mean 15±1.1mm) corresponded to a central area of substrate of 28.2±9.2mm^2. (p<0.001 for correlation of ST segment elevation and correlation of central area of substrate, with a value > 0.90 of ROC curve).

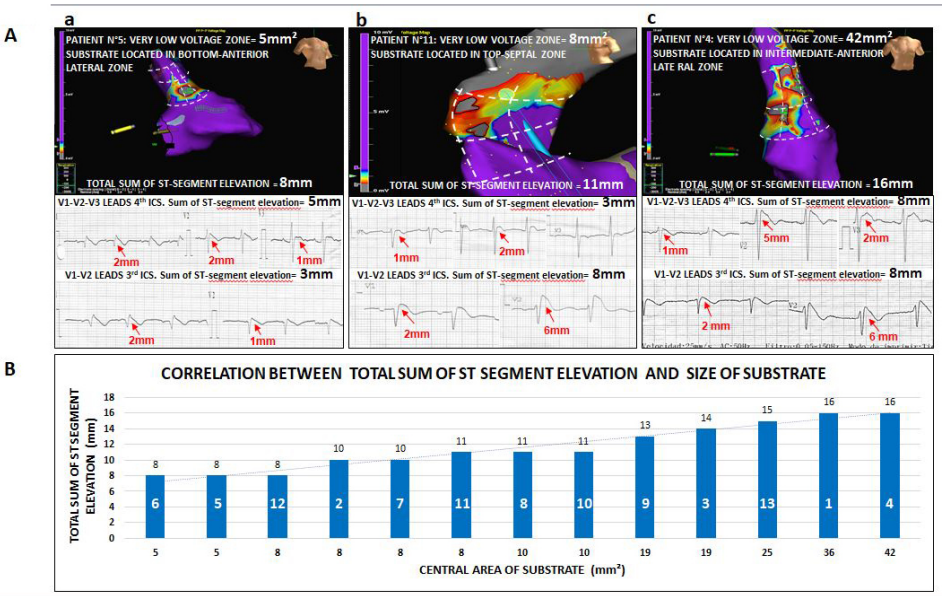

Figure 2: Correlation between ST segment elevation and substrate localization. A. With the administration of sodium channel blocker, a greater magnitude of total sum of ST segment elevation corresponds to a greater very low voltage area of substrate, and allows its location in the longitudinal plane of RVOT. **a)** The location of the substrate in the bottom zone of RVOT correspond to a greater sum of ST segment elevation in 4th ICS vs 3rd ICS (5mm vs. 3mm). **b)** The location of the substrate in the top zone of RVOT correspond to a greater sum of ST segment elevation in 3rd ICS vs 4th ICS (8mm vs. 3mm). **c)** The location of the substrate in the intermediate zone of RVOT corresponds to a sum of ST segment elevation of equal magnitude in 3rd ICS and 4th ICS (8mm and 8mm). **B.** The graph shows the correlation between linear increase of total sum of ST segment elevation and substrate size. The blue bars indicate each Patient.

Patient	ST-Segment Elevation (mm) V1-V2 LEADS 3rd ICS	V1-V2-V3 LEADS 4th ICS	Total Sum Of St-Segment Elevation (Mm)	Size Of Low Voltage Area (mm^2)	Location Of Substrate In The Longitudinal And Transverse Plane
1	V1= 2 V2= 4	V1= 2 V2= 7 V3= 1	16	36	Bottom Anterior-Lateral
2	V1= 3 V2= 4	V1= 1 V2= 2 V3= 0	10	8	Top Aterior-Lateral
3	V1= 2 V2= 3	V1= 3 V2= 5 V3= 1	14	19	Bottom Aterior-Lateral
4	V1= 2 V2= 6	V1= 1 V2= 5 V3= 2	16	42	Intermediate Anterior-Lateral
5	V1= 2 V2= 1	V1= 2 V2= 2 V3= 1	8	5	Bottom Anterior-Lateral
6	V1= 2 V2= 2	V1= 0 V2= 2 V3= 2	8	5	Intermediate Anterior
7	V1= 1 V2= 3	V1= 1 V2= 4 V3= 1	10	8	Bottom Septal
8	V1= 3 V2= 5	V1= 1 V2= 2 V3= 0	11	10	Top Anterior
9	V1= 2 V2= 3	V1= 3 V2= 4 V3= 1	13	19	Bottom Anterior-Septal
10	V1= 5 V2= 3	V1= 1 V2= 2 V3= 0	11	10	Top Anterior
11	V1= 2 V2= 6	V1= 1 V2= 2 V3= 0	11	8	Top Septal
12	V1= 2 V2= 3	V1= 1 V2= 2 V3= 0	8	8	Top Anterior-Septal
13	V1= 3 V2= 4	V1= 3 V2= 5 V3= 0	15	25	Intermediate Anterior
Mean And SD	3±1.3	2.3±1.5	<13 (n=8, 61.5%) 9.6±1.3	7.7±1.8	
			≥13 (n=5, 38.5%) 15±1.1	28.2±9.2	
P Value			<0.001	<0.001	

TABLE 2: CORRELATION BETWEEN SUM OF ST-SEGMENT ELEVATION, LOCATION AND SIZE OF LOW VOLTAGE AREA

Location of substrate in the longitudinal plane

As shown in Figure 2-A and Table 2, three variants were found: 1) When the substrate was located in the top region of RVOT, it corresponded with the greater sum of ST-segment elevation in V1-V2 leads in the 3rd ICS (n=5, 38.5%); 2) When the substrate was located in the bottom region of RVOT (n=5, 38.5%), it corresponded with the greater sum of ST-segment elevation in V1-V2-V3 leads in the 4th ICS; 3) When the substrate was located in the intermediate region of RVOT (n=3, 23%), the sum of ST-segment elevation was of equal magnitude in the 3rd and 4th ICS (n=3, 23%).

Location of substrate in the transverse plane

As shown in Figure 3, two variants were found: 1) When the substrate was located in the anterior-lateral region of RVOT (n=5, 38.5%), the HV interval measured to DI lead (mean 70.6±24 ms) was longer than the HV interval to V2 lead (mean 50±15ms). This was accompanied with widening of the QRS complex in its initial and final parts (widening of QRS left and right) in V1 and V2

lead. We defined this as "mixed delay of depolarization of RVOT". Simultaneously, TDT and RDT were prolonged, because early depolarization of RVOT occurs. 2) When the substrate was located only in the anterior part (30.8%), or anterior-septal (15.4%) or exclusively in the septal region (15.4%) of RVOT, the HV interval measured to DI and V2 leads showed no increase in its duration (mean 42.6±6ms and 41±6.5ms, respectively) and the widening of QRS was only rightward (widening QRS rightward). Moreover, the endocardial EGM of RVIT and RVOT, and beginning of QRS complex in DI-V1-V2 lead they were activated simultaneously, indicating that there is no RDT delay, while TDT of RVOT was prolonged of dynamic manner. We defined this as "end delay depolarization of RVOT".

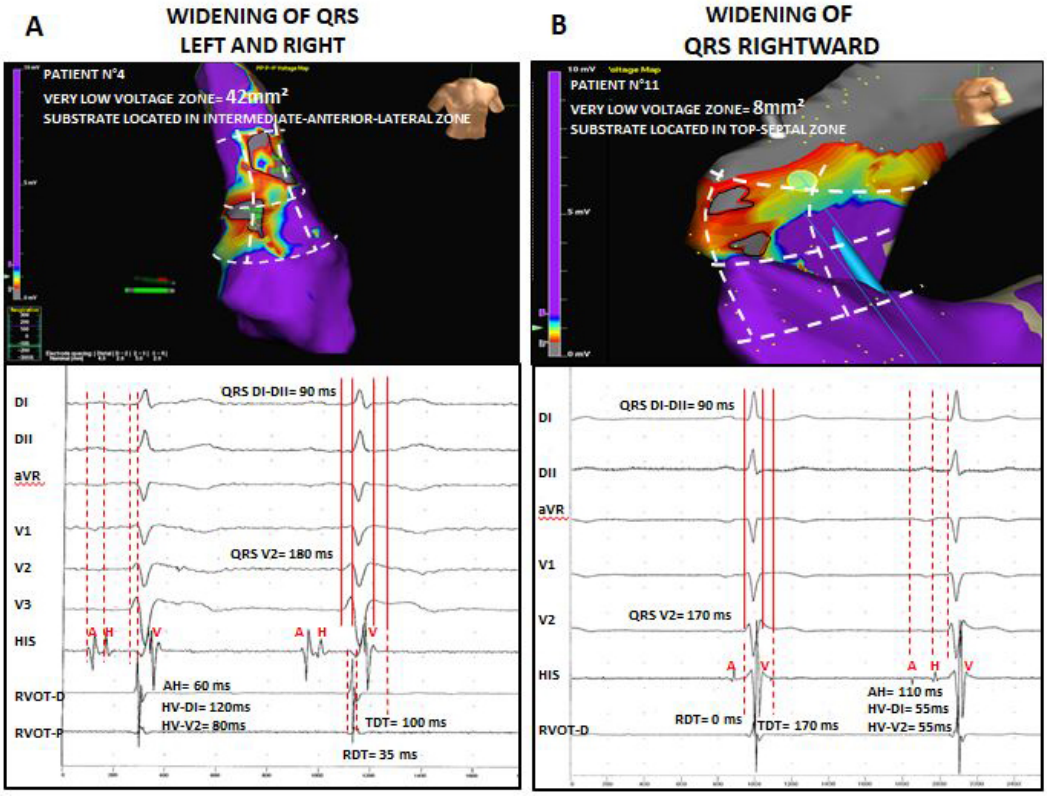

Figure 3: Location of substrate in transverse plane of RVOT.A. The N° 4 patient displays a substrate located in the anterior-lateral zone of RVOT which corresponds to a widening of QRS complex to left and right. The HV interval to DI lead is longer (120ms) that the HV interval to V2 lead (80ms), while RDT and TDT are prolonged. B. The N° 11 patient displays a substrate located in the septal zone of RVOT which correspond only to a widening of end QRS complex. The HV interval to DI and V2 leads is equal (55ms) and only TDT is Prolonged

Interestingly, as shown in Figure 4-A and B, two patients who had a substrate of exclusively septal location, showed end-QRS notching or slurring pattern. When the substrate was located in the bottom-septal zone of RVOT (patient N° 7) only end-QRS notch in aVL lead and slurred S-wave in DII, DIII and a VF leads was observed. Whereas, when the substrate was located in the top-septal zone of RVOT (patient N° 11) an end-QRS slur in DI and aVL leadswas observed.

Effects of RFA on the ECG BrS pattern and substrate

Once the endocardial arrhythmogenic substrate was accurately identified/quantified and tagged on the electroanatomic voltage maps, endocardial RFA applications were delivered.

Endocardial RFA resulted normalization of the ECG BrS pattern, disappearance of end-QRS notching or slurring and suppression of inducibility in all patients during a mean follow-up of 35±15.5 months (Figure 4). Thirty days after RFA a flecainide testing did not develop ECG BrS pattern. Seven patients who entered to procedure with spontaneous type 1 ECG pattern showed ECG normalization at the end of the procedure. Immediately after RFA was applied, varying degrees of changes in ST segment were observed. With following applications of RFA the ECG pattern progressively decreased (Supplementary Figure 3). After RFA local abnormal diastolic EGMs completely disappear and systolic EGMs were replaced by residual very low voltage areas. The mean time of procedure and fluoroscopy were 112±24.5 and 13.7±5.5 minutes, respectively (Table I). Of note, during the procedure 8 patients (61.5%) developed a complete and transient right bundle branch block (RBBB) that lasted for a few minutes. Only a patient developed a persistent RBBB (7.7%).

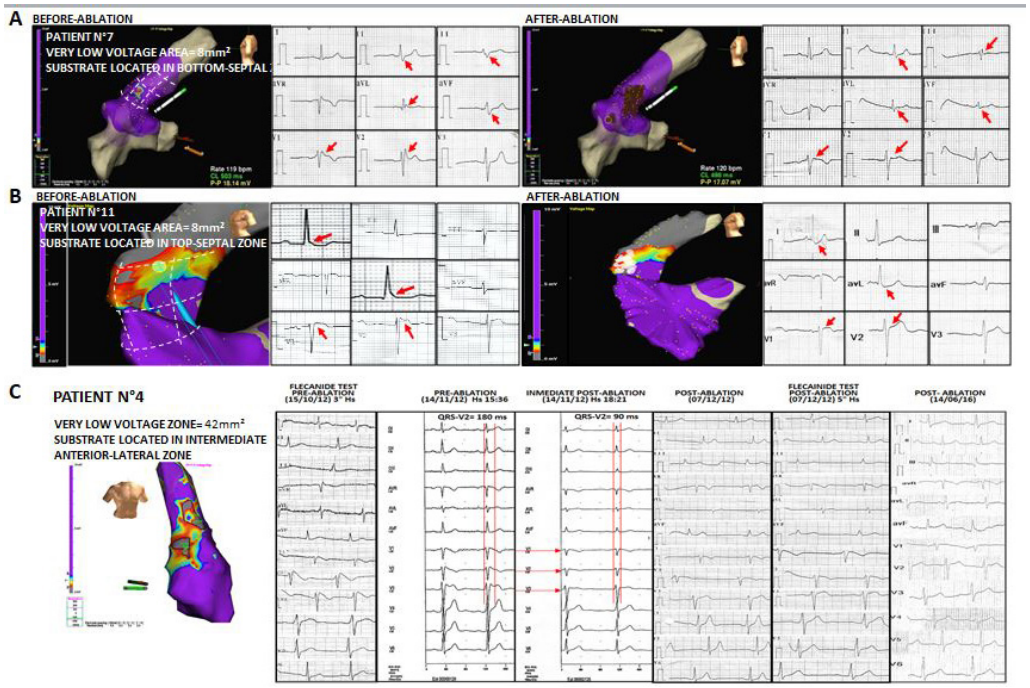

Figure 4: RFA effects on the ECG. A. The N° 7 patient displays a substrate located in bottom septal zone of RVOT. Before RFA a type 2 ECG BrS pattern, end-QRS notch in aVL lead and slurred S-wave in DII, DIII and aVF leads are present. Itdisappears after RFA (red arrows). **B.** The N° 11 patient displays a substrate in the top septal zone of RVOT. Before RFA a type 1 ECG BrS pattern and end-QRS slur in DI and aVL leads are present. It disappears after RFA (red arrows). **C.** Before RFA the flecainide test showed a type 1 ECG BrS pattern. The QRS complex duration in V1 and V2 leads was 180ms, while in DI was 90ms (red lines). Immediately after ablation, the duration of QRS complex in V2 lead decreased to 90 ms and ECG BrS pattern disappears (red arrows). After RFA the flecainide test not induced type 1 ECG BrS pattern. The ECG at 3.8 years follow-up persist Normal

Correlation between functional and ultrastructural substrate

In two patients endo-myocardial biopsies were obtained and correlated with the functional substrate.

In the Figure 5-A, the patient N°13 in (a), (d) and (g) shows electro-anatomic and voltage map (functional substrate) with a central area of substrate of 25mm² located in the intermediate-anterior zone of the RVOT and the bioptome connected to the navigation system. In (b) and (c) on the right side can be seen the ultrastructural substrate which corresponds to normal zone with mitochondria, myofibrils, and a Purkinje cell of normal characteristics. In contrast, in the peripheral zone of substrate (e and f) note that when approaching to pathological area, cytoplasmic vacuolization, myofibrillar and mitochondrial disorganization with myofibrillar residue can be observed. The (h) and (i) corresponds to the central zone, which depicts strong vacuolization and cell destruction with intense cytoplasmic disorganization and myofibrillar residue. In the Figure 5-B, the patient N° 11 in (a), (d) and (g) shows the voltage and electro-anatomic map (functional substrate) with central area of substrate of 8mm² located in the top-septal zone of the RVOT. In (b) and (c) on the right side can be seen the ultrastructural substrate which corresponds to normal zone with normal characteristics of mitochondria, myofibrils, and a Purkinje cell. The approaching to pathological area in the peripheral zone of substrate (e and f), myofibrillar disorganization, citoplasmic vacuolization, swelling and disappearance of mitochondrial crests, myofibrillar rests and remains of erythrocytes were observed. The (h) and (i) corresponds to the central zone showing strong vacuolization and cell destruction and myofibrillar residue.

Fat replacement, lymphocytic infiltration, Chagasic myocarditis, collagen tissue or apoptotic bodies were not observed.

Follow-Up

Postprocedure, predischarge, and follow-up 12-lead ECG confirmed the absence of BrS ECG pattern before and after flecainide test in all patients (Figure 4). The patients were asymptomatic and free of arrhythmic events in the 24-hour ambulatory ECG monitoring and in follow-up the ICD interrogation after a median follow-up of 44.7±15.5 months.

Two patients (15%) had a near-syncope with prodrome at 24 of 46 months and at 18 of 36 months of follow-up respectively, without arrhythmias in ICD interrogation.

Complications

A patient underwent endomyocardial biopsy had mild pericardial effusion and only a patient developing a persistent RBBB.

Discussion

Population characteristics and inducibility

Patients with a Brugada type 1 ECG pattern may suffer SCD [3]. The risk of SCD in patients without ICD is 2 per 1000 patients per year [19]. But unfortunately, the possibility of survival out of hospital is low if the first symptom is the SCD. In the follow-up, the arrhythmic events occur in patients who presented spontaneous type 1 ECG BrS pattern and syncope of presumed arrhythmic origin, so both are considered high-risk factors [3]. VF inducibility rate is the highest in those patients with syncope of unknown origin (80%) [21]. In our patients the endocardial RFA of diastolic electrical activity and abnormal systolic EGMs suppressed the type 1 ECG BrS pattern and inducibility, making the patients asymptomatic, as was previously reported for the epicardial RFA [17,18].

The induction with PVS up to two premature beats is independent predictors of poor prognosis with a high negative predictive value and was associated with increased risk, but has a controversial prognostic value. The lack of induction does not necessarily portend a low risk and hence clinical factors are the most important determinants [22-25]. VF inducibility rate is highest in patients with BrS and syncope of unknown origin (80%), the lowest in asymptomatic patients (61.5%), and intermediate in patients with vasovagal syncope (70.5%) [24,25]. However, it is important to note that these observations correspond to the pre-ablation era of BrS and therefore, the PVS could be a good predictor of outcome after RFA [26].

Approximately one-third of BrS patients present syncope. Syncope constitute an important diagnostic and therapeutic challenge in BrS. Some cases of syncope may be related to VF that terminates spontaneously. Vagal syncope is probably the most frequent cause of syncope in the BrS and vagal hypertony may facilitate the onset of spontaneous VF in BrS [27,28]. Also symptoms suggesting of vagal syncope may also be observed in syncope of cardiac origin [29]. In this study, two patients (15%) after RFA had near-syncope with vaso-vagal prodrome and without arrhythmias in the ICD interrogation [21].

BrS is eight times more prevalent in males, probably for higher testosterone levels and a more prominent transient outward current (Ito). Males are at increased risk for developing a spontaneous type 1 ECG BrS pattern and VF during PVS. Nevertheless, because the majority of the asymptomatic patients are also male the gender is not an independent predictor of arrhythmic events [9,30]. It is striking that eight of our patients (61.5%) were females. Five of these had between 38 and 58 years of age and menopausal symptoms, so we might suspect that lack of estrogens could induce the expression of phenotype.

Mapping, ablation and mechanisms of VF

Endocardial and epicardial RFA has been proposed as a new strategy to prevent SCD and VT/VF in BrS patients of high risk. Nademanee K. *et al.* found prolonged and fractionated late potentials in the anterior zone of epicardium of RVOT. The RFA at these sites normalized the ECG BrS pattern and prevented VT/VF in all but one patient during a follow-up of 20±6 months [17]. Recently, Brugada J. and Pappone C. in fourteen inducible patients reported abnormal EGMs only in epicardium of the anterior free wall of right ventricle and in RVOT. RFA eliminated both ECG BrS pattern and inducibility, with a median follow-up of 5 months [18]. However, consistently we find a substrate in the endocardium of RVOT and RFA eliminates abnormal EGMs, ECG BrS pattern and inducibility during a median follow-up of 34.7±15.5 months in all our patients. We saw the substrate not only in the anterior zone of RVOT but also in septal and lateral regions, but never in the posterior region. It is important to note, that epicardial mapping may not recognized a substrate located in the septal zone of RVOT. In addition, during epicardial mapping the interposition of fat tissue between the epicardium and the exploratory catheter could decrease the amplitude of the potentials recorded, giving false areas of low voltage.

Two theories suggest that an abnormal repolarization (local re-excitation by phase 2 reentry in the epicardium) or a defect of the depolarization (disturbances in depolarization of RVOT can cause conduction delay) may be responsible of phenotype and VF [31-34]. However, in BrS the arrhythmias are usually polymorphic VT or VF, and these cannot be supported by macro-reentry mechanisms. VF depends of a firing focus initiated by early or delayed after-depolarization or a micro-re-entry [35]. Surviving cells surrounded by fibrosis has demonstrated to be responsible of slow conduction and reentry in inhomogeneous scars [36]. The residual electrical activity within scar was reported as delayed or isolated EGMs, late potentials or diastolic EGMs and their elimination during sinus rhythm was effective to prevent VT/VF [21]. In peripheral zone of substrate when a sufficient degree of cell damage was reached such as we found with TEM and resting potentials are reduced the polymorphic VT/VF may occur. This event could be originated through a firing focus or by multiple wavelets from a reentrant microcircuit, and would explain the "diastolic electrical activity" observed in our patients.

Sunsaneewitayakul *et al.* reported that endocardial RFA on the late depolarization zones modified the ECG BrS pattern in 3 patients and suppress the VF storm in 4 patients, during follow-up of 12-30 months [16]. Similarly, we obtained suppression of inducibility, normalization of BrS ECG pattern and early repolarization pattern with endocardial RFA of areas with late depolarization, diastolic electrical activity and abnormal systolic EGMs; probably by substrate homogenization and transmural lesion of the thin wall of RVOT (mean 3mm). In addition, we found pre-systolic potentials as was previously reported by Haissaguerre et al. [15]. The Purkinje fibers in RVOT showed in our study by TEM could be involved in the origin of pre-systolic potentialsand in genesis

of early-onset PVCs that can trigger VT or VF, by spontaneous depolarization or micro-reentry circuit in the Purkinje network [37]. The elimination of ECG BrS pattern with endocardial RFA supports the depolarization theory rather than the repolarization theory.

During the procedure, 61.5% of the patients developed a complete and transient right bundle branch block (RBBB). This could simply be due to the traumatic effect of ablation catheter. But in both biopsied patients, we found Purkinje fibers in the most peripheral zone of the substrate in the RVOT (Figure 5). Some investigators have denied the existence of a specialized system of conduction in the RVOT. Extensive ablation of the substrate especially in regions below the RVOT could affect conduction in the right branch or could simply be a vagal reflex by ablation of autonomic nerve endings. Recently Morita *et al,* (145 patients who experienced syncope or had VF events) proposes ECG risk markers for the initial and recurrent episodes of VF in symptomatic patients with BrS. The f-QRS, inferolateral early repolarization and complete RBBB were associated with occurrence of ventricular tachyarrhythmia in the symptomatic patients [38].

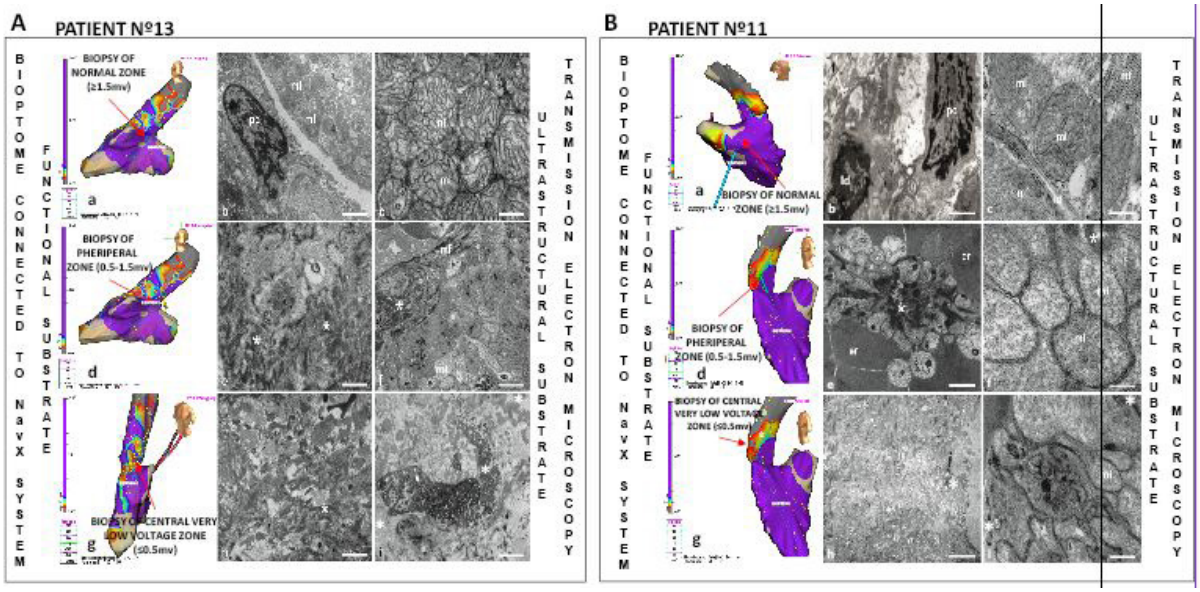

Figure 5: Correlation between functional and ultrastructural substrate. On the left side the electro-anatomic and voltage map (functional substrate) and bioptome connected to the navigation system are shown. On the right side the ultrastructural substrate is shown. Scale Barr: 3.33, 2.2 and 1.42 µm; mitochondria (mi); myofibrils (mf); Purkinje cell (pc); myofibrillar rests (*); lipofusin deposit (ld); intercalated disk (id); remains of erythrocytes (er)

New ECG observations in relation to the substrate

In the BrS the mechanism of ST segment elevation has been explained by the repolarization theory (decreased of Na+ and/or Ca^{++} channel function with a prominent Ito current in epicardium of RVOT generates a transmural voltage gradient), or the depolarization theory (disturbances in depolarization of RVOT can be cause of delayed conduction and ST segment elevation) [18,19]. Nevertheless, the ECG changes are actually explained by the theorem of solid angle, where a substrate larger increases the magnitude of the ST segment elevation [39]. Brugada J. and Pappone C. during electro-anatomic mapping with administration of a sodium channel blocker showed a increases the size of the low voltage are [18]. In concordance, our observations suggest that the total sum of ST segment elevation during flecainide testing would approximately determine the substrate size. In addition, the ECG-leads with greater ST segment elevation would locate the substrate in the RVOT.

Was report that 11% of the patients with BrS have early repolarization pattern in the inferior-lateral leads and a more severe phenotype [40]. Our observations suggest that a location of substrate in septal region of RVOT, with beginning of the depolarization at the endocardium correlated with the presence of end-QRS notching or slurring pattern in inferior or/and lateral leads by slow conduction. Theendocardial RFA of substrate produced their disappearance. Normally the epicardium is electropositive with respect to electronegative endocardium creating a current flow of endocardium to epicardium. When activation spreads from endocardium to epicardium, in a context of slow conduction, the J wave coincides with the notch in the epicardial AP mediated by the Ito current and it is recorded by ECG. Conversely, when the activation beginning in the epicardium the J wave disappears hidden by the QRS complex [41]. Consequently, our observations lead us to think that the J wave depends more of late depolarization that early repolarization as was suggested by other authors.

Functional and ultrastructural findings

In the present study we found a clear correlation between ultrastructural and functional alterations in the endocardium of RVOT in two patients. Coronel *et al.* show in a heart explanted of a BrS patient, fibrosis with epicardial fat infiltration as well

as conduction slow without transmural repolarization differences [42]. Furthermore, interstitial fibrosis, fat tissue and myocyte disorganization with reduced gap junction expression in the presence of fractionated and unfractionated low voltage systolic EGMs in the endocardium and epicardium of RVOT was reported with optical microscopy [43-45]. Our findings, together with the aforementioned, support the probability of a transmural substrate. In these patients when approaching the pathological areas progressive cell damage was observed. In the central zone of substrate low voltage systolic EGMs coincided with strong cell destruction and cytoplasmic disorganization. The peripheral zone of substrate with cell damage, mitochondrial swelling and myofibrillar residue without apoptotic bodies coincided with late potentials, diastolic and/or presystolic activity. These findings support mitochondrial energy loss as possible non-apoptotic progressive tissue damage and death cell. Our results suggest the interesting possibility that substrate could be generated by an abnormal expression of neural crest cells localized in RVOT during cardiac development.

Limitations

The inclusion of thirteen patients may be considered a small size sample. However, our results are consistent allowing us to reach reliable conclusions. Genetic studies were not performed. However, it is unlikely that this could affect our observations because mutations have been described in over 23 genes.

None patients had a history of SCD or spontaneous VT/VF. However, all had high-risk criteria.

ECG BrS pattern disappearance may be questioned because to spontaneous variation. However, after endocardial RFA its persistent normalization with a negative flecainide tests at 30 days, strongly suggest a stable elimination of ECG BrS pattern. A sodium channel blocker was not used during mapping and we did not perform epicardial mapping. Although the results of mapping and RFA were good, we do not perform epicardial mapping and do not ignore the possibility that a portion of the substrate can remain present after ablation.

Only two patients accepted to undergo endo-myocardial biopsiesas research objective. However, the findings in both were equal and consistent.

Conclusion

In patients with BrS of high risk the substrate RFA may be a potential option of treatment. We successfully ablated the substrate of BrS from the endocardium based on the electrophysiological and ultrastructural findings. Our data together with the observations of other researchers suggest a transmural substrate, contributing to future definition of the arrhythmogenic substrate in BrS. As many phenotypes are involved in BrS, it is not unthinkable that different substrates may exist in BrS. ECG analysis during administration of a sodium channel blocker allows approximately determined the size and location of substrate. Careful endocardial mapping allows identify late potentials, presystolic and diastolic EGMs as a new risk marker to guide an endocardial substrate RFA, probably with the same results what a more complex epicardial ablation.

Conflict of Interest

The authors declare none conflict of interest

supplymentary

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