ST-Segment Elevation and Fractionated Electrograms in Brugada Syndrome Patients Arise from the Same Structurally Abnormal Subepicardial RVOT Area but Have a Different Mechanism

Running title: ten Sande et al.; Pathological basis of ST-segment elevation in BrS

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Abstract:

**Background** - Brugada syndrome (BrS) is characterized by a typical ECG pattern. We aimed to determine the pathophysiologic basis of the ST-segment in the BrS-ECG with data from various epicardial and endocardial right ventricular activation mapping procedures in 6 BrS patients and 5 non-BrS controls.

**Methods and Results** - In 7 patients (2 BrS, 5 controls) with atrial fibrillation an epicardial 8x6 electrode grid electrode (inter-electrode distance 1mm) was placed epicardially on the RV outflow tract (RVOT) prior to Video Assisted Thoracoscopic Surgical Pulmonary Vein Isolation (VATS-PVI). In two other BrS patients endocardial, epicardial RV (CARTO) and body surface mapping (BSM) was performed. In two additional BrS patients we performed decremental pre-excitation of the RVOT prior to endocardial RV mapping. During VATS-PVI and CARTO mapping, BrS patients (n=4) showed greater activation delay and more fractionated electrograms in the RVOT region than controls. Ajmaline administration increased the region with fractionated electrograms as well as ST-segment elevation. Pre-excitation of the RVOT (n=2) resulted in ECGs that supported the current to load mismatch hypothesis for ST-segment elevation. BSM mapping showed that the area with ST-segment elevation, anatomically correlated with the area of fractionated electrograms and activation delay at the RVOT epicardium.

**Conclusions** - ST-segment elevation and epicardial fractionation/conduction delay in BrS patients are most likely related to the same structural subepicardial abnormalities, but the mechanism is different. ST-segment elevation may be caused by current-to-load mismatch, whereas fractionated electrograms and conduction delay are expected to be caused by discontinuous conduction in the same area with abnormal myocardium.

**Key words:** Brugada syndrome, electrophysiology, mapping, activation delay
Introduction

Brugada syndrome (BrS) is associated with a familial occurrence of sudden cardiac death (SCD) by ventricular tachycardia and/or ventricular fibrillation (VT/VF). BrS is diagnosed when the typical ECG pattern (BrS-ECG i.e., coved ST-segment elevation followed by a negative T wave) occurs in at least one right precordial lead positioned at the 2nd, 3rd or 4th intercostal space either spontaneously or after provocation by intravenous administration of class I antiarrhythmic drugs. Occurrence of this BrS-ECG can wax and wane, and often precedes onset of VT/VF.

In about 20-30% of patients, a mutation in the gene encoding the sodium channel, the L-type Ca2+-channel or the channel carrying the transient outward current (Ito) is found. Despite earlier studies in which BrS has been described in patients with structurally normal hearts, there is now increasing evidence that subtle structural abnormalities exist in the right ventricle outflow tract (RVOT) of BrS patients and that these may impair impulse propagation and could act as an arrhythmogenic substrate. The pathophysiologic basis of BrS remains incompletely understood. Three hypotheses, each with a specific mechanistic basis, have been proposed to explain the BrS-ECG: (1) repolarization disorder hypothesis, based on transmural dispersion in repolarization of the right ventricle/right ventricular outflow tract (RV/RVOT); (2) depolarization disorder hypothesis, based on activation delay in the RV/RVOT subepicardium; (3) electrotonic current hypothesis, based on activation block at sites of current-to-load mismatch in the RV/RVOT subepicardium or combinations of the above. Some animal models which include monophasic action potential recordings at the epicardium and endocardium, and clinical studies in Brugada syndrome patients give some support for the repolarization disorder hypothesis, other clinical studies provide evidence in favor of the depolarization disorder hypothesis, in particular, the recording of fractionated and late potentials. These recordings
imply structurally abnormal myocardium in the RV/RVOT region and, as such, also support the electrotonic current hypothesis. Current-to-load mismatch may occur in areas with isthmus sites and at sites of abrupt expansion of myocardial bundles, e.g., where myocardial tissue is intermingled with collagen fibers or adipose tissue. Such structural abnormalities have been found in the RV/RVOT region of the hearts of Brugada syndrome patients. We have demonstrated that the BrS-ECG could only be reproduced when both the structural abnormalities in the RV/RVOT and either a reduced sodium or calcium current or an increased transient outward current were present. These findings support the concept that structural abnormalities together with modulated ion currents play an important role in the pathomechanism of BrS. Because both the depolarization disorder hypothesis and the electrotonic current hypothesis require an abnormal myocardial substrate, we proposed that 1) the myocardial areas which exhibit local ST-elevation coincide with areas of structural changes and fractionated electrograms, 2) the size of the myocardial area with fractionated electrograms is augmented by sodium channel blockade, 3) myocardial areas of BrS patients may contain fractionated electrograms even at times they have no BrS-ECG, 4) ST-elevation extends beyond the duration of fractionated electrograms and 5) ablation of the tissue with fractionated electrograms also attenuates ST-segment elevation because both phenomena occur in the same structurally abnormal area.

We therefore, 1) recorded conduction delay and fractionated electrograms at the epicardial RV/RVOT of patients undergoing a VATS procedure for AF, 2) performed epicardial and endocardial mapping of the RV/RVOT and epicardial ablation of regions with marked fractionated electrograms, 3) administered the sodium channel blocker ajmaline to modulate ST-segment elevation, 4) performed premature stimulation of the RV/RVOT at different intervals...
after onset QRS during sinus rhythm to shift ST-segment elevation. Because fractionation/conduction delay and ST-segment elevation presumably are caused by the same substrate, we further hypothesize that there is an anatomic correlation between the epicardial areas of delayed RV/RVOT activation and/or fractionated electrograms and ST-segment elevation on the body surface map (BSM).

Methods

This study was performed in accordance with the Declaration of Helsinki and written informed consent was obtained from all patients.

Three studies were performed.

Study 1 Multiple epicardial RVOT unipolar electrograms were simultaneously acquired to assess the presence of fractionated electrograms, their related pathways and activation delay in BrS patients and control patients who underwent VATS-PVI (proposal 1,3,4).

Study 2 Endocardial and epicardial mapping and RVOT ablation was performed in two BrS patients to evaluate the effect of ablation of epicardial areas with fractionated electrograms on ST-segment elevation and to correlate epicardial fractionation with ST-segment elevation, before and after infusion of ajmaline (proposal 2-5).

Study 3 Premature stimulation of the RVOT at different times after onset of QRS during sinus rhythm (SR) was performed in two other BrS patients in the course of diagnostic electrophysiological evaluation. With this method we advanced the ST-segment elevation, allowing us to distinguish between the electrotonic current hypothesis and repolarization disorder hypothesis for ST-segment elevation (proposal 3-5).

Procedures

BrS was diagnosed using the consensus criteria\(^3\). Controls were non-BrS who underwent VATS-
PVI for AF\textsuperscript{27}. Signals were analyzed using a custom made program\textsuperscript{28} based on MATLAB (The MathWorks, Inc., Natick, MA, USA) or CARTO 3 Navigation System (Biosense Webster Inc., Diamond Bar, CA, USA). Unipolar electrograms of poor quality (noise, movement artifacts) were excluded. Cardiac magnetic resonance (CMR) was performed in all BrS patients without implantable cardioverter defibrillator (ICD) or computerized tomography (CT) scan in those with ICD.

**Study 1. Epicardial RVOT mapping during VATS-PVI**

VATS-PVI was performed under general anesthesia. Prior to PVI, and after introduction of the various trocars, a custom made 8x6 electrode grid (interelectrode distance 1mm) was placed under visual guidance on the RVOT to obtain epicardial unipolar electrograms. A 256-channel mapping system (BioSemi, ActiveTwo, Amsterdam, The Netherlands, 24 bit dynamic range, 122.07 nV LSB, total noise 0.5 μV) was used with a sampling frequency of 2048 Hz (bandwidth DC - 400 Hz (-3dB)). An indifferent reference electrode was inserted in the skin at one of the incision sites. Activation time (AT) was defined as the point of maximal negative \( \frac{dV}{dt} \) (\( \leq -0.1 \) V/sec) of the initial deflection of the unipolar electrogram. Total activation duration (TAD) of the myocardium underlying the grid was defined as the interval between the earliest and latest activation in milliseconds (ms). To distinguish local from remote deflections, the timing of the maximal negative \( \frac{dV}{dt} \) of each deflection was compared with the Laplacian transformation at that same time \textsuperscript{29}. An electrogram was considered to be fractionated if multiple deflections with \( \frac{dV}{dt} \leq -0.01 \) V/s corresponded to Laplacian deflections. Every local electrogram was manually investigated for fractionation. Fractionation was quantified as the mean number of deflections per electrode per grid. Fractionation duration was measured as the interval between the first and last deflection in ms of a fractionated complex.
Study 2. CARTO mapping of endocardial and epicardial right ventricle for epicardial ablation

Antiarrhythmic drugs were withheld for at least five half-lives before the electrophysiology study. Prior to the invasive procedure BSM using a 64-electrode system was obtained and a CT scan was made to obtain a 3D heart-thorax reconstruction. Electro-anatomical endocardial and epicardial mapping of the RV was obtained during sinus rhythm with CARTO 3 and a 3.5 mm tip quadripolar ThermoCool SmartTouch mapping catheter (Biosense Webster Inc.) as described earlier\textsuperscript{12}. The same activation and fractionation criteria were applied as in study 1. Repolarization time (RT) was defined as the point of maximal positive dV/dt in the T wave of the unipolar electrogram\textsuperscript{30, 31}. The activation recovery interval (ARI), a surrogate for action potential duration, was the interval between AT and RT. To avoid interference by remote signals, bipolar signals obtained from the mapping catheter (signal at the tip – signal from the first ring electrode) were also analyzed. First, endocardial mapping was performed. Thereafter, pericardial puncture was performed according to Sosa et al.\textsuperscript{32} and baseline epicardial mapping was performed. Sites displaying fractionated electrograms were marked on the CARTO map. Subsequently, ajmaline was administered i.v. and epicardial mapping was repeated. Guided by the location of fractionated electrograms, radiofrequency ablation of these sites was performed. Radiofrequency ablations were performed at a power from 30 to 40 W, with the maximum temperature set at 43°C. After ablation of abnormal (fractionated) sites, the epicardial RV was remapped and the absence of abnormal electrograms was used as endpoint for the ablation.

Study 3. Endocardial CARTO 3 mapping of RV and stimulation of RV/RVOT

In 2 patients undergoing diagnostic electrophysiological assessment for risk stratification for SCD in BrS, a programmed electrical stimulation protocol was performed including premature
stimulation of the endocardial RV/RVOT region. A decapolar catheter was placed in the coronary sinus for atrial stimulation. The mapping catheter was placed in the RVOT for ventricular stimulation and was performed at different intervals after the onset of the QRS complex during SR. Following a drive train of 8 atrial stimuli at a cycle length (CL) of 800 ms, a single ventricular extrastimulus – initially timed at the end of the last atrially stimulated and atrioventricular conducted QRS complex - was delivered. The ventricular coupling interval was decreased in subsequent sequences with 10 ms intervals, allowing progressive pre-excitation of the RVOT. This was repeated until fusion between AV conducted ventricular activation and RVOT paced ventricular activation was no longer present and the last QRS complex was fully paced from the ventricular myocardium.

Results

Eleven individuals were studied (Table 1); six BrS patients and five controls. Age at time of studies was 45±16 years in BrS patients and 57±9 years in control patients. Five of six BrS patients were symptomatic, including two with an ICD for secondary prevention (documented VT/VF). Non-invasive cardiac imaging of the BrS patients (CMR n=4, CT scan n=2) revealed no overt structural abnormalities, in particular, no signs of myocardial fibrosis on CMR. In two patients a mutation in SCN5A was identified (c.2635T>C and c.3228+2delT).

Study 1: Epicardial right ventricle mapping prior to VATS-PVI

In seven patients (BrS n=2, controls n=5) unipolar ventricular electrograms were recorded during SR (n=3) or AF (n=4). In all patients, no ST-segment elevation on the ECG was seen on the day of the procedure. Patient 1 was admitted after a VF episode accompanied by a spontaneous BrS-ECG during fever. Patient 2 had a drug induced BrS-ECG. BrS patients had longer TAD than controls (21 vs. 9 ms, Table 2), more extensive fractionation (number of deflections 4.5 vs. 2),
and longer fractionation duration (48 vs. 8 ms). Heart rate during the procedure (cycle length 560 to 1200 ms) was unrelated to TAD or fractionation. One control (no. 3 in Table 2) showed long fractionation duration compared to the other controls, although it was shorter than in the BrS patients and not associated with a longer TAD. Both BrS patients revealed clear late potentials; these were not seen in controls (Fig.1).

**Study 2: CARTO mapping of endocardial and epicardial right ventricle for epicardial ablation**

In two patients (no. 3 and 4, Table 1), endocardial and epicardial RV mapping was performed (Fig. 2; epicardial activation (panel A) and fractionation (panel B) map at baseline and during ajmaline administration) prior to the mapping and ablation procedures. Patient 3 was diagnosed with BrS after an in hospital cardiac arrest based on VF and a type I BrS-ECG. In this patient a mutation in the SCN5A gene was found. A type 1 BrS-ECG was present at the beginning of the procedure. This patient had experienced several VT/VF episodes with multiple ICD shocks per year. Body surface voltage map showed the tallest ST-segment elevation at electrode E3, which was located immediately over the RVOT (Fig. 3, circled electrogram in panel C). The electrophysiology study was performed under general anesthesia with rocuronium, propofol and sufentanil. Figure 4B (left) shows the patients’ ECGs with type 1 BrS-ECG during baseline, at maximal ajmaline level and after epicardial ablation. Ajmaline increased ST-segment elevation markedly. After epicardial ablation of the area with fractionated electrograms, typical ST-segment elevation almost disappeared as compared to the ST-segment elevation prior to ajmaline administration. One month after epicardial ablation, ST-segment elevation was still absent. During CARTO mapping, the RV endocardial unipolar voltage map showed no abnormalities (data not shown) and endocardial RV electrograms showed only a small area of fractionated
signals. Endocardial ARIs were 395±13 ms on average at the RV free wall and 370±6 ms at the RVOT. In contrast, epicardial RV mapping showed an area of low voltage and fractionated signals extending from the subpulmonary area to mid wall RV (Fig. 2B, left panel). Epicardial ARIs were 360±12 ms at the RV free wall and 310±11 ms at the RVOT. Administration of ajmaline increased the area with fractionated electrograms (Fig. 2B, right panel). After epicardial ablation, ventricular arrhythmias occurred less frequently, although, the patient experienced appropriate ICD shocks six weeks after ablation, after which quinidine therapy was reinstated.

Patient 4 had 1 mV ST-segment elevation in the right precordial leads at baseline and BrS was diagnosed after episodes of polymorphic VT and a type 1 BrS-ECG after ajmaline administration (he had type 2 BrS-ECG at baseline). The patient also had coronary artery disease. The electrophysiology study was performed under general anesthesia with sufentanil and thiopental. The effects of ajmaline administration and epicardial ablation on ST-segment elevation was less prominent than in patient 1 (Fig. 4B, right panel). There was a moderate increase in ST-segment elevation with ajmaline and ST-elevation was slightly lower after ablation. The endocardial unipolar electrograms of the RVOT showed no fractionated signals (data not shown). Epicardial mapping showed fractionated electrograms at the superior, mid, and posterior site of the RVOT. ARIs were 310±18 ms on average at the epicardial RVOT and 405±9 ms at the epicardial RV free wall. After ajmaline administration, the area with abnormal electrograms expanded towards the anterior and inferior RVOT. This area was additionally marked and successfully ablated. After epicardial ablation of the fractionated signals, late potentials and fractionation diminished (Fig. 4A). Following the procedure the patient remained free of ventricular arrhythmias during 12 months of follow-up. Ultimate fractionated electrograms were 270 ms and 220 ms (patient 3 and 4 respectively) after onset QRS, and
occurred far before ST-segment elevation ended.

To reveal changes in V1 caused by ajmaline, we subtracted the ECG during baseline from the ECG during peak ajmaline (Fig.4C) in both patients. This procedure revealed that, after the deflection that exposes the QRS difference, a pulse shaped deflection arises with a width of 220 to 310 ms. This indicates that the effect of ajmaline on the ST-segment operates over this time interval. The pulse shaped pattern mimics the configuration of an action potential as expected for an electrotonic component. The arrow in the signal marks the time of the latest epicardial late potential.

**Study 3: Pre-excitation of the RVOT**

In two BrS patients (no. 5 and 6) pre-excitation of the RVOT was performed during a diagnostic electrophysiologic study. Panel A of Figure 5 shows leads V1 during SR alone and during SR and RVOT pre-stimulation at different delays after onset QRS (numbers indicate the delay in ms). Note that there is ST-segment elevation and a negative T-wave in both the basic and premature activations. Sharp deflections in the signals are the stimulus artefacts (indicated by numbers of measured coupling intervals). Panel B shows the subtraction of the electrograms in V1 during SR with stimulation and the SR electrogram in V1 alone (matched at onset QRS). Striking in all these differential signals is the pattern-wave that follows directly after the stimulus artefact. The width of the wave (arrows) becomes wider with increasing prematurity of the stimulus after onset QRS. By premature stimulation of the RVOT, ST-segment elevation (irrespective of its mechanism) occurs earlier in time and partly within the QRS complex, but is not removed as is evident from Figure 5. Premature stimulation of the RVOT region therefore only slightly altered the QRS configuration. As a result, the difference of a stimulated and a SR complex is mainly the difference of the ST-segment elevated signal during SR and virtually the
same signal during stimulation but now shifted in time. The resulting pulse shaped signal increases in width with prematurity of stimulation and is compatible with our hypothesis that ST-segment elevation is caused by an electrotonic signal due to current to load mismatch (further explanation in supplement, Fig.1). In both patients the ST-segment morphology of the RVOT pre-excited complex changed in a similar fashion.

Discussion

This study shows that ST-segment elevation and fractionated electrograms/activation delay in BrS patients arise both from the same (structurally abnormal) subepicardium of the RV/RVOT region (Fig 6). Study 1 shows that local electrograms in the RVOT are more fractionated in BrS patients than in controls. However, they may also be present in control patients, but are not related to ST-segment elevation (neither in control, nor in BrS patients). Study 2 demonstrates that ST-elevation in one patient disappeared when areas with fractionated local electrograms were ablated. The area of fractionation increased after sodium channel blockade. In the other patient, the typical BrS-ECG was absent despite extensive fractionation. Study 3 shows that the difference of a pre-stimulated and a SR complex is mainly the difference of the ST-segment elevated signal during SR and virtually the same signal during stimulation, but now shifted in time. The resulting difference wave (fig.5B) increases in width with prematurity of stimulation. This is consistent with our hypothesis that ST-segment elevation is caused by an electrotonic signal due to current to load mismatch. This study with various mapping and stimulation protocols shows that BrS patients have longer activation delay, more and longer fractionated epicardial electrograms and late potentials in the RV/RVOT compared to controls and that local fractionation is not solely responsible for the ST-elevation in the BrS-ECG. We additionally show that the area with ST-segment elevation on the BSM-ECG corresponds with the area of
fractionated epicardial electrograms in the RV/RVOT.

The mechanism causing the typical ST-segment elevation in the right precordial leads in BrS patients has still not completely been elucidated. Three hypotheses have been proposed. The repolarization disorder hypothesis is based on transmural voltage gradients caused by heterogeneity in action potential duration between the RV epicardium and endocardium, resulting in dispersion of repolarization measured in canine wedge preparations. According to the depolarization disorder hypothesis late activation of the RVOT is the underlying mechanism. The electrotonic current hypothesis explains ST elevation by electrotonic currents caused by current to load mismatch in a structurally abnormal subepicardium of the RV/RVOT area. There is growing evidence that mild structural abnormalities in BrS patients, that are not detectable on conventional imaging modalities, result in discontinuous conduction. In our study population, BrS patients showed clear fractionated electrograms at the RVOT epicardium compared to controls, which indicate discontinuous electrical impulse conduction that occurs if patchy fibrosis is present in the myocardium. Supplemental Figure 1 demonstrates the different hypotheses on cellular level. The first column (action potentials) shows 3 combinations of schematic epicardial and endocardial action potentials that could cause an electrotonic component for ST-segment elevation. The upper left panel shows an endocardial action potential only. Activation toward the epicardium is blocked due to current to-load-mismatch in the structurally abnormal subepicardium (electrotonic current hypothesis). The left middle and lower panels show a gradient in APD from endocardium to epicardium and a strong spike and dome configuration for the epicardial action potential respectively (repolarization disorder hypothesis). The middle column displays the configuration of the electrotonic signals that are generated due to the difference in epi- and endocardial action.
potentials as illustrated at the left. The signals are low pass filtered (3dB at 60Hz), to cope with
their electrotonic feature. The third column shows the difference between the electrotonic
component and its time shifted equivalent for the 3 electrotonic components. The electrotonic
components are shifted over 15, 30 and 45 units (ms), simulating premature stimulation at
different coupling intervals. The pulse shaped signals in the upper right panel best correspond
with the recorded signals from Figure 5. Signals in the middle right panel start later and are more
sinusoidal, whereas signals in the lower right panel have a biphasic pulse shaped form. The
figure illustrates that results obtained during premature stimulation at different coupling intervals
(Fig.5, top row of Supplemental Fig.1) fit best with the electrotonic current hypothesis.

Figure 4 shows that if the ajmaline level increases, the ST-segment elevation increases.
We expect that this occurs because ajmaline increases the number of sites with conduction
block26. Subtracting electrograms recorded during different ajmaline levels gives the change in
the signal caused by the increase in the ajmaline concentration. Figure 4C shows that subtraction
reveals a wide signal compatible with an electrotonic component generated by the duration of the
action potential in the activated area. If the electrotonic current was based on a shorter APD at
the epicardium compared to the endocardium, electrotonic current would only flow during the
time the difference in APD is present and thus during a shorter period. In addition this would
occur later after depolarization. A role for activation delay as a single mechanism for ST segment
elevation is also unlikely, because late potentials are not present at the end of the pulse signal as
Figure 4C illustrates. In addition, late potentials with a delay > 160 ms were present in only
0.04% of the electrograms with fractionation. When the substrate was modulated by catheter
ablation the ST-segment elevation diminished corroborating the study performed by Nademanee
et al12. Nademanee et al. previously identified low voltage areas with fractionated electrograms
and severe activation delay at the anterior epicardial aspect of the RVOT, and diminishing of preexistent ST-segment elevation after ablation of this area. Recently, Szél et al.\textsuperscript{34} also observed fractionated electrograms in the RV epicardium, but they suggested that this is due to a heterogeneous epicardial loss of dome and local re-excitation via a concealed phase 2 re-entry, challenging abnormal depolarization or structural abnormalities as a mechanism. However, the data in our patients suggest differently. Fractionated signals were clearly associated with diastolic potentials in the BrS patients undergoing VATS-PVI (see Fig.1), whereas diastolic potentials were not observed in control patients except 1 (control 3). This is not surprising because structural subepicardial abnormalities have been observed in the RVOT of healthy pig hearts. To lead to ST-elevation, however, additional electrophysiological changes, e.g. reduced sodium current, are necessary.

Normally, the RV/RVOT area is activated relatively late during sinus rhythm. The effect of alteration of activation of the endocardial RVOT on ST-segment elevation was investigated by premature RVOT stimulation in two BrS patients. Pre-excitation of the right ventricular apex was described earlier by Chiale et al.\textsuperscript{35} Their study describes a maneuver to unmask the BrS-ECG by pre-excitation of the RV apex when a right bundle branch block (RBBB) pattern is present on the surface ECG. Our study followed a different line of reasoning: instead of unmasking ST-segment elevation, we sought to study whether ST-segment elevation in patients without intraventricular conduction block could be caused by current to load mismatch of the RVOT. To test this, we advanced activation of the RVOT by pacing the RVOT instead of the RV apex. An electrotonic component that would cause ST-segment elevation must also start late after onset QRS. If, however, the RV/RVOT area during sinus rhythm is activated prematurely by stimulation, the electrotonic current will start earlier as well. Indeed the electrotonic current
(elevation of the signal) started directly after the stimulus at every coupling interval (Fig.5). To reveal the electrotonic component (and discard the activation component), the electrogram during sinus rhythm was subtracted from the stimulated one. By doing so, mainly the difference between the electrotonic components during SR alone and of SR with stimulation remains (small additional changes may be present due to altered activation in RV). The earlier the RV/RVOT is stimulated after onset of QRS of the sinus beat, the earlier the electrotonic component arises and the wider the component will be (Fig.5). Although subtle structural abnormalities have been described in hearts of BrS patients, during postmortem analyses\textsuperscript{9,11} or in explanted hearts\textsuperscript{10,16}, we could not document these in our patients. The standard imaging techniques may not have been sensitive enough to detect these subtle changes in RVOT architecture. However, based on the available data the presence of subclinical structural changes in these patients is likely\textsuperscript{11}. In addition, ablation aimed at anatomically abnormal tissue was effective as a therapy.

**Methodological considerations and limitations**

The number of patients is small. However, we used three different types of experiments in 2 of which the patients served as their own control (study 2 and 3) and these studies point to a same mechanism for the electrophysiological characteristics of Brugada syndrome. Analysis of the electrograms of the epicardial grid was performed during spontaneous SR or conducted AF and heart rate may have influenced the TAD. However, we did not observe differences in AT between patient with AF or SR and with different cycle lengths.

**Conclusion**

ST-segment elevation and fractionated electrograms/activation delay in BrS patients are most likely related to the (structurally abnormal) subepicardium of the RV/RVOT region. Such abnormalities may cause conduction block due to current-to-load mismatch at tissue...
discontinuities resulting in ST-segment elevation. Our currently presented data support this hypothesis. The same structural abnormalities may cause fractionated electrograms and conduction delay if excitability is appropriate. These electrophysiological parameters can be modulated by sodium channel blockers like ajmaline.

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Conflict of Interest Disclosures: Dr. De Groot is supported by a personal VIDI grant from NWO/ZonMw 016.146.310. Dr. Tan is supported by a personal VICI grant from NWO/ZonMw 918.86.616.

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Table 1: Patient characteristics

<table>
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**Table 2:** Electrophysiological parameters

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<th>Rhythm and CL in ms</th>
<th>AT in ms</th>
<th>No. of deflections</th>
<th>Fractionation duration in ms</th>
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<tbody>
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<td>1. BrS</td>
<td>SR, 660</td>
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<td>5</td>
<td>31</td>
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<td>2. BrS</td>
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<td>4</td>
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<td>25</td>
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<td>4. Control</td>
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<td>5. Control</td>
<td>AF, 600</td>
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<td>6. Control</td>
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<td>3</td>
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<td>7. Control</td>
<td>SR, 1200</td>
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AF: atrial fibrillation, AT: activation time, BrS: Brugada syndrome, CL: average cycle length, ms: milliseconds, SR: sinus rhythm
Figure Legends:

**Figure 1:** Activation maps with corresponding electrograms obtained by the 8x6 grid during VATS-PVI. Two examples of activation maps (control and BrS patient). Colors correspond with activation time in milliseconds with regard to earliest activation within the grid. Red is earliest and blue latest activation. Asterisks are locations of the 8x6 electrode grid where unipolar electrograms at the left were recorded prior to PVI. Panel A: Activation map of a control patient (middle panel). Purple dots in tracings correspond with the steepest negative dV/dt. Deflections prior and post the initial activation are remote as shown by the second activation map where no propagation is seen (and therefore representing remote activity). Panel B: Color map of the propagation of the main activation (middle panel) and of the late potentials (right panel) seen in a BrS patient. mV: millivolts. Purple dots in tracings correspond with the steepest negative dV/dt whereas red dots are the late potentials after initial activation. The secondary deflections propagate and therefore represent local activity.

**Figure 2:** Epicardial RV CARTO map of patient no. 3 displaying activation (upper panel) and fractionation duration (lower panel) before (left panel) and after ajmaline (right panel) administration. Color bars show activation and fractionation duration (ms). Red areas indicate short activation time or electrograms of short duration and blue areas indicate longer activation time or fractionation duration. Note that during administration of ajmaline the area of longer fractionation duration is expanding and becomes more heterogeneous.

**Figure 3:** Body surface ST-segment potential map of patient no. 3. A. A torso with epicardial
RV CARTO map of fractionated electrograms (green areas are highly fractionated) as in figure 2 with the positions of the BSM electrodes. B. ST-segment potentials measured at the indicated BSM electrodes. Red a higher potential (1 mV) and green zero potential. Black dots represent the electrode positions. C. the electrocardiograms corresponding with the black dots within the white rectangle on the body surface potential map in panel B and the torso in panel A. The area with the highest ST-segment potential (red) including electrocardiogram E3 are immediately overlying the area of RVOT fractionation (A).

**Figure 4A** Epicardial RV CARTO map of patient no. 4 displaying activation at baseline (left panel), during ajmaline (middle panel) administration and after epicardial ablation of the fractionated signals (right panel). Left to the CARTO images are the corresponding electrograms. Blue dots are recording points and red points indicate ablation sites. Please note that color bars have different ranges. B Twelve lead ECG of one of the BrS patients who underwent epicardial ablation. The ECG shows a type 1 Brugada syndrome during baseline, maximal ajmaline level and after epicardial ablation of areas with outspoken fractionated electrograms. Note that ST-segment elevation increases with ajmaline and virtually disappears after ablation. The ECGs of the second patient (right) are less outspoken, but differences are also present. C Tracings are the difference between V1 during baseline and V1 at maximal ajmaline level of two Brugada syndrome patients (no. 3 and 4). Patient 3 has a type 1 and patient 4 a type 2 Brugada syndrome. In both signals the deflection marking the difference in QRS (V) is followed by a pulse shaped deflection with a duration of approximately 220 and 300 ms respectively. Arrows mark the time of latest epicardial late potentials in the patients. A: atrial complex, Stim.: stimulation artefact.
**Figure 5A:** Tracings are V1 of a type 1 Brugada syndrome patient during sinus rhythm (SR) and during SR with RVOT stimulation at different times after onset of the QRS complex (S1 – S4). Delay between onset QRS and the stimulus is indicated by a number left from the dashed line. Sharp deflections right from the dashed lines are the stimulus artefacts. **B:** Tracings are the difference between the stimulated complexes in S1 till S4 in panel A) and the SR complex. Sharp deflections are stimulus artefacts, which are followed by a pulse shaped signal. The width of the pulses are indicated by an arrow, and increases with prematurity of the stimulus. See text for discussion.

**Figure 6:** Schematic drawing of intra and extracellular signals recorded near an isthmus site where activation in myocardial tissue proximal from the isthmus is blocked toward myocardium distal from the isthmus. Activation in the proximal area generates an action potential (intracellular) at the recording site. Due to activation block, the distal area is not activated and the intracellular signal is a flat line (intracellular). The upper tracing shows the extracellular signal, which consists of a stimulus artefact, a remote deflection of the activation front in the proximal area and an action potential shaped deflection caused by electrotonic current flowing through the isthmus. At the right site schematics of myocardial tissue subdivided in multiple myocardial bundles by electrically in-excitable barriers. Bundles are interconnected at different sites by an isthmus. Activation has to follow a tortuous route between the barriers, which results in activation delay because of the increased path length. Fractionated electrograms occur because of the asynchronous activation between the barriers. Black dots are recording sites.
A
ST-Segment Elevation and Fractionated Electrograms in Brugada Syndrome Patients Arise from the Same Structurally Abnormal Subepicardial RVOT Area but Have a Different Mechanism

SUPPLEMENTAL MATERIAL
Suppl.fig 1

Action potentials

Electrotonic component
AP endo – epi, low pass filtered

Electrotonic component
Electrotonic component shifted

[Graphs showing various waveforms and curves with annotations]
Supplemental Figure 1. Simulation of epicardial and endocardial action potentials to differentiate between the repolarization and current to load mismatch hypothesis for ST-segment elevation on the base of the signals obtained by premature stimulation of the RVOT. Signals in the upper right panel correspond best with the recorded signals in figure 6B as for morphology and timing. Stimulus artefacts are not indicated in the simulated signals.