A New Electrocardiographic Marker of Sudden Death in Brugada Syndrome: The S-Wave in Lead I

A New ECG Marker of Sudden Death in Brugada Syndrome: the S wave in lead I.

Leonardo Calò, MD, FESC*, Carla Giustetto, MD#, Annamaria Martino, MD*, Luigi Sciarra, MD*, Natascia Cerrato, MD#, Marta Marziali, MD*, Jessica Rauzino, MD†, Giulia Carlino, MD||,Ermenegildo de Ruvo, MD*, Federico Guerra, MD, FESC§, Marco Rebecchi, MD*,Chiara Lanzillo, MD, PhD*,Matteo Anselmino, MD#, Antonio Castro, MD‡, Federico Turreni, MD‡,Maria Penco, MD||, Massimo Volpe, MD, FESC, FAHA†, Alessandro Capucci, MD, FESC, FACC, FHRS§, Fiorenzo Gaita, MD#

*Division of Cardiology, Policlinico Casilino, ASL Rome B; † Division of Cardiology, Department of Clinical and Molecular Medicine, St. Andrea Hospital, Sapienza University, Rome, Italy; ‡ Division of Cardiology, Policlinic Sandro Pertini, ASL Rome B; § Cardiology Clinic, Ospedali Riuniti Umberto I- Lancisi – Salesi, Ancona; || Department of Cardiology, University of L'Aquila, Italy; #Division of Cardiology, University of Torino, Department of Medical Sciences, "Città della Salute e della Scienza" Hospital, Torino.

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Correspondence to Leonardo Calò, MD, FESC, Division of Cardiology, Policlinico Casilino, ASL Rome B, Via Casilina 1049, 00169 Rome, Italy. Fax +39 06 23188410; tel. +39 06 23188406; email leonardo.calo@tin.it

Structured Abstract

Background **–** Risk stratification in asymptomatic patients remains by far the most important and yet unresolved clinical problem in the Brugada syndrome (BrS).

Objectives**–** Aim of this study was to analyze the usefulness of electrocardiographic (ECG) parameters as markers of sudden cardiac death (SCD) in BrS.

Methods **–** We analyzed data from 347 consecutive patients (78.4% male; mean age: 45 ± 13.1 years) with spontaneous type 1 BrS ECG phenotype. Characteristics of 12-lead surface ECG perfomed at the first clinic visit were analyzed to predict ventricular fibrillation (VF)/SCD during follow-up.

Results - During the follow-up period (48 ± 38.6 months), 276 (79.5%) patients remained asymptomatic, 39 (11.2%) developed syncope and 32 (9.2%) (VF)/SCD. The patients who presented VF/SCD had a lower prevalence of mutation of the SCN5A gene (P=0.009), and a higher prevalence of positive EPS (P<0.0001), family history of SCD (P=0.03), and atrial fibrillation episodes (P<0.0001). The most powerful marker for VF/SCD was a significant S wave (≥ 0.1 mV and/or ≥40 msec) in lead I, that showed a sensitivity of 90.6 and 96.9%, a specificity of 62.2 and 61.1% with a negative predictive value of 98.5% and 99.5%, and a positive predictive value of 19.6% and 20.5%, respectively. Considering the strong correlation found between amplitude, duration and amplitude-duration area of S wave in lead I, only S duration ≥40 ms was chosen for the multivariate analysis, on the basis of the best Akaike information. In the multivariate analysis, duration of S wave in lead I ≥40 msec (HR=39.1), and AF (HR=3.7) were independent predictors of VF/SCD during follow-up. Electroanatomical mapping in 12 patients showed an endocardial activation time significantly longer in those with S wave in lead I, mostly due to a significant delay in the anterolateral RVOT.

Conclusions **-** The presence of a wide and/or large S wave in lead I showed to be a powerful predictor of life-threatening ventricular arrhythmias in BrS.

Key words: Brugada syndrome, prognosis, arrhythmia, electrocardiography.

Selected abbreviations: AF, atrial fibrillation; BrS, Brugada syndrome; CMR, cardiac magnetic resonance; EPS, electrophysiological study; ICD, implantable cardioverter-defibrillator; RBBB, right bundle branch block; RV, right ventricle; RVOT, right ventricular outflow tract; SCD, sudden cardiac death; VF, ventricular fibrillation; VT, ventricular tachycardia.

Introduction

Brugada syndrome (BrS) is characterized by ST-segment elevation in the right precordial leads and an increased risk of ventricular fibrillation (VF) and sudden cardiac death (SCD) (1-2). The real incidence of SCD in these patients is uncertain and controversy exists with regard to the risk stratification in asymptomatic subjects (3-12).

A number of electrocardiographic (ECG) markers of ventricular depolarization and repolarization have been reported to identify high-risk patients with BrS (5-12), although conclusions regarding their clinical impact have been inconsistent. Some studies (13-21) have suggested that the pathophysiologic basis of this syndrome is a conduction delay in the right ventricular outflow tract (RVOT).

The S wave in lead I is generated by the so called third vector that is directed upward and somewhat to the right and backward (22). This vector is determined by the electrical activation of the basal region of both ventricles and the depolarization of the RVOT. A prominent S wave in lead I is typically present in cases of congenital heart disease, valvular heart disease and cor pulmonale that cause right ventricular enlargement and fibrosis (22). Thus, we hypothesized that a deep and/or large S wave in lead I in BrS revealed a conduction delay over the RVOT and can be used to identify high-risk patients.

The purpose of this study, conducted in a large population of patients with BrS, was to verify the usefulness of the previously proposed ECG markers of SCD and to analyze the potential role of S wave in lead I as a new prognostic ECG parameter to predict VF/SCD during follow-up.

Methods

Study population. Of a study population of 655 subjects affected by BrS, we analyzed data from 347 consecutive patients (78.4% male; mean age: 45 ± 13.1 years) with spontaneous type 1 BrS ECG phenotype (coved ST segment elevation > 2mm in at least one right

precordial lead). These subjects were prospectively enrolled in four Italian 3rd level cardiology centers since 1999 (Policlinic Casilino, Rome; "Città della Salute e della Scienza" Hospital, Torino; Policlinic Sandro Pertini, Rome; Cardiology Clinic, Ospedali Riuniti Umberto I-Lancisi – Salesi, Ancona).

The study was approved by the local institutional review boards of each participating institution and each subject gave consent to participate in the study.

All subjects after enrollment were prospectively followed with periodic cardiologic visits comprehensive of rest 12-lead ECG, performed at least every year or in case of symptoms. We have not considered in this study BrS patients with history of VF/aborted SCD at presentation. Family medical history was achieved at the first clinical visit and considered positive when at least one first-degree family member died suddenly with a type 1 Brugada ECG pattern or before the age of 45 years in the absence of known heart disease. All patients underwent trans-thoracic echocardiography and Holter ECG monitoring. Genetic testing and cardiac magnetic resonance (CMR) were carried out at discretion of physicians, in line with each center clinical practice.

Electrocardiographic analysis. ECGs were recorded at a paper speed of 25 mm/s and at a standard gain of 1 mV/cm. Two independent cardiologists (CL and MA) examined and interpreted all ECG tracings by using lens and discrepancies were resolved by consensus. The ECG recorded at patient inclusion in the study was used for the analysis. The ECGs were analyzed with no patient on antiarrhythmics drugs.

Heart rate and QRS axis were manually calculated. QRS duration, PR interval, JT and QT interval were measured in II and V6 leads with calipers by physicians who were blinded to history data. A PR interval >200 ms and a QRS duration >120 ms were considered abnormal (23). Right bundle branch block (RBBB), left bundle branch block, left anterior fascicular block and left posterior fascicular block were defined in accordance with current guidelines (23). The presence of fragmented QRS, chracterized by fragmentation within the QRS

complex, with ≥4 spikes in a single lead or ≥8 spikes in leads V1, V2 and V3 (5) as well as evidence of an epsilon wave in V1 lead, were investigated. Considering that the fibrosis in patients with non-ischemic cardiomyopathy typically involves the epicardial RVOT in addition to the basal LV, the Tzou criteria (24), including V1 R>0.15mV, V6 S >0.15mV and V6 S:R> 0.2mV, were also analyzed.

The presence of an S wave in lead I, II and III was examined. Amplitude (mV) from the isoelectric line to the nadir of the S wave and duration (ms) from the beginning to the end of the S wave in lead I, II and III were measured with calipers. The area (mm^2) of the S wave was calculated by the product of the amplitude and duration.

The corrected QT (QTc) interval in lead II was calculated by Bazett's method. The corrected JT interval was obtained by subtracting the QRS duration from the QTc interval in leads II and V6 (12). The Tpeak–Tend interval in leads V2 and V6 was defined as the interval from the peak of a positive T-wave or the nadir of a negative T-wave to the end of T-wave (12). An early repolarization pattern was defined as an elevation of the J-point of at least 1 mm above the baseline level, in ≥2 consecutive leads, either as QRS slurring or notching, in the inferior (II, III, aVF) or lateral (I, aVL, and V4 to V6) leads (11).

Electrophysiological study and electroanatomical mapping. Electrophysiological study (EPS) was performed, in accordance to current guidelines (25), with a protocol including ventricular premature stimulation at the apex and at the outflow tract at two pacing cycle lengths (600 and 400 ms) with up to two or three extrastimuli. EPS was defined positive when VF leading to collapse and requiring shock was induced. Twelve patients undergoing to EPS were randomly selected for detailed electroanatomical mapping of the RV. This was performed during normal sinus rhythm with either a 4-mm-tip Navistar®, or a 3.5-mm-tip NaviStar®-ThermoCool®, or a 3.5-mm-tip NaviStar®-ThermoCool® SorroundFlow™, or a 3.5-mm-tip NaviStar®-ThermoCool® SmartTouch™ catheter (Biosense Webster Inc, Diamond Bar, CA), using the CARTO®3 EP Navigation System (Biosense Webster Inc.),

which enables the creation of maps based on different parameters simultaneously, like local activation time, bipolar signal voltage and unipolar signal voltage maps.

In the bipolar maps, tissue was defined normal when the voltage amplitude was ≥1.5 mV, scar tissue with voltage <0.5 mV, and low-voltage electroanatomical border zone with voltage >0.5 and <1.5 mV (26). In the unipolar maps, an electroanatomical normal tissue was defined when the voltage amplitude was ≥5.5 mV, scar tissue with voltage <3.5 mV, and low-voltage electroanatomical border zone with voltage >3.5 and <5.5 mV (27). Abnormal electrograms were defined as electrograms that have (1) low voltage (≤1 mV); (2) split electrograms or fractionated electrograms with multiple potentials and ≥2 distinct components, with >20 ms isoelectric segments between peaks of individual components; and (3) wide duration (>80 ms) or late potentials, with distinct potentials extending beyond the end of the QRS complex. RVOT for electroanatomical mapping is defined superiorly by the pulmonic valve and inferiorly by the RV inflow tract and the top of the tricuspid valve (28).

Automatic implantable cardioverter defibrillator (ICD) was implanted in accordance with current guidelines (25). In patients with ICD, analysis of arrhythmias and of appropriate shocks was also performed.

Classification of clinical events. BrS individuals were divided in three groups according to clinical events during follow-up: asymptomatic, syncope and VF/SCD. The group of patients who remained asymptomatic included subjects who developed syncope presumed to be of neurally-mediated origin, without documentation of ventricular arrhythmias by resting ECG and/or Holter monitoring. The group VF/SCD included subjects who experienced SCD, aborted SCD, spontaneous VF or sustained polymorphic ventricular tachycardia or with VF/fast ventricular tachycardia (>200 bpm) episodes recorded by the implanted ICD. In the group with syncope, we considered only the episodes of loss of consciousness supposed to be caused by ventricular tachyarrhytmias after exclusion of other causes, such as neurally mediated syncope (6).

Statistical Analysis. Categorical variables were summarized as frequencies and percentages and were analyzed by the chi-square test. Continuous variables were summarized as mean ± standard deviation. Differences between groups were evaluated by Student T test or Wilcoxon Runk sum, as appropriate, for continuous variables. The ANOVA test was adopted for comparison between more than 2 groups. Receiver operating characteristic (ROC) curve were drawn to identify the optimal discriminative cut-off values for variables that differed between the groups in the prediction of VF/SCD during follow-up. Curve point with the highest sum of specificity and sensitivity was labeled as the optimized cut-off point and used in the OR, sensitivity, and specificity analyses. Univariate analysis was performed to individuate predictors associated with VF/SCD. Multivariate analysis using Cox proportional hazard regression analysis was also performed to individuate independent risk factors for VF/SCD. Selection of variables for inclusion in the multivariate model was made on the basis of a stepwise (backward; P removal= 0.1) approach. The colinearity test was performed in case of predictors that could be interrelated one'another. If a strong correlation was found between variables, only one of them was chosen, on the basis of the best Akaike information criterion for inclusion of any single variable in the model.

The effect of independent risk factors on MAE during follow-up was evaluated using the log-rank test and was described using a Kaplan-Meier curve. Interobserver variability was assessed using the kappa statistic and proportion agreement. Fleiss's agreement scale was used to interpret Kappas considering values over 0.75 as excellent, 0.40 to 0.75 as fair to good, and below 0.40 as poor. A two-tailed *P*-value of <0.05 was considered to be statistically significant. Statistics were performed using R software (R-3.1.2 for Windows) and were confirmed by an indipendent statistic who used a different software (StataCorp LP,4905 Lakeway drive College Station, Texas, USA for Windows).

Results

Study population. The demographic and clinical characteristics of the study population are summarized in Table 1. Genetic testing was carried out in 107 (30.8%) subjects and showed SCN5A mutation in 32 (29.9%) cases. CMR was performed in 22 (6.3%) patients and showed structurally normal hearts, apart from 1 case of mild left ventricular hypertrophy, 1 mild hypokinesis of the RV apex and 1 case of scar in the apical segment of the inter-ventricular septum. ICD implantantion was performed in 98 patients. Eighteen patients (5.2%) had history of persistent/ paroxysmal atrial fibrillation (AF). At presentation, 14 patients had history of syncope and 18 had history of symptomatic AF. One patient was symptomatic both for syncope and AF.

During the follow-up period of 48±38.6 months, 276 (79.5%) patients remained asymptomatic, 39 (11.2%) developed syncope, and 32 (9.2%) had VF/SCD. Twenty-two patients presenting with neurally-mediated syncope were classified as asymptomatic.

Among subjects that developed VF/SCD during follow-up, 3 died suddenly, 14 suffered aborted SCD and 15 had appropriate ICD shocks due to of VF episodes. Two of these 32 patients had syncope at presentation.

Table 2 summarizes the clinical characteristics of subgroups. In particular, the patients that presented VF/SCD during follow-up had more frequently a positive EPS (P<0.0001), family history of SCD (P=0.03), and AF episodes (P<0.0001) than patients who developed syncope or remained asymptomatic (Table 2).

Electrocardiographic findings. Interobserver variability for ECGs parameters indicated a good agreement. Online-only Data Supplement Table 1 shows in details for each ECGs parameters the interobserver variability.

Electrocardiographic findings of the study population are presented in Table 3. Mean amplitude of R wave in lead V1 and S wave in lead V6 did not significantly differ among patients with VF/SCD, syncope and that remained asymptomatic during follow-up (Table3).

The prevalence of V1R >0.15 mV, V6 S >0.15 mV and V6 S/R > 0.2 mV was similar in the three groups (Table 3). Mean amplitude and duration of S wave in lead II and III were similar among patients in the different groups (Table 3).

Overall, S wave in lead I was present in 205 patients (59.1%), including all but one of those suffering VF/SCD (96.9%). Mean amplitude of S wave in lead I was higher among patients who developed VF/SCD (0.21±0.08 mV), than those who developed syncope (0.082±0.07 mV, *P<0.0001*) and remained asymptomatic (0.077±0.06 mV, *P <0.0001*) (Figure 1, panel A). Similarly, mean duration of S wave in lead I was longer in the group VF/SCD (52.8±20.1 ms), than in syncope (20.8±20.3 ms, *P*<0.001) and asymptomatic group (20.2±20.1 ms, *P*<0.001) (Figure 1, panel B). Moreover, mean amplitude-duration area of S wave was higher in patients with VF/SCD (2.9±1.7 mm2), than those with syncope (0.92±0.91 mm2) and remained asymptomatic (0.77±0.76 mm2) (*P*<0.001 (Figure 1, panel C). No relationship has been observed between the degree of ST-elevation and the S-wave characteristics in lead I.

ROC curves for amplitude, duration and amplitude-duration area of S wave in lead I were calculated to have an optimized cut-off point in the prediction of VF/SCD during follow-up. The optimized cut-off point for amplitude of S in lead I was 0.075 mV, for S wave duration was 25 ms, and for the product of depth and duration was 0.69 mm2 (Figure 2). For utilization in clinical practice, these cutoff values were approximated to ≥ 0.1mV (amplitude), ≥40 ms (duration) and ≥1 mm2 (amplitude-duration area) respectively, and were distinctly used to identify a "significant" S wave in lead I.

The S wave amplitude ≥0.1 mV, duration ≥40 ms and area ≥1 mm2 in lead I had a sensitivity of 90.6%, 96.9%, 96.9%, respectively; a specificity of 62.2%, 61.1%, 69.5%, respectively; a negative predictive value of 98.5%, 99.5% and 98.7%, respectively; a positive predictive value of 19.6%, 20.5% and 23.2% respectively; and a diagnostic accuracy of 64.8%, 65.1% and 71.5%, respectively, for VF/SCD during follow-up.

Figure 3 and 4 present some ECGs of BrS patients with and without a significant S wave in lead I.

Clinical and electrocardiographic characteristics associated with S wave in lead I. Clinical, genetic, and ECG data of patients with and without significant S wave in lead I were analyzed (Table 4). The patients with significant S wave in lead I were relatively younger and more often symptomatic for VF/SCD. ECGs parameters did not differ between groups, a part from larger QRS duration in lead V2 and II, a higher incidence of complete RBBB and first atrio-ventricular block in patients with significant S wave in lead I.

Electrophysiologic study and electroanatomical mapping. Electrophysiological study was performed in 186 patients (53.6%) and resulted positive in 77 (41.4%). RV electroanatomical maps (210±73 points) were performed in 8 patients with S wave in lead I and in 4 patients without S wave. In all patients, activation started in the lower septum and subsequently diverged toward the tricuspid annulus and RVOT. The mean endocardial activation time was significantly longer in BrS patients with S wave in lead I compared with patients without S wave (102.0±41.2 versus 51.5±31.4 ms, P <0.05). Within the group with S wave in lead I, significant delays were evident in the anterolateral RVOT, representing a line of conduction delay of 41.2±24.3 versus 8.4±3.7 ms over this region (Figure 5). Fragmented electrograms exhibiting relatively low voltage, prolonged duration, and late polyphasic potentials were significantly more present in the group of patients with S wave in lead I (7 patients vs 1 patient). These abnormal electrograms and the areas of low voltage were exclusively localized over the anterior aspect of the RVOT.

The mean voltage in the RVOT was lower in the patients with S wave (1.6±0.8 versus 3.7±1.4 mV, P <0.05), particularly in the anterolateral region (0.9±0.4 versus 3.5±1.2 mV, P <0.05). In the patients with S wave in lead I the mean area of abnormal bipolar and unipolar voltage was 4.8±3.6 cm^2 and 11.3±6.8 cm^2, respectively; whereas, in those without S wave the mean area of abnormal bipolar and unipolar voltage was 0.6±1.2 cm^2 and 3.7±6.7 cm^2,

respectively. The details of electroanatomical maps are available in the online-only Data Supplement Table 2. Figure 5 shows Electroanatomical mapping in patients with S wave and without S wave.

Clinical and electrocardiographic characteristics associated to VF/SCD. Univariate and multivariate analysis for prediction of VF/SCD during follow-up are reassumed in Table 4. A strong correlation was found between amplitude, duration and amplitude-duration area of S wave in lead I (R 0.73 for correlation between amplitude and duration of S wave in lead I, P <0.000001; R 0.88 for correlation between amplitude and amplitude-duration area of S wave in lead I, P <0.000001; R 0.77 for correlation between duration and amplitude-duration area of S wave in lead I, P <0.000001). Considering the strong correlation found between these variables, only S duration ≥40 ms was chosen, on the basis of the best Akaike information criterion for inclusion of any single variable in the model. Multivariate analysis showed that the following 2 parameters were independent risk factors for VF/SCD: S duration ≥40 ms and AF (Table 5).

The central illustration shows Kaplan-Meier analysis of freedom from FV/SCD events during follow-up in patients with S wave in lead I versus those without S wave. The former patients had a significantly worse prognosis than the others (P <0.0001). A similar trend was found for S wave in lead I with amplitude ≥0.1 mV (P <0.0001), duration ≥40 ms (P< 0.0001) and area ≥1 mm2 (P<0.0001).

Discussion

Main findings. This study has been conducted in a large population of BrS patients with a long term follow-up (48±38.6 months), to analyze the usefulness of electrocardiographic parameters as markers of SCD. The following results were observed. (1) The most powerful marker for VF/SCD was a significant S wave (≥ 0.1 mV and/or ≥40 msec) in lead I, that showed a sensitivity of 90.6 and 96.9%, a specificity of 62.2 and 61.1% with a negative

predictive value of 98.5% and 99.5%, and a positive predictive value of 19.6% and 20.5%, respectively. (2) In the multivariate regression analysis duration of S wave in lead I ≥40 msec (HR=39.1), and AF (HR=3.7) were independent predictors of VF/SCD during follow-up. (3) Electroanatomical mapping mapping in 12 patients showed that the endocardial activation time was significantly longer in BrS patients with S wave in lead I as compared to the patients without S. This difference in activation time was mostly due to a significant delay in the anterolateral RVOT.

In our study, a deep and/or large S wave in lead I resulted a predictor of malignant ventricular arrhythmias in BrS. The presence of a significant S wave in lead I could be related to a delayed activation in the RVOT. In fact, ventricular depolarization, described as QRS complex on ECG, occurs in three consecutive phases that give rise to the generation of three vectors. The third vector, generating the S wave in lead I, represents the depolarization of basal parts of the septum and RV, particularly the pulmonary conus region. A large and prominent S wave in lead I and V6 in adults is one of the diagnostic criteria for RBBB (23). However, an $S_IS_{II}S_{III}$ pattern and an $S_IR_{II}R_{III}$ pattern with a QRS < 0.12 seconds can be produced by right ventricular enlargement or zonal right ventricular block (22). Furthermore, some rare type of distal RBBB, without impairment of conduction over the main right bundle branch, can be observed in patients with tetralogy of Fallot after transatrial or transventricular repair, in cardiomyopathy with chronic lung disease and in atrial septal defect, where the stretching of Purkinje fibers and/or muscle, causes delayed activation of the RVOT. Horowitz et al. (29) found that some patients after repair of tetralogy of Fallot had RBBB caused by vertical ventriculotomy along the RVOT. In these patients, the activation delays with fragmented endocardial electrograms were restricted to the anterobasal region of the RVOT and produced wide slurred S waves in lead I and V6. In an experimental study (30), the damage to part of the right ventricular specialized conductive tissue distal to the anterior papillary muscle determined a very slight increase in QRS duration with the greatest

modification in lead I where the ventricular complex changes from a QR to an RS configuration. More recently, the vectorcardiographic analysis of patients with BrS type 1 showed peculiar characteristics in comparison with healthy individuals with incomplete and complete RBBB (31). This study clearly demonstrated that the wide S wave in left lead does not indicate a typical RBBB morphology but represents a right end conduction delay in the RVOT. Thus, it can be hypothesized that the localization and the amount of delayed activation in larger or small mass of ventricular tissue in the RVOT could be related to the area of S wave in lead I and produce variable morphology and the presence or absence of RBBB in these patients.

Role of right ventricular conduction delay in BrS. The depolarization hypothesis as arrhythmogenic substrate of BrS is supported by a number of histologic, imaging, ECG and electrophysiological observations. In 1989, Martini et al. (32) described the presence of histopathological changes in patients with resuscitated VF, apparent absence of heart disease, and an ECG pattern reminiscent of BrS. Some years later, Corrado et al. (13) found 14% of SCD in type I BrS population and all these patients had arrhythmogenic right ventricular dysplasia except one without evidence of structural heart disease, suggesting an overlap between BrS and ARVC. Coronel et al. (14) published a combined electrophysiological, genetic, histopathologic, and computational study of a patient with clinical evidence of BrS who underwent heart transplantation for incessant VF. In this patient, conduction slowing based on interstitial fibrosis caused the ECG signs and was the origin of VF. A subsequent study confirmed the presence of concealed structural abnormalities by endomyocardial biopsy in BrS patients (33). It has been suggested that the cause of these myocardial structural abnormalities such as severe reactive fibrosis and altered gap junction proteins expression, can be related to a reduced SCN5A expression (34). Recently, it has been observed that BrS is associated with epicardial surface and interstitial fibrosis and reduced gap junction expression in the RVOT (35). Furthermore, Zhang et al. (36) using noninvasive ECG imaging showed

that slow discontinuous conduction and steep dispersion of repolarization were present in the right ventricular outflow tract of BrS patients, whereas the control group with only right bundle-branch block had delayed activation in the entire right ventricle, without ST-segment elevation, fractionation, or repolarization abnormalities on electrograms.

Several studies, using ECG, late potentials, and electrophysiologic mapping, have reported depolarization abnormalities and conduction delay in patients with BrS (3,5-10,13-21,35,36). First degree atrio-ventricular block has been associated with SCD or ICD appropriate therapies in BrS (6). Prolonged QRS duration in the precordial leads and fragmented QRS have been shown to be markers for future major cardiac events (3,5-8), even if a prolonged QRS duration has not been found to be of prognostic value in a recent review (37). Moreover, epsilon-like waves were observed in about 10% of BrS patients. Finally, abnormal late potentials were found in BrS patients and their presence seems to indicate increased arrhythmic risk (9).

Delayed activation at the RVOT was reported on endocardial and epicardial mapping (13-21,35). Nagase et al. (15) found the presence of late potentials on the signal-averaged ECG and demonstrated the correlation between these late potentials and delayed abnormal electrograms in the epicardium of the RVOT. Postema et al. (16) observed that BrS is characterized by wide and fractionated electrograms at the RV endocardium. The same group - by using ECG, vectorcardiogram and body surface potential map - confirmed that the dominant pathophysiologic mechanism for type-1 ECG is related to local depolarization abnormalities in the RV (17). Lambiase et al. (18), by high-density mapping in BrS patients, demonstrated that zones of significant regional delays were present in the anterolateral free wall of the RVOT and these areas were critical in VF initiation. More recently, it has been observed that the conduction delay in RV was significantly larger in patients with documented VF than those with syncope and without any symptoms (19) and the induction of VF depended on the severity of the depolarization abnormality (20). The role of these areas of

slow conduction as key marker of SCD in BrS is reinforced by the observation that electroanatomic map of RV in BrS patients with recurrent VF episodes showed a prominent delayed depolarization with low-voltage and fractionated electrograms (21,35). These electrograms were exclusively present over the anterior epicardial region of the RVOT and their ablation determined prevention of VF and in the major part of the patients the disappearance of the Brugada ECG pattern (21,35).

In our study, electroanatomical mapping showed that the endocardial activation time (mostly due to a significant delay in the anterolateral RVOT) was significantly longer in patients with S wave in lead I when compared to those without S. Fragmented electrograms exhibiting relatively low voltage, prolonged duration, and late polyphasic potentials were significantly more present in the group with S wave in lead I.

Conclusions. In the last decade, several markers have been proposed for the risk stratification of BrS. The "depolarization theory" has been reinforced by our observations that highlight the role of right ventricular conduction delay in this syndrome. The presence of a wide and/or large S wave in lead I, due to delayed activation in the RVOT, showed to be a powerful predictor of life-threatening ventricular arrhythmias. This substrate could favor reentrant ventricular tachyarrhythmias and can be used as a potential novel marker of SCD risk stratification in BrS patients.

Study Limitations. Our study presents some limitations. First, we have analyzed CMR imaging in only 22/346 patients (6.3%) and endocardial electroanatomic mapping in only 12/346 patients (3.5%). Some investigations (14,16,18) reported endocardial abnormal electrograms, whereas other (5,15,19,21,35) found areas of slow conduction only over the RVOT epicardium. In future studies, it could be very important to obtain CMR imaging with a more detailed characterization of fibrosis such as T1 mapping and to perform high density epicardial and endocardial electroanatomic mapping with the aim to better understand the substrate in BrS, the role of right ventricular conduction delay and the relationship with ECG.

Second, several prognostic parameters (including markers of conduction delay) have been proposed over the years in BrS. However, none of them proved to be useful in larger studies. This could also be the case for the S-wave in lead I. Therefore an independent confirmation cohort is necessary to confirm the value of the current study. Noteworthy, since the cut-points of the S wave in lead I were identified and evaluated on the same data set, they will require validation in a separate sample of healthy subjects.

Third, a potential limitation is that the ECG analysis could be influenced by the orientation of the RVOT since it could change the terminal vector of the QRS, particularly in some cases with significant deviation.

Another limitation is that our centers are arrhythmologic institutions. Therefore, we cannot exclude some form of selection bias like referral of patients with higher risk at baseline to our centers.

Finally, EPS and genetic screening was performed in only 54% and 31% of the included individuals, respectively. The group with VF/SCD had a lower prevalence of SCN5A mutation (17%) and more EPS inducibility (65%). In BrS patients with aborted SCD, Eckardt et al. (4) reported 12% of SCN5A mutation and 62% of VF induction was 62%. However, we have to bear in mind that only 25-30% of patients with BrS have a known genotype, implying that additional still not identified genes may be linked to this disease. Probably, BrS is a disease of connexome (35,38) and the genes involved can include structures such as desmosomes, gap junctions and sodium channel complex. Therefore, the relationship between the ECG, the genes, and the EP inducibility should be revisited in the next future.

Perspectives

Competency in Medical Knowledge: A number of electrocardiographic (ECG) markers of ventricular depolarization and repolarization have been reported to identify high-risk patients with Brugada syndrome. The presence of a wide and/or large S wave in lead I, a simple ECG marker, due to delayed activation in the right ventricle outflow tract, could be a powerful predictor of life-threatening ventricular arrhythmias

Translational Outlook: The presence of a wide and/or large S wave in lead I in Brugada syndrome, expression of a delayed activation in the right ventricle outflow tract, can be used as a potential novel marker of sudden cardiac death risk stratification

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Figure legends

Figure 1. Distribution of S wave in lead I in subgroups of patients with VF/SCD, syncope and asymptomatic. **Panel A:** Amplitude of S wave in lead I. **Panel B:** Duration of S wave in lead I. **Panel C:** Product of amplitude and duration of S wave in lead I. The box width is proportional to the square-root of the number of observations in the group. The bottom and top of the box represent the first and the third quartiles; the band inside the box represents the median value; the ends of the whiskers represent respectively the lowest datum within 1.5 inter quartile range of the lower quartile and the highest datum within 1.5 inter quartile range of the upper quartile. Outliers are presented as small circles.

Figure 2. Panel A: Receiver operating characteristic (ROC) curve for amplitude of S wave in lead I**. Panel B:** ROC curve for duration of S wave in lead I. **Panel C:** ROC curve for the product of amplitude and duration of S wave in lead I.

Figure 3. ECGs of BrS patients showing spontaneous coved-type pattern and a significant S wave in lead I.

Figure 4. ECGs of BrS patients showing spontaneous coved-type pattern without a significant S wave in lead I.

Figure 5. Electroanatomical mapping using CARTO. Antero-lateral view of activation, voltage and some electrograms in a patient with significant S wave in lead I **(Panel A, B, C)** and without S wave **(Panel D, E, F)**. **Panel A and D** show a single QRS at a paper speed of 200 mm/sec, including the 12 surface leads (the arrow indicates the S-wave in lead I in panel

A). The last 3 channels show the local QRS as recorded with the mapping catheter (unipolar recording in blue and distal and proximal bipolar recording in yellow). **Panels B and E** show the activation time in a patient with and a patient without S-wave (B and E, respectively). On the activation map, a colour-coded scale from red to purple represents earliest-to-latest activation, **Panels C and F** show the voltage map in a patient with and a patient without S-wave (B and E, respectively). On the voltage map, purple represents normal endocardium (amplitude ≥1.5 mV); red, dense scar (amplitude ≤0.5 mV); and range between purple and red, border zone (signal amplitudes between 0.5 and 1.5 mV). The patient with significant S wave showed over the antero-lateral region of RVOT abnormal electrograms, late activation displayed in purple in the LAT map and low voltage, as displayed in red in the bipolar-voltage map.

Central illustration. Kaplan-Meier analysis of freedom from VF/SCD events during follow-up in patients with S wave in lead I versus those without S wave in lead I.