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

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

Background—The differential diagnosis between idiopathic and cardiomyopathy-related right ventricular outflow tract (RVOT) ventricular arrhythmias (VAs) is crucial. Signal-averaged ECG (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—Twenty-four consecutive patients (median age, 50 years [42–59]; 12 men) with RVOT-VAs (10 with frequent [≥1000/24 hours] premature ventricular contractions, 14 with ventricular tachycardias) 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 <40 mV (LAS40), and root-mean-square voltage in the 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 ms [92–132] versus 83 ms [82–84], P=0.01) and LAS40 (39 ms [36–51] versus 19 ms [18–21], P=0.02), and lower RMS40 (18 µV [9–26] versus 33 µV [32–33], P=0.04). A significant linear correlation was found between the extension (cm²) of the RVOT scar and all 3 SAECG parameters (r_s=0.76, P<0.001 for the fQRSd; r_s=0.73, P<0.001 for the LAS40; and r_s=−0.72, P<0.001 for the RMS40). Using the established 2 of 3 criteria (ie, late potentials), SAECG diagnosed cardiomyopathy-related RVOT-VAs with high positive (100%) but low negative (38%) predictive values and missed 7 of 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. (Circ Arrhythm Electrophysiol. 2012;5:475-483.)

Key Words: right ventricular outflow tract tachycardia ■ signal-averaged ECG ■ three-dimensional electroanatomic mapping

Right ventricular outflow tract ventricular arrhythmias (RVOT-VAs) are the most common variant of nonischemic VAs. The majority of RVOT-VAs have an idiopathic origin with a benign clinical course; however, they may also represent an early manifestation of potentially life-threatening structural heart diseases, such as myocarditis or arrhythmogenic right ventricular cardiomyopathy (ARVC). Accordingly, syncope and sudden cardiac death have been reported in patients presenting with RVOT-VAs and an apparently normal heart. An accurate distinction between idiopathic and cardiomyopathy-related RVOT-VAs carries important clinical and prognostic implications. 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 ECG (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 (ie, 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 3-dimensional electroanatomic voltage mapping (EAM) with EAM-guided endomyocardial biopsy allows reliable localization and quantification of 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 histological substrate identified through EAM with EAM-guided biopsy in a series of patients with RVOT-Vas.

**Methods**

We studied 24 patients (median age, 50 years [42–59]; 12 men) with RVOT-VAs according to standard 12-lead ECG criteria.\(^11\) All patients underwent a complete cardiovascular examination including history, physical examination, 24-hour Holter monitoring, SAECG, 2-dimensional echocardiography, and gadolinium contrast-enhanced cardiac magnetic resonance (CMR) (not performed in 2 patients because of 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.\(^12\) Diagnosis of myocarditis was established according to standardized histological and immunohistochemistry criteria (see below).

A structurally normal heart (ie, 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; if they needed antiarrhythmic therapy at the time of SAECG and EAM; or if the noise level of the SAECG was ≥0.5 mV.

**Signal-Averaged ECG**

The SAECG was obtained with an Arrhythmia Research Technology-101 or 1200 System, with bidirectional Butterworth filtering (40–250 Hz), as previously described.\(^13\) 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.

**Cardiac Magnetic Resonance**

CMR was performed with a 1.5-T Signa Excite 2 scanner (General Electric Medical Systems, Milwaukee, WI), using a cardiac 8-channel, phased-array coil, with vector ECG gating at end-expiration. Morphological evaluation of the cardiac chambers and presence of intramyocardial fatty infiltration were obtained by black-blood double- and triple-inversion recovery fast spin-echo sequences (repetition time, 2 R-R intervals; echo time, 34 ms; slice thickness, 8 mm; image matrix, 256×256; and field of view, 30–36 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, 224–288; field of view, 30–36 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 minutes after intravenous administration of 0.2 mmol/kg gadodiamide (Omniscan, Amersham Health, Princeton, NJ). Late gadolinium enhancement was reported when it was detected in more than one imaging plane, using cross-plane localizers to confirm the position.

Postprocessing was performed on an Advantage Windows Workstation using MASS software (Medis, Leiden, The Netherlands). This software was used to view images using standardized window and level settings. The same software was also used for measurement of RV end-diastolic and end-systolic diameter. CMR 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), 3-dimensional EAM, and EAM-guided endomyocardial biopsy. In particular, RV angiography was performed before EAM to provide RV silhouette in 2 views, thus improving the anatomic accuracy of EAM. On the basis of EAM, endomyocardial biopsies were withdrawn from areas presenting electric abnormalities, as previously shown.\(^10\)

**Three-Dimensional Electroanatomic Mapping**

All patients underwent high-density RV 3-dimensional electroanatomic voltage mapping with the CARTO system (Biosense-Webster, Diamond Bar, CA). Mapping points were sampled with a 7F, 3.5-mm irrigated tip Navi-Star Thermocool catheter (Biosense-Webster) to generate an accurate 3-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.\(^15\) Adequate catheter contact was confirmed by concordant catheter tip motion with the cardiac silhouette 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 3 stability criteria automatically detected by the CARTO 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 CARTO-incorporated surface area calculation tool was used to measure the extension of RV electroanatomic scars. The anatomic distribution of the pathological areas was evaluated dividing the RV voltage map into 5 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

RV 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 biopomte (Cordis, Johnson & Johnson, Miami FL). 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 the case of normal EAM, endomyocardial biopsies were withdrawn from conventional sites including apex and interventricular septum.

Two to 3 samples were processed for histology and immunohistochemistry. For histology, multiple 5-µm-thick sections were cut and stained with hematoxylin and eosin, Miller elastic Van Gieson, or Masson trichrome and examined by light microscopy. Immunohistochemistry for the characterization of inflammatory infiltrates was performed using the following antibodies: CD3, CD8, CD45RO, and CD68, (Dako Corporation, Glostrup, Denmark), as previously described.10,16–18 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.16–18 In patients presenting histological evidence of fibro-fatty replacement, a histomorphometric analysis was performed on Masson 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:19 in particular, a T-lymphocyte infiltration (>7/mm²) in the presence of cytotoxic (CD8+) and activated (CD45RO+) lymphocytes was considered diagnostic.20 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.21

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 exact test. Bivariate linear correlations analyses were assessed with the Spearman 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,
and positive and negative predictive values with their 95% confidence interval (CI). The best cutoff 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 with the STATA 11.2 software package (Stata Corporation, College Station, TX).

**Results**

**Clinical Features and Noninvasive Findings**

Clinical characteristics and noninvasive findings of the patient population are summarized in Table 1. Spontaneous RVOT-VAs (ie, 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 (ie, $>100/24$ hours) premature ventricular contractions (PVCs) in 10 (42%) patients. Overall, 17 (71%) patients presented 1 clinical, ECG, or imaging abnormality suggestive of ARVC. Among these, 10 (42%) patients presented ECG depolarization (ie, epsilon wave or localized prolongation of the QRS complex in right precordial leads) or repolarization (ie, inverted T waves beyond lead V₆) abnormalities, 5 (21%) major abnormalities at noninvasive imaging evaluation (2-dimensional echocardiography or CMR), and 2 (8%) had family history of ARVC. No association between different types of presenting RVOT arrhythmia (sustained versus nonsustained VT versus frequent PVC) and presence of baseline major abnormalities at noninvasive evaluation was found ($P=0.14$ for multiple comparison). Seven patients (29%) had a 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 1 area with contiguous bipolar electrograms with voltage values $<0.5$ mV (scar tissue) surrounded by a larger zone with signal amplitudes 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 of 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 cm² (12–46).

**Endomyocardial Biopsy Findings and Final Diagnosis of the RVOT-VAs Substrate**

In 11 of 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, and 3, and 4). In the remaining 8 of 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 or 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, noninvasive 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 (ie, frequent PVCs or nonsustained VT), whereas 6 of 19 (32%) patients with cardiomyopathy-related RVOT-VAs presented with sustained VT ($P=0.28$ for comparison).

**SAECG and the Histological 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 ms [82–84] versus 106 ms [92–132], $P=0.01$) and LAS40 (19 ms [18–21] versus 39 ms [36–51], $P=0.02$) and significantly higher values of RMS40 (33 µV [32–33] versus 18 µV [9–26]).
No significant differences in SAECG parameters were found between patients with ARVC-related RVOT-V As and those with myocarditis (fQRSd, 106 ms [91–132] versus 106 ms [96–127], $P=0.83$; LAS40, 39 ms [36–51] versus 42 ms [36–68], $P=0.33$; RMS40, 18 µV [15–28] versus 18 µV [8–25], $P=0.48$, respectively).

A significant linear correlation was found between all SAECG parameters and the extension of RVOT cardiomyopathic involvement (ie, 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%, and 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 of 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 cm² [9–17] versus 5 cm² [4–6], $P=0.01$), and late potentials were absent in 7 of 9 (78%) patients with RVOT scar <8 cm². Accordingly, at ROC analysis, an extension of electroanatomic scar ≥8 cm² was found the best

Table 3. Task Force Diagnostic Criteria in the 11 Patients With Biopsy-Proven ARVC

<table>
<thead>
<tr>
<th>Patient No.</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</td>
<td>M: None</td>
<td>No</td>
<td>M: None</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td></td>
<td>m: None</td>
<td>m: None</td>
<td></td>
<td>m: NSVT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other: Dyssynchronous contraction of the RVOT, LGE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>No</td>
<td>M: None</td>
<td>Fibro-fatty tissue</td>
</tr>
<tr>
<td></td>
<td>m: None</td>
<td>m: None</td>
<td></td>
<td>m: Sust. VT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other: QRS (V1–V3) ≥110 ms</td>
<td>Other: Mild RVOT dilatation, LGE</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3</td>
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<td>M: None</td>
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<td>Fibro-fatty tissue</td>
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<tr>
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<td>m: None</td>
<td></td>
<td>m: Freq. PVCs</td>
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<td>Other: None</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>m: None</td>
<td></td>
<td>m: Freq. PVCs</td>
<td></td>
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<td>Other: QRS (V1–V3) ≥110 ms</td>
<td>Other: Mild RVOT dilatation at echocardiography</td>
<td></td>
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<td>5</td>
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<td>m: Sust. VT</td>
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<tr>
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<td>M: Significant RV dilatation and dysfunction</td>
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<td>m: Freq. PVCs</td>
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<td>Other: RV free wall hypokinesia</td>
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<td></td>
<td>m: NSVT</td>
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</tbody>
</table>

The Task Force Diagnostic Criteria in the 11 Patients With Biopsy-Proven ARVC

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; M, major criteria; m, minor criteria; other, finding not included among current criteria; NSVT, nonsustained ventricular tachycardia; RVOT, right ventricular outflow tract; LGE, late gadolinium enhancement; Sust., sustained; Freq., frequent; PVCs, premature ventricular contractions.

*Cardiac magnetic resonance not performed because of claustrophobia.
†Per study inclusion criteria, all ventricular arrhythmias had a left bundle-branch block morphology and inferior axis (ie, RVOT origin, minor criteria).

$P=0.04$ (Figure 1). No significant differences in SAECG parameters were found between patients with ARVC-related RVOT-VAs and those with myocarditis (fQRSd, 106 ms [91–132] versus 106 ms [96–127], $P=0.83$; LAS40, 39 ms [36–51] versus 42 ms [36–68], $P=0.33$; RMS40, 18 µV [15–28] versus 18 µV [8–25], $P=0.48$, respectively).
When analyzed according to established cutoff values (ie, fQRSd >114 ms; LAS40 >38 ms, and RMS40 <20 µV), all 3 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 cutoff value of ≥100 ms, whereas the established cutoff values for LAS40 and RMS40 were confirmed as the best cutoff values also at ROC analysis.

Adopting a cutoff value to define abnormal fQRSd of ≥100 ms, the recalculated sensitivity and negative predictive value of late potentials (ie, 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, whereas 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
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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 (ie, 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 pathological involvement of the RVOT by cardiomyopathic substrates. Of note, epsilon waves at the 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 with those without epsilon waves. This finding is in line with seminal experiences on endocardial mapping of epsilon waves in ARVC.22

The presence of late potentials was strikingly associated with cardiomyopathy-related RVOT-VAs, and such association was observed independently from the underlying histological substrate identified through EAM-guided endomyocardial biopsy (ie, ARVC or myocarditis). The clinical overlap between these 2 clinical entities has been well reported in recent years10,23,24; 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.25 Moreover, late potentials were associated with a higher prevalence of RV dilatation and dysfunction, which is consistent with previous studies.9 Although the presence of late potentials was strikingly associated with underlying RVOT cardiomyopathic substrates, absence of SAECG abnormalities did not reliably rule out RVOT pathological involvement, since 7 of 9 patients with a small RVOT scar (ie, <8 cm²) 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.15

At ROC analysis, the diagnostic performance of individual SAECG parameters adopting established cutoff 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 cutoff value to define abnormal fQRSd from 114 to 100 ms. Although the results of our ROC analysis should be interpreted with caution due to the small sample size of our patient population, they may also suggest that different cutoff values for the fQRSd may be necessary to improve the diagnostic performance of SAECG in the setting of RVOT-VAs. In this regard, it is important to emphasize that the current cutoff values for late potentials have been derived from studies in patients with ischemic cardiomyopathy after acute myocardial infarction.14

Figure 3. A, Performance of late potentials (≥2 abnormal signal-averaged ECG [SAECG] parameters) in diagnosing cardiomyopathy-related right ventricular outflow tract ventricular arrhythmias (RVOT-VAs) according to established cutoff values of SAECG parameters (filtered averaged QRS [fQRSd] >114 ms; low amplitude signal duration <40 mV [LAS-40] >38 ms, and root-mean-square voltage in the last 40 ms of the QRS [RMS40] <20 µV). B, Performance of late potentials in diagnosing cardiomyopathy-related RVOT-VAs according to cutoff values of SAECG parameters derived from receiver operating characteristic analysis (fQRSd ≥100 ms; LAS40 >38 ms, and root-mean-square voltage in the last 40 ms of the QRS [RMS40] <20 µV).
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 pathological substrates, at least when adopting current cutoff 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 noninvasive 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 (eg, ablation, implantable cardioverter-defibrillator, 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 cutoff value for defining abnormal fQRSd of ≥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 (eg, family history of sudden death, depolarization/repolarization abnormalities at the 12-lead ECG) are usually sent from 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 (eg, sarcoidosis, Chagas cardiomyopathy, or myocardial infarction) warrants further investigation.

Conclusions
In patients with RVOT-VA, abnormal SAECG parameters reflect the presence of cardiomyopathic involvement of the RVOT and should prompt 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-VA. With the current cutoff values to define abnormal SAECG parameters, most patients with small RVOT areas of cardiomyopathic involvement (ie, <8 cm²) are missed. The diagnostic sensitivity of SAECG in detecting cardiomyopathy-related RVOT-VA 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.

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findings may have important clinical implication particularly in segmental forms of ARVC and in patients with focal myocarditis. The positive predictive value of SAECG in detecting underlying cardiomyopathic substrates was very high, although SAECG was not able to correctly diagnose patients with small areas of RVOT involvement. These findings may be considered when the clinical suspicion is high.

The differential diagnosis between idiopathic and cardiomyopathy-related right ventricular outflow tract arrhythmias (RVOT-VAs) is a clinical challenge for cardiologists, as they may represent the early manifestation of concealed cardiomyopathies that can unpredictably lead to sudden cardiac death in the absence of overt structural RV abnormalities. Signal-averaged ECG (SAECG) is a quick and inexpensive diagnostic tool to disclose the presence of pathologically slow conduction areas in the RV (ie, late potentials) due to underlying cardiomyopathic substrates. The present study elucidates the pathophysiologic basis of SAECG abnormalities in patients with RVOT-VAs, showing that they correlate with the extent of the pathological involvement of the RVOT by cardiomyopathic substrates. Such an association appeared independent from the underlying pathological substrate, as it was confirmed both in patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) and in those with myocarditis. The positive predictive value of SAECG in detecting underlying cardiomyopathic substrates was very high, although SAECG was not able to correctly diagnose patients with small areas of RVOT involvement. These findings may have important clinical implication particularly in segmental forms of ARVC and in patients with focal myocarditis and mild or absent RV abnormalities. In patients with RVOT-VAs, the detection of abnormal SAECG parameters during the noninvasive workup 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 areas of RVOT involvement by cardiomyopathic substrates, and more advanced tests including electroanatomic mapping with biopsy should be considered when the clinical suspicion is high.

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Correlation Between Signal-Averaged ECG 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

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