Correlation Between Signal-Averaged Electrocardiogram and the Histologic Evaluation of the Myocardial Substrate in Right Ventricular Outflow Tract Arrhythmias

Running title: Santangeli et al.; SAECG and the diagnosis of RVOT arrhythmias substrate

Pasquale Santangeli, MD1; Maurizio Pieroni, MD, PhD4,6; Antonio Dello Russo, MD, PhD5;
Michela Casella, MD, PhD5; Gemma Pelargonio, MD; PhD4;
Luigi Di Biase, MD, PhD, FHR1,2,3; Andrea Macchione, MD1;
J. David Burkhard, MD, FACC, FHR1; Fulvio Bellocci, MD4; Pietro Santarelli, MD4;
Claudio Tondo, MD, PhD5; Andrea Natale, MD, FACC, FHR, FESC1,2

1Texas Cardiac Arrhythmia Institute, St. David’s Medical Center, Austin, TX; 2Dept of Biomedical Engineering, University of Texas, Austin, TX; 3Dept of Cardiology, University of Foggia, Foggia; 4Catholic University of the Sacred Heart, Rome; 5Cardiac Arrhythmia Research Centre, Centro Cardiologico Monzino, Milan; 6Department of Cardiovascular Diseases, San Donato Hospital, Arezzo, Italy

Corresponding author:
Andrea Natale, MD, FACC, FHR, FESC
Executive Med Director, TX Cardiac Arrhythmia Inst at St. David’s Medical Ctr, Austin, TX,
Consulting Prof, Division of Cardio, Stanford Univ, Palo Alto, CA, Clinical Assoc Prof of Med,
Case Western Reserve Univ, Cleveland, OH, Director, Interventional Electrophysiology, Scripps Clinic, San Diego, Sr Clinical Director, EP Services, CA Pacific Med Ctr, San Francisco, CA
3000 N. I-35, Suite 720; Austin, TX 78705
Tel: +15215448186
Fax: +15125448184
E-mail: dr.natale@gmail.com

Abstract:

Background - The differential diagnosis between idiopathic and cardiomyopathy-related right ventricular outflow tract (RVOT) ventricular arrhythmias (VAs) is crucial. Signal-averaged electrocardiogram (SAECG) abnormalities are frequent in cardiomyopathy-related RVOT-VAs, although their pathophysiologic basis and diagnostic value in this setting are undefined. We tested the association between SAECG and the myocardial substrate underlying RVOT-VAs.

Methods and Results - 24 consecutive patients (median age 50 [42-59] years, 12 men) with RVOT-VAs (10 with frequent [≥1,000/24h] PVCs, 14 with VTs) underwent SAECG with 40 Hz filtering and electroanatomic mapping (EAM) with EAM-guided biopsy for characterization of the RVOT-VAs substrate. A filtered averaged QRS (fQRS) was obtained and analyzed for fQRS duration, low amplitude signal duration below 40 mV (LAS40) and root mean square voltage in last 40 ms of the QRS (RMS40). Standard definition of EAM scar was used. EAM-guided biopsy diagnosed ARVC in 11 (46%), myocarditis in 8 (33%), and idiopathic RVOT-VAs in 5 (21%) patients. Patients with cardiomyopathy-related RVOT-VAs had ≥1 EAM scar (median 2 [1-2], all with RVOT scar). EAM of patients with idiopathic RVOT-VAs was normal. Patients with cardiomyopathy-related RVOT-VAs had significantly longer fQRS (106 [92-132] ms vs. 83 [82-84] ms, P = 0.01) and LAS40 (39 [36-51] ms vs. 19 [18-21] ms, P = 0.02), and lower RMS40 (18 [9-26] μV vs. 33 [32-33] μV, P = 0.04). A significant linear correlation was found between the extension (cm²) of the RVOT scar and all three SAECG parameters (rₘ = 0.76, P < 0.001 for the fQRS; rₛ = 0.73, P < 0.001 for the LAS40; and rₚ = -0.72, P < 0.001 for the RMS40). Using the established 2 of 3 criteria (i.e., late potentials) SAECG diagnosed cardiomyopathy-related RVOT-VAs with high positive (100%) but low negative (38%) predictive values, and missed 7/9 (78%) patients with RVOT scar <8 cm².

Conclusions - In patients with RVOT-VAs, abnormal SAECG parameters reflect the presence of extensive cardiomyopathic involvement of the RVOT. However, a negative SAECG does not reliably rule out cardiomyopathy-related RVOT-VAs in the presence of a small RVOT scar.

Key words: right ventricular outflow tract tachycardia, signal-averaged electrocardiogram, three dimensional electroanatomic mapping.
Introduction

Right ventricular outflow tract ventricular arrhythmias (RVOT-VAs) are the most common variant of nonischemic VAs.\(^1,2\) The majority of RVOT-VAs has an idiopathic origin with a benign clinical course;\(^2\) however, they may also represent an early manifestation of potentially life-threatening structural heart diseases, such as myocarditis or arrhythmogenic right ventricular cardiomyopathy (ARVC).\(^3\) Accordingly, syncope and sudden cardiac death have been reported in patients presenting with RVOT-VAs and an apparently normal heart.\(^4-6\) An accurate distinction between idiopathic and cardiomyopathy-related RVOT-VAs carries important clinical and prognostic implication. Unfortunately, such distinction is often challenging especially in early and segmental forms of ARVC, or in focal myocarditis, which may selectively affect the RVOT without other evidence of RV involvement. The signal-averaged electrocardiogram (SAECG) is a quick and inexpensive test to disclose areas of slow and fragmented conduction in the RV associated with underlying cardiomyopathic substrates.\(^7\)

Accordingly, SAECG abnormalities (i.e., late potentials) are frequently encountered in patients with cardiomyopathy-related RVOT-VAs.\(^7-9\) However, the pathophysiologic significance and diagnostic reliability of SAECG abnormalities in the setting of cardiomyopathy-related RVOT-VAs are still undefined.

Recent studies have shown that three-dimensional electroanatomic voltage mapping (EAM) with EAM-guided endomyocardial biopsy allow to reliably localize and quantify affected RV segments in patients with different variants of cardiomyopathy-related VAs.\(^10\)

The aim of this study was to establish the value of the SAECG in the differential diagnosis between idiopathic and cardiomyopathy-related RVOT-VAs, by testing the association Between SAECG abnormalities and the RVOT histologic substrate identified through EAM with
EAM-guided biopsy in a series of patients with RVOT-VAs.

Methods

We studied 24 patients (median age 50 [42-59] years, 12 males) with RVOT-VAs according to standard 12-lead ECG criteria.\textsuperscript{11} All patients underwent a complete cardiovascular examination including history, physical examination, 24-h Holter monitoring, SAECG, two-dimensional echocardiography, and gadolinium contrast-enhanced cardiac magnetic resonance (CMR) (not performed in 2 patients due to claustrophobia).

Diagnosis of ARVC was established according to current diagnostic criteria defined by the European Society of Cardiology and International Society and Federation of Cardiology Task Force.\textsuperscript{12} Diagnosis of myocarditis was established according to standardized histologic and immunohistochemistry criteria (see below).

A structurally normal heart (i.e., idiopathic RVOT-VAs) was defined on the basis of normal resting ECG, normal dimension and function (global and regional) of the left ventricular (LV) and RV chambers as determined by echocardiography and CMR, absence of late gadolinium enhancement on CMR, and normal EAM and EAM-guided biopsy.

Patients were excluded from this study if: they had atrial fibrillation or pacemaker rhythm at the time of SAECG; they needed antiarrhythmic therapy at the time of SAECG and EAM; the noise level of the SAECG was \( \geq 0.5 \) mV.

Signal-averaged electrocardiogram

The SAECG was obtained with an Arrhythmia Research Technology-101 or 1200 System, with bidirectional Butterworth filtering (40 to 250 Hz), as previously described.\textsuperscript{13} The following quantitative SAECG variables of the filtered QRS were evaluated: 1) total duration (fQRSd), 2)
duration of the low-amplitude signals (<40 mV) in the terminal portion (LAS40), and 3) root-mean-square voltage of the last 40 ms (RMS40). Between 300 and 500 QRS complexes were averaged for each recording to reach a noise level <0.5 mV. Ventricular late potentials were considered positive when ≥2 of the following criteria were fulfilled: 7, 14, 15 1) fQRSd >114 ms, 2) LAS40 >38 ms, and 3) RMS40 <20 μV.

**Cardiac magnetic resonance**

Cardiac magnetic resonance was performed with a 1.5-T Signa Excite 2 scanner (General Electric Medical Systems, Milwaukee, Wisconsin) using a cardiac 8-channel phased-array coil, with vector ECG gating at end-expiration. Morphological evaluation of the cardiac chambers and presence of intra-myocardial fatty infiltration were obtained by black-blood double- and triple-inversion recovery fast spin-echo sequences (repetition time 2 RR intervals, echo time 34 ms, slice thickness 8 mm, image matrix 256 to 256, and field of view 30 to 36 cm) along axial, short-axis, and horizontal long-axis planes. Functional assessment was carried out using bright-blood high-resolution steady-state free precession sequence (repetition time 3.4 ms, echo time 1.5 ms, flip angle 50°, image matrix 224 to 288, field of view 30 to 36 cm) in axial, vertical long-axis, horizontal long-axis, and short-axis stack. Finally, late gadolinium enhancement images were acquired using an inversion recovery prepared breath-hold gradient-echo sequence obtained 20 min after intravenous administration of 0.2 mmol/kg gadodiamide (Omniscan, Amersham Health, Princeton, New Jersey). Late gadolinium enhancement was reported when it was detected in more than one imaging plane, using cross-plane localizers to confirm the position.

Post-processing was performed on an Advantage Windows Workstation using MASS software (Medis, Leiden, the Netherlands). This software was used to view images using standardized window width and level settings. The same software was also used for
measurement of RV end-diastolic and end-systolic diameter. Cardiac magnetic resonance analysis was performed by an expert radiologist with Society of Cardiovascular Magnetic Resonance level-3 experience, who was blinded to the clinical, SAECG, and endomyocardial biopsy data.

**Invasive study**

All patients were submitted to coronary and left and right ventricular angiography (right and left anterior oblique views), three-dimensional EAM, and EAM-guided endomyocardial biopsy. In particular RV angiography was performed before EAM to provide RV silhouette in two views, thus improving the anatomical accuracy of EAM. On the basis of EAM, endomyocardial biopsies were withdrawn from areas presenting electrical abnormalities, as previously shown.\(^{10}\)

**Three-dimensional electroanatomic mapping**

All patients underwent high-density RV three-dimensional electroanatomic voltage mapping with the CARTOTM system (Biosense-Webster, Diamond Bar, CA). Mapping points were sampled with a 7-Fr 3.5-mm irrigated tip Navi-Star ThermocoolTM catheter (Biosense-Webster, Diamond Bar, CA) to generate an accurate three-dimensional electroanatomic map of the RV, reflecting the shape evidenced by angiography. High-density mapping was obtained in sinus rhythm, and the voltage maps were edited setting the point density (fill threshold) at 15 mm and manually eliminating intracavitary points.\(^{10}\) Adequate catheter contact was confirmed by concordant catheter tip motion with the cardiac silhouettes on fluoroscopy and by adherence of voltage map to frozen angiographic right ventricular shape. In addition, to avoid low voltage recordings due to poor contact, the following tools were used: 1) the signal had to satisfy the three stability criteria automatically detected by the CARTOTM system in terms of cycle length, local activation time and beat-to-beat difference of the location of the catheter (<2%, <3 ms, and
<4 mm, respectively); 2) both bipolar and unipolar signals were simultaneously acquired to confirm true catheter contact through the analysis of local electrograms (in particular the shape of the unipolar electrograms); 3) in the presence of a low voltage area, at least 3 additional points were further acquired at the same site to confirm the reproducibility of the voltage measurement. The color display to identify normal and abnormal voltage myocardium ranged from red, indicating electroanatomic scar tissue (amplitude <0.5 mV), to purple, indicating electroanatomic normal tissue (amplitude ≥1.5 mV). Intermediate colors represented the electroanatomic border zone (signal amplitudes between 0.5 and 1.5 mV). The CARTOTM-incorporated surface area calculation tool was used to measure the extension of RV electroanatomical scars. The anatomical distribution of the pathological areas was evaluated dividing the RV voltage map into five areas: 1) the outflow tract; 2) the free (anterolateral) wall; 3) the apex; 4) the inferior/posterior wall (including the inferior and posterior basal segments); and 5) the septal wall. According to previous studies, “electroanatomic scar” was defined as an area including at least 3 adjacent points with bipolar signal amplitude <0.5 mV; the reference value for normal endocardium was set at 1.5 mV as previously described for the identification of normal and scarred areas.10

Endomyocardial biopsy, histology and immunohistochemistry

Right ventricular endomyocardial biopsies (4-5 samples from each patient) were obtained via the femoral vein with the use of a preformed long sheath and a disposable bioptome (Cordis, Johnson and Johnson, FL, USA). Once EAM was completed, the mapping catheter’s tip was directed against abnormal voltage areas and the distal end of the sheath was placed close to it. Sheath position was checked in right and left anterior oblique view and then biopsies were withdrawn from wall segments with abnormal voltage, as previously shown.10 In case of normal
EAM, endomyocardial biopsies were withdrawn from conventional sites including apex and interventricular septum.

Two to three samples were processed for histology and immunohistochemistry. For histology, multiple 5-μm-thick sections were cut and stained with hematoxylin-eosin, Miller’s elastic Van Gieson, Masson’s trichrome, and examined by light microscopy. Immunohistochemistry for the characterization of inflammatory infiltrates was performed using the following antibodies: CD3, CD8, CD45RO, CD68, (Dako Corporation, Glostrup, Denmark), as previously described. To quantify the inflammatory infiltrates, CD8+ and CD45RO+ positive lymphocytes were counted per high-power field (400-fold magnification) in all available fields, and the mean number was calculated, as previously described. In patients presenting histologic evidence of fibro-fatty replacement, a histomorphometric analysis was performed on Masson’s trichrome-stained sections to calculate the extent of myocardial atrophy and fibro-fatty replacement. Images obtained at 5× magnification with a digital camera (Leica DFC 420C, Leica microsystems, Switzerland) were stored as TIFF files and analyzed with a dedicated imaging software (Leica Application Suite v3.0, Leica microsystems, Switzerland) to calculate the percent area occupied by adipose tissue, replacement fibrosis and residual myocardium. The diagnosis of myocarditis was based on Dallas criteria and immunohistochemistry: in particular a T-lymphocyte infiltration (>7/mm2) in the presence of cytotoxic (CD8+) and activated (CD45RO+) lymphocytes, was considered diagnostic. The diagnosis of ARVC was made on the basis of extensive fibro-fatty myocardial atrophy with a percentage of fat >3% and fibrous tissue >40% associated with amounts of residual myocytes <45% of the specimen at morphometric analysis.

**Statistical analysis**
Between-group comparisons were assessed by the unpaired t-test or Mann-Whitney U test, as appropriate, and proportions were compared by Fisher’s exact test. Bivariate linear correlations analyses were assessed with the Spearman’s rank correlation coefficient. The diagnostic performance of late potentials and, separately, of different SAECG parameters in diagnosing cardiomyopathy-related RVOT-VAs was evaluated computing the sensitivity, specificity, positive and negative predictive values with their 95% confidence interval (CI). The best cut-off value of each SAECG parameter for the diagnosis of cardiomyopathy-related RVOT-VAs was assessed by means of a receiver operating characteristic (ROC) analysis. Data are reported as median (interquartile range), unless differently indicated. A level of \( P < 0.05 \) was considered for statistical significance. Statistical analyses were done by STATA 11.2 software package (Stata Corporation, College Station, Texas, USA).

Results

Clinical features and non-invasive findings

Clinical characteristics and noninvasive findings of the patient population are summarized in Table 1. Spontaneous RVOT-VAs (i.e., left bundle branch block pattern and inferior axis) were documented in all patients, and included sustained monomorphic ventricular tachycardia (VT) in 6 (25%), multiple runs of nonsustained VT in 8 (33%), and frequent (i.e., >1,000/24h) premature ventricular contractions (PVCs) in 10 (42%) patients. Overall, 17 (71%) patients presented either one clinical, ECG or imaging abnormality suggestive of ARVC. Among these, 10 (42%) patients presented ECG depolarization (i.e., epsilon wave or localized prolongation of the QRS complex in right precordial leads) or repolarization (i.e., inverted T waves beyond lead V1) abnormalities, 5 (21%) major abnormalities at non-invasive imaging evaluation (two-dimensional
echocardiography or CMR), and 2 (8%) had family history of ARVC. No association between different types of presenting RVOT arrhythmia (sustained vs. nonsustained VT vs. frequent VPBs) and presence of baseline major abnormalities at non-invasive evaluation was found (P = 0.14 for multiple comparison). Seven patients (29%) had history of unexplained syncope and arrhythmia-related symptoms (mainly palpitations) were present in 20 patients (83%).

**Electroanatomic voltage mapping results**

Table 2 reports the results of the invasive study (EAM and EAM-guided endomyocardial biopsy). Nineteen patients (79%) had an abnormal voltage map, presenting at least one area (median 2 [1-2]) with contiguous bipolar electrograms with voltage values < 0.5 mV (scar tissue) surrounded by a larger zone with signal amplitudes comprised between 0.5 and 1.5 mV indicating abnormal myocardium. The RVOT was involved in all 19 patients with abnormal EAM, and the RV free wall represented the second most frequently affected segment (10/19 cases, 53%). Focal involvement of the RVOT was present in 5/19 (26%) patients, with a median scar extension of 10 (9-17) cm². The remaining 14 patients presented a more diffuse RV involvement (2 RV segments in 11 cases, and 3 RV segments in 3 cases), corresponding to a median scar extension of 28 (12-46) cm².

**Endomyocardial biopsy findings and final diagnosis of the RVOT-VAs substrate**

In 11/19 (58%) patients with abnormal EAM, the presence of myocardial atrophy and fibro-fatty replacement at EAM-guided endomyocardial biopsy definitely established the diagnosis of ARVC according to current diagnostic criteria (Tables 2 to 4). In the remaining 8/19 (42%) cases with abnormal EAM, histology showed the presence of inflammatory infiltrates associated with necrosis of adjacent myocytes, consistent with the diagnosis of active myocarditis according to Dallas criteria (Tables 2 and 4). In all these patients immunohistochemistry showed
inflammatory infiltrates to be mainly represented by activated and cytotoxic T lymphocytes. No patient showed histological features of sarcoidosis, granulomatous and/or giant cell myocarditis. With the exception of being slightly older, patients with ARVC did not differ from those with myocarditis in terms of other clinical and instrumental findings.

Finally, all 5 patients with normal EAM displayed also normal histology at endomyocardial biopsies, which were withdrawn from conventional sites including the RV apex and the interventricular septum. In all these patients, non-invasive evaluation showed also no abnormality, and a final diagnosis of idiopathic RVOT-VAs was definitely established. Of note, all patients with idiopathic RVOT-VAs presented with nonsustained VAs (i.e., frequent PVCs or nonsustained VT), whereas 6/19 (32%) patients with cardiomyopathy-related RVOT-VAs presented with sustained VT (P = 0.28 for comparison).

**Signal-averaged ECG and the histologic substrate of RVOT-VAs**

Overall, ventricular late potentials at SAECG were present in 11 patients (46%), all with cardiomyopathy-related RVOT-VAs (7 ARVC and 4 myocarditis, P = 0.041 for comparison with idiopathic RVOT-VAs). Of note, patients with late potentials were more likely to have history of syncope (P = 0.023), and RV morpho-functional abnormalities at CMR (*Table 1*).

Patients with idiopathic RVOT-VAs had significantly shorter duration of the fQRS complex (83 [82-84] ms vs. 106 [92-132] ms, P = 0.01) and LAS40 (19 [18-21] ms vs. 39 [36-51] ms, P = 0.02), and significantly higher values of RMS40 (33 [32-33] μV vs. 18 [9-26] μV, P = 0.04) (*Figure 1*). No significant differences in SAECG parameters were found between patients with ARVC-related RVOT-VAs and those with myocarditis (fQRSd 106 [91-132] ms vs. 106 [96-127] ms, P = 0.83; LAS40 39 [36-51] ms vs. 42 [36-68] ms, P = 0.33; RMS40 18 [15-28] μV vs. 18 [8-25] μV, P = 0.48, respectively).
A significant linear correlation was found between all SAECG parameters and the extension of RVOT cardiomyopathic involvement (i.e., cm² of RVOT scar), with the most significant association being observed for the fQRSd ($r_s = 0.76$, $P < 0.001$) and for the LAS40 ($r_s = 0.73$, $P < 0.001$) (Figure 2). Of note, SAECG parameters were not associated with presence of scar in other RV segments (Table 2).

Overall, presence of late potentials at SAECG diagnosed cardiomyopathy-related RVOT-VAs with a sensitivity of 58% (95% CI 38% to 78%), a specificity of 100%, a very high positive predictive value (100%), but relatively low negative predictive value (38% [95% CI 19% to 58%]), since late potentials were absent in 8/19 (42%) patients with cardiomyopathy-related RVOT-VAs (4 ARVC and 4 myocarditis). The presence of late potentials was associated with higher extension of electroanatomic scar in the RVOT ($13 [9-17]$ cm² vs. $5 [4-6]$ cm², $P = 0.01$), and late potentials were absent in 7/9 (78%) patients with RVOT scar <8 cm². Accordingly, at ROC analysis, an extension of electroanatomic scar ≥8 cm² was found the best predictor of positive late potentials (sensitivity 91%, specificity 92%).

When analyzed according to established cut-off values (i.e., fQRSd >114 ms; LAS40 >38 ms, and RMS40 <20 μV), all three individual SAECG parameters showed high specificity (100%) for the diagnosis of cardiomyopathy-related RVOT-VAs. However, the sensitivity ranged from 37% (95% CI 18% to 56%) for the fQRSd, to 58% (95% CI 38% to 78%) for the LAS40 and RMS40. The sensitivity of the fQRSd reached 74% (95% CI 56% to 91%) without affecting the 100% specificity adopting a cut-off value of ≥100 ms, while the established cut-off values for LAS40 and RMS40 were confirmed the best cut-off values also at ROC analysis.

Adopting a cut-off value to define abnormal fQRSd of ≥100 ms, the recalculated sensitivity and negative predictive value of late potentials (i.e., at least 2 abnormal SAECG
parameters) to diagnose cardiomyopathy-related RVOT-VAs reached 84% (95% CI 70% to 99%) and 63% (95% CI 43% to 82%), respectively, while the specificity and positive predictive value remained high (100%) (Figure 3).

Discussion

The differential diagnosis between idiopathic and cardiomyopathy-related RVOT-VAs is a major clinical challenge for cardiologists; RVOT-VAs may represent the early manifestation of concealed cardiomyopathies that can unpredictably lead to sudden cardiac death in the absence of overt structural RV abnormalities.4-6 The SAECG is a quick and inexpensive diagnostic tool to disclose the presence of pathologically slow conduction areas in the RV (i.e., late potentials) due to underlying cardiomyopathic substrates.7, 12, 15 Although SAECG abnormalities have been described in patients with cardiomyopathy-related RVOT-VAs,7, 10, 15 their pathophysiologic basis and diagnostic relevance are still undefined.

The present study elucidates the pathophysiologic basis of SAECG abnormalities in patients with RVOT-VAs, showing that they correlate with the extent of the pathologic involvement of the RVOT by cardiomyopathic substrates. Of note, epsilon waves at surface ECG, which represent the macroscopic manifestation of late potentials at SAECG, were found in 3 patients in the ARVC group who had significantly larger RVOT scars compared to those without epsilon waves. This finding is in line with seminal experiences on endocardial mapping of epsilon waves in ARVC.22

Presence of late potentials was strikingly associated with cardiomyopathy-related RVOT-VAs, and such association was observed independently from the underlying histologic substrate identified through EAM-guided endomyocardial biopsy (i.e., ARVC or myocarditis). The
clinical overlap between these two clinical entities has been well-reported in recent years;\textsuperscript{10, 23, 24}
the present study shows that such overlap may extend also to SAECG abnormalities, which
could be caused by either slow conduction due to fibro-fatty tissue, as it is the case for ARVC, or
to underlying myocardial inflammation, as it is the case for myocarditis. Importantly, a
significantly higher prevalence of late potentials was found among patients with history of
previous syncope, further supporting the concept that SAECG abnormalities in these patients
underlie potentially life-threatening cardiomyopathic substrates. Indeed, syncope has been
consistently demonstrated an ominous predictor of sudden cardiac death in patients with RV
cardiomyopathy.\textsuperscript{25} Moreover, late potentials were associated with a higher prevalence of RV
dilatation and dysfunction, which is consistent with previous studies.\textsuperscript{9,10} While the presence of late
potentials was strikingly associated with underlying RVOT cardiomyopathic substrates, absence
of SAECG abnormalities did not reliably rule out RVOT pathologic involvement, since 7/9
patients with a small RVOT scar (i.e., <8 cm\textsuperscript{2}) actually displayed normal SAECG. These
findings account for a high specificity and positive predictive value, but relatively low sensitivity
and negative predictive value of SAECG in diagnosing cardiomyopathy-related RVOT-VAs,
which is in line with recent data on SAECG in ARVC.\textsuperscript{15}

At ROC analysis, the diagnostic performance of individual SAECG parameters adopting
established cut-off values appeared optimal for the LAS40 and RMS40, but suboptimal for the
fQRSd. However, the diagnostic sensitivity of the fQRSd increased from 37\% (95\% CI 18\% to
56\%) to 74\% (95\% CI 56\% to 91\%) after decreasing the cut-off value to define abnormal fQRSd
from 114 ms to 100 ms. Although the results of our ROC analysis should be interpreted with
cautions due to the small sample size of our patient population, they may also suggest that
different cut-off values for the fQRSd may be necessary to improve the diagnostic performance
of SAECG in the setting of RVOT-VAs. To this regard, it is important to emphasize that the
current cut-off values for late potentials have been derived from studies in patients with ischemic
cardiomyopathy after acute myocardial infarction.14

Clinical implications

The significant correlation between SAECG abnormalities and cardiomyopathic involvement of
the RVOT, whatever the underlying pathological substrate, may have important clinical
implication, particularly in segmental and early forms of ARVC and in younger patients with
ventricular arrhythmias due to focal myocarditis and mild or absent RV abnormalities. On the
basis of the observed high positive predictive value, the detection of abnormal SAECG
parameters during the noninvasive workup of patients with RVOT-VAs should raise the
suspicion of underlying cardiomyopathic substrates and point to further investigation. On the
other hand, absence of late potentials does not reliably rule out the presence of small RVOT
scars reflecting underlying pathologic substrates, at least when adopting current cut-off values
for defining abnormal SAECG parameters.

In these cases, EAM with EAM-guided endomyocardial biopsy appears important to
reach a definite diagnosis of substrate, especially in the presence of peculiar clinical features
such as family history of ARVC, sustained VAs, or typical ECG depolarization/repolarization
abnormalities. Indeed, in our study no other non-invasive diagnostic tool, including contrast-
enhanced cardiac magnetic resonance, was able to distinguish between patients with ARVC-
related VAs and those with myocarditis. The definite diagnosis of the substrate underlying
RVOT-VAs might have important clinical consequences on the therapeutic approach (e.g.,
ablation, ICD, drugs), prognosis, and familial screening (indicated in the presence of a diagnosis
of ARVC).
On the other hand, our study suggests also that the sensitivity and negative predictive value of the SAECG may significantly increase when adopting a cut-off value for defining abnormal fQRSd of $\geq 100$ ms, and this finding warrants a prospective validation in properly designed studies.

**Study limitations**

This study included a relatively small sample of patients who underwent an extensive diagnostic study protocol, including EAM and EAM-guided endomyocardial biopsy. As such, caution should be exercised in generalizing our findings to a larger and unselected cohort of patients with RVOT-VAs, especially the computations on the diagnostic performance of the SAECG in diagnosing cardiomyopathy-related RVOT-VAs.

It is also important to emphasize that our Institution is a tertiary center for the study of arrhythmic manifestations of cardiomyopathies, and many patients with clinical suspicion of underlying cardiomyopathic substrates (e.g., family history of sudden death, depolarization/repolarization abnormalities at the 12-lead ECG) are usually sent from other other Institutions or referring physicians. Therefore, a possible referral bias may also have influenced the features of study population.

Finally, our cohort of patients with cardiomyopathy-related RVOT-VAs consisted only of patients with ARVC and myocarditis. Whether our results may be generalized also to patients with RVOT-VAs and underlying myocardial substrates other than that reported in our series (e.g., sarcoidosis, Chagas cardiomyopathy, or myocardial infarction) warrants further investigation.

**Conclusions**

In patients with RVOT-Vas, abnormal SAECG parameters reflect the presence of
cardiomyopathic involvement of the RVOT, and should prompt to perform further diagnostic investigations, including EAM with EAM-guided biopsy, to identify the underlying myocardial substrate. Our findings clarify the pathophysiologic basis of SAECG abnormalities in such patients, and provide an explanation to the observed high specificity but low sensitivity of late potentials in diagnosing cardiomyopathy-related RVOT-VAs. With the current cut-off values to define abnormal SAECG parameters, most patients with small RVOT areas of cardiomyopathic involvement (i.e., <8 cm²) are missed. The diagnostic sensitivity of SAECG in detecting cardiomyopathy-related RVOT-VAs may significantly increase (16/19 [84%] patients correctly diagnosed) considering a value of the fQRSd ≥100 ms as abnormal. Such findings, if confirmed in larger series, could lead to redefine the relevance of SAECG in the differential diagnosis between idiopathic and cardiomyopathy-related RVOT-VAs.

Funding Sources: This study was partially supported by a Telethon Foundation Grant (GGP10186 to M.P.).

Conflict of Interest Disclosures: Dr. Andrea Natale has received consultant fees or honoraria from Biosense Webster, Boston Scientific, Medtronic, Biotronic, and LifeWatch. Dr. Claudio Tondo has served as a member of the advisory board of Biosense Webster and has been a consultant for and received lecture fees from St. Jude Medical. Dr. Luigi Di Biase has received consultant fees from Biosense Webster and Hansen Medical.

References:


Table 1. Clinical characteristics and noninvasive findings of the overall sample and according to results of the SAECG.

<table>
<thead>
<tr>
<th></th>
<th>Overall Sample (n = 24)</th>
<th>Positive LPs (n = 11)</th>
<th>Negative LPs (n = 13)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (median (IQR))</td>
<td>50 (42-59)</td>
<td>51 (47-64)</td>
<td>45 (39-54)</td>
<td>0.10</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>12 (50)</td>
<td>5 (45)</td>
<td>7 (54)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Presenting RVOT-VA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained VT (%)</td>
<td>6 (25)</td>
<td>5 (45)</td>
<td>1 (8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Nonsustained VT (%)</td>
<td>8 (33)</td>
<td>3 (27)</td>
<td>5 (38)</td>
<td>0.68</td>
</tr>
<tr>
<td>Frequent PVCs (&gt;1,000/24h)</td>
<td>10 (42)</td>
<td>3 (27)</td>
<td>7 (54)</td>
<td>0.24</td>
</tr>
<tr>
<td>Family history of ARVC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARVC (%)</td>
<td>2 (8)</td>
<td></td>
<td>2 (15)</td>
<td>0.48</td>
</tr>
<tr>
<td>Clinical symptoms (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac arrest (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>Syncope (%)</td>
<td>7 (29)</td>
<td>6 (55)</td>
<td>1 (8)</td>
<td>0.023</td>
</tr>
<tr>
<td>Palpitations (%)</td>
<td>17 (71)</td>
<td>8 (73)</td>
<td>9 (69)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>No symptoms (%)</td>
<td>4 (17)</td>
<td>0 (0)</td>
<td>4 (31)</td>
<td>0.09</td>
</tr>
<tr>
<td>ECG abnormalities (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right precordial QRS duration ≥110 ms</td>
<td>9 (38)</td>
<td>7 (64)</td>
<td>2 (15)</td>
<td>0.033</td>
</tr>
<tr>
<td>Epsilon wave (%)</td>
<td>3 (13)</td>
<td>3 (27)</td>
<td>0 (0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Inverted T waves beyond lead V1</td>
<td>4 (17)</td>
<td>3 (27)</td>
<td>1 (8)</td>
<td>0.30</td>
</tr>
<tr>
<td>CMR abnormalities (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV dilatation (%)</td>
<td>6/22 (27)</td>
<td>5/9 (55)</td>
<td>1/3 (8)</td>
<td>0.023</td>
</tr>
<tr>
<td>RV global dysfunction (%)</td>
<td>5/22 (23)</td>
<td>5/9 (56)</td>
<td>0/13 (0)</td>
<td>0.005</td>
</tr>
<tr>
<td>RV delayed enhancement, %</td>
<td>7/22 (32)</td>
<td>5/9 (56)</td>
<td>2/13 (15)</td>
<td>0.07</td>
</tr>
<tr>
<td>RV ejection fraction, %</td>
<td>58 (50-59)</td>
<td>49 (49-59)</td>
<td>59 (58-60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>60 (56-65)</td>
<td>60 (55-66)</td>
<td>60 (59-64)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Values expressed as median (interquartile range) or n (%). LPs = ventricular late potentials; RVOT-VA = right ventricular outflow tract ventricular arrhythmia; VT = ventricular tachycardia; PVC = premature ventricular contraction; CMR = cardiac magnetic resonance; RV = right ventricular; LV = left ventricular.

*Comparison between “Positive LPs” and “Negative LPs”

Table 2. Invasive findings of the overall sample and according to results of the SAECG.

<table>
<thead>
<tr>
<th></th>
<th>Overall Sample (n = 24)</th>
<th>Positive LPs (n = 11)</th>
<th>Negative LPs (n = 13)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electroanatomic scar</td>
<td>19 (79)</td>
<td>11 (100)</td>
<td>8 (62)</td>
<td>0.041</td>
</tr>
<tr>
<td>N. of EAM scars (%)</td>
<td>2 (1-2)</td>
<td>2 (1-2)</td>
<td>2 (0-2)</td>
<td>0.69</td>
</tr>
<tr>
<td>Localization of EAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outflow tract (%)</td>
<td>19 (79)</td>
<td>11 (100)</td>
<td>8 (62)</td>
<td>0.041</td>
</tr>
<tr>
<td>Free wall (%)</td>
<td>10 (42)</td>
<td>6 (55)</td>
<td>4 (31)</td>
<td>0.41</td>
</tr>
<tr>
<td>Inferior/Posterior wall</td>
<td>6 (25)</td>
<td>1 (9)</td>
<td>5 (38)</td>
<td>0.17</td>
</tr>
<tr>
<td>Septal wall (%)</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Apex (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>Endomyocardial biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARVC (%)</td>
<td>11 (46)</td>
<td>7 (64)</td>
<td>4 (31)</td>
<td>0.22</td>
</tr>
<tr>
<td>Myocarditis (%)</td>
<td>8 (33)</td>
<td>4 (36)</td>
<td>4 (31)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Normal myocardium (%)</td>
<td>5 (21)</td>
<td>0 (0)</td>
<td>5 (38)</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Values expressed as median (interquartile range), or n (%). LPs = ventricular late potentials; N. = number; EAM = electroanatomic mapping.

*Comparison between “Positive LPs” and “Negative LPs”
Table 3. Task Force diagnostic criteria in the 11 patients with biopsy-proven ARVC.

<table>
<thead>
<tr>
<th>Pt. #</th>
<th>ECG abnormalities</th>
<th>Morpho-functional abnormalities</th>
<th>Family history (M)</th>
<th>Arrhythmias*</th>
<th>Biopsy (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M: Inverted T waves &gt;V1. m: None.</td>
<td>M: None. m: None. Other: Dyssynchronous contraction of the RVOT, LGE.</td>
<td>No</td>
<td>M: None m: NSVT</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>2</td>
<td>M: Inverted T waves &gt;V1. m: None. Other: QRS (V1-V3) ≥110 ms.</td>
<td>M: None. m: None. Other: Mild RVOT dilatation, LGE.</td>
<td>No</td>
<td>M: None m: Sust. VT</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>3</td>
<td>M: None. m: None. Other: None.</td>
<td>M: None. m: None. Other: None.</td>
<td>Yes</td>
<td>M: None m: Freq. PVCs</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>4*</td>
<td>M: Inverted T waves &gt;V1. m: None. Other: QRS (V1-V3) ≥110 ms.</td>
<td>M: None. m: None. Other: Mild RVOT dilatation at ECHO.</td>
<td>No</td>
<td>M: None m: Freq. PVCs</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>5</td>
<td>M: Epsilon wave. m: None. Other: QRS (V1-V3) ≥110 ms.</td>
<td>M: None. m: None. Other: Mild global RV dilatation.</td>
<td>No</td>
<td>M: None m: Sust. VT</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>6</td>
<td>M: None. m: None. Other: QRS (V1-V3) ≥110 ms.</td>
<td>M: Significant RV dilatation and dysfunction. m: None. Other: LGE.</td>
<td>No</td>
<td>M: None m: Freq. PVCs</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>7</td>
<td>M: Epsilon wave. m: None. Other: QRS (V1-V3) ≥110 ms.</td>
<td>M: None. m: None. Other: Mild global RV dysfunction, LGE.</td>
<td>No</td>
<td>M: None m: Freq. PVCs</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>8</td>
<td>M: Inverted T waves &gt;V1. m: None. Other: None.</td>
<td>M: None. m: None. Other: RV free wall hypokinesia.</td>
<td>No</td>
<td>M: None m: Sust. VT</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>9*</td>
<td>M: Epsilon wave. m: None. Other: QRS (V1-V3) ≥110 ms.</td>
<td>M: None. m: None. Other: Mild RVOT enlargement at ECHO.</td>
<td>No</td>
<td>M: None m: Sust. VT</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>10</td>
<td>M: None. m: None. Other: None.</td>
<td>M: None. m: None. Other: None.</td>
<td>Yes</td>
<td>M: None m: Sust. VT</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td>11</td>
<td>M: None. m: None. Other: QRS (V1-V3) ≥110 ms.</td>
<td>M: None. m: RV inferior wall akinesia and mild RV dilatation. Other: None.</td>
<td>No</td>
<td>M: None m: NSVT</td>
<td>Fibro-fatty tissue</td>
</tr>
</tbody>
</table>

M = major criteria; m = minor criteria; Other = finding not included among current criteria. *Cardiac magnetic resonance not performed for claustrophobia. +Per study inclusion criteria, all ventricular arrhythmias had a left bundle-branch block morphology and inferior axis (i.e., RVOT origin, minor criteria). RVOT = right ventricular outflow tract; VT = ventricular tachycardia; Sust. = sustained; NSVT = nonsustained VT; PVCs = premature ventricular contractions; LGE = late gadolinium enhancement.
Table 4. Clinical features of the 19 patients with cardiomyopathy-related RVOT-VAs.

<table>
<thead>
<tr>
<th></th>
<th>ARVC  (n = 11)</th>
<th>Myocarditis  (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>59 (48-68)</td>
<td>44 (38-53)</td>
<td>0.04</td>
</tr>
<tr>
<td>Sex, male</td>
<td>4 (36)</td>
<td>5 (63)</td>
<td>0.37</td>
</tr>
<tr>
<td>Presenting RVOT-VA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained VT</td>
<td>4 (36)</td>
<td>2 (25)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Nonsustained VT</td>
<td>2 (18)</td>
<td>3 (38)</td>
<td>0.60</td>
</tr>
<tr>
<td>Frequent PVCs (&gt;1,000/24h)</td>
<td>5 (45)</td>
<td>3 (38)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Family history of ARVC</td>
<td>2 (18)</td>
<td>0 (0)</td>
<td>0.49</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>Syncope</td>
<td>5 (45)</td>
<td>2 (25)</td>
<td>0.63</td>
</tr>
<tr>
<td>Palpitations</td>
<td>8 (73)</td>
<td>4 (50)</td>
<td>0.38</td>
</tr>
<tr>
<td>No symptoms</td>
<td>1 (9)</td>
<td>3 (38)</td>
<td>0.26</td>
</tr>
<tr>
<td>ECG abnormalities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right precordial QRS duration ≥110 ms</td>
<td>7 (64)</td>
<td>2 (25)</td>
<td>0.17</td>
</tr>
<tr>
<td>Epsilon wave</td>
<td>3 (27)</td>
<td>0 (0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Inverted T waves beyond lead V1</td>
<td>4 (36)</td>
<td>0 (0)</td>
<td>0.10</td>
</tr>
<tr>
<td>CMR abnormalities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV dilatation</td>
<td>3/9 (33)</td>
<td>3/8 (38)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>RV global dysfunction</td>
<td>3/9 (33)</td>
<td>7/8 (25)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>RV delayed enhancement, %</td>
<td>4/9 (44)</td>
<td>3/8 (38)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>RV ejection fraction, %</td>
<td>53 (50-58)</td>
<td>56 (49-58)</td>
<td>0.68</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>60 (56-64)</td>
<td>60 (56-65)</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Values expressed as median (interquartile range) or n (%). LPs = ventricular late potentials; RVOT-VA = right ventricular outflow tract ventricular arrhythmia; VT = ventricular tachycardia; PVC = premature ventricular contraction; CMR = cardiac magnetic resonance; RV = right ventricular; LV = left ventricular.

Figure Legends:

**Figure 1.** SAECG parameters in patients with cardiomyopathy-related and idiopathic RVOT-VAs. Vertical bars represent milliseconds (ms) for the fQRSd and LAS40, and microvolts (µV) for the RMS40.

**Figure 2.** Linear correlation between mean bipolar voltages in the right ventricular outflow tract and signal averaged electrocardiogram variables (fQRSd and LAS40). rs = Spearman’s correlation coefficient.

**Figure 3.** Panel A: performance of late potentials (≥2 abnormal SAECG parameters) in diagnosing cardiomyopathy-related RVOT-VAs according to established cut-off values of SAECG parameters (fQRSd >114 ms; LAS40 >38 ms, and RMS40 <20 µV). Panel B: performance of late potentials in diagnosing cardiomyopathy-related RVOT-VAs according to cut-off values of SAECG parameters derived from ROC analysis (fQRSd ≥100 ms; LAS40 >38 ms, and RMS40 <20 µV).
Correlation Between Signal-Averaged Electrocardiogram and the Histologic Evaluation of the Myocardial Substrate in Right Ventricular Outflow Tract Arrhythmias

Pasquale Santangeli, Maurizio Pieroni, Antonio Dello Russo, Michela Casella, Gemma Pelargonio, Luigi Di Biase, Andrea Macchione, J. David Burkhardt, Fulvio Bellocci, Pietro Santarelli, Claudio Tondo and Andrea Natale

Circ Arrhythm Electrophysiol. published online March 15, 2012;
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/early/2012/03/15/CIRCEP.111.967893

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circep.ahajournals.org//subscriptions/