ORIGINAL RESEARCH

Endomyocardial Biopsy: The Forgotten Piece in the Arrhythmogenic Cardiomyopathy Puzzle

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BACKGROUND: Endomyocardial biopsy (EMB) is part of 2010 Task Force Criteria (TFC) for arrhythmogenic right ventricular cardiomyopathy (ARVC). However, its usage has been curtailed because of its low presumed diagnostic yield, and it is now a poorly used tool. This study aims to analyze the contribution of EMB to the final diagnosis of ARVC.

METHODS AND RESULTS: We included 104 consecutive patients evaluated for a suspicion of ARVC, who were referred for EMB. Patients with suspected left dominant pattern were excluded from the primary analysis. Subjects were initially stratified according to TFC without considering EMB. After EMB, patients were reclassified accordingly, and the reclassification rate was calculated. EMB yielded a diagnostic finding in 92 patients (85.5%). After including EMB evaluation, 20 (43%) more patients “at risk” received a definite diagnosis of ARVC. Overall, 59 patients received a definite diagnosis of ARVC, 34% only after EMB. EMB appeared to be the better-performing exam with respect to the final diagnosis (β, 2.2; area under the curve, 0.73; P<0.05). The reclassification improvement after EMB measured 28%. TFC score increased from 3.5±1.3 to 4.3±1.4 (P<0.001). Notably, active inflammation was present in 6 (10%) patients. Minor complications were reported in only 2% of the cohort. In patients with suspected left-dominant disease, conventional TFC performed poorly.

CONCLUSIONS: Electroanatomic voltage mapping–guided EMB was safe and yielded an optimal diagnostic yield. It allowed upgrading of the diagnosis of nearly one-third of the patients considered “at risk.” Classical TFC without EMB performed poorly in patients with the left dominant form of ARVC.

Key Words: arrhythmogenic cardiomyopathy ■ cardiac magnetic resonance ■ electroanatomic mapping ■ endomyocardial biopsies ■ right ventricular arrhythmogenic cardiomyopathy ■ task force criteria

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an underdiagnosed clinical entity characterized by life-threatening ventricular arrhythmias and a progressive fibrous of fibro-fatty replacement of the myocardium.1 ARVC diagnosis is probably the most challenging in the field of inherited cardiomyopathies because of the absence of a unique diagnostic criterion or test, variable expressivity, and incomplete penetrance. At present, ARVC diagnosis is based on a scoring system known as the 2010 Task Force Criteria (TFC).2,3 Endomyocardial biopsy (EMB) represents 1 of the 6 “pieces” in the puzzle of ARVC
diagnosis. However, the role of EMB in the diagnosis of ARVC is still controversial because of its low sensitivity. This is testified by the low number of EMBs being reported in recent ARVC registries and is also supported by current guidelines and societies statements. Yet the early stage of the disease may often go unrecognized by noninvasive evaluation, and EMB also allows to recognize arrhythmogenic cardiomyopathy (ACM) phenocopies (myocarditis, sarcoidosis, or idiopathic dilated cardiomyopathy) apart. Additionally, growing evidences support the existence of an arrhythmogenic left ventricular cardiomyopathy (ALVC), for which no specific validated diagnostic criteria exists yet. For all these reasons, EMB’s role is far from being useless in this setting.

This paper aims to analyze the diagnostic performance of 2010 TFC in a cohort of patients with suspected ARVC. Furthermore, we also aim to assess the diagnostic performance of electroanatomic voltage mapping (EVM) guided EMB and its safety in patients with ARVC.

METHODS

Study Population
We included all consecutive patients with a suspicion of ARVC according to 2010 TFC admitted to 2 tertiary referral centers for cardiac arrhythmias (Monzino Cardiology Center, Milan, Italy; and Marche Polytechnic University, Ancona, Italy) between November 2010 and May 2020. Patients with a suspected left-dominant pattern were excluded from the primary analysis. The study protocol was approved by the Ethical Committee of the Monzino Cardiology Center (R1115/20-CCM1179) in compliance with institutional standards, national legal requirements, and the Declaration of Helsinki. All patients agreed to participate in the study, providing informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Data Collection
Data were retrospectively collected from medical records and included clinical history and diagnostic tests necessary to fulfill the TFC: ECG, Holter recordings, cardiac magnetic resonance (CMR) imaging, echocardiography, genetic testing, and family history. Other clinically relevant diagnostic tests (eg, coronary angiograms, exercise stress tests, and electrophysiology study) were upon discretion of the managing physician. Genetic analysis was always performed by next-generation sequencing (NGS Illumina NextSeq, with the TruSight Cardio Sequencing Kit). Specifically, we screened patients for pathogenic variants in a prefixed panel of desmosomal (ie, plakophilin-2 [PKP2], plakoglobin [JUP], desmoglein-2 [DSG2], desmocollin-2 [DSC2], and desmoplakin [DSP]) and nondesmosomal genes (TMEM43, RYR2, PLN, SCN5A, and LMNA) that were previously reported to be associated with the disease.

Nonstandard Abbreviations and Acronyms

| Abbreviation | Definition |
|--------------|------------|
| ACM          | arrhythmogenic cardiomyopathy |
| ALVC         | arrhythmogenic left ventricular cardiomyopathy |
| ARVC         | arrhythmogenic right ventricular cardiomyopathy |
| EMB          | endomyocardial biopsy |
| EVM          | electroanatomic voltage mapping |
| TFC          | Task Force Criteria |
Endomyocardial Biopsy

EMB was performed in accordance with international guidelines. In particular, EMB was required (1) when TFC without EMB were insufficient to achieve a definite diagnosis; and (2) when, although a definite diagnosis of ACM was reached, the possibility of phenocopies was high, in particular in patients without genetic predisposition. Figure 1 depicts the EMB algorithm. A detailed description of EMB is reported in Data S1.

Diagnostic Classification

According to recent guidelines, arrhythmogenic cardiomyopathy is defined as “an arrhythmogenic heart muscle disorder not explained by ischemic, hypertensive or valvular heart disease.” Yet the same terminology is often used referring to either left or biventricular forms of arrhythmogenic cardiomyopathy. Not to be misinterpreted, we specify that when generally referring to both ALVC and ARVC, we will use the term ACM, which does not include infiltrative diseases, channelopathies, noncompaction cardiomyopathy, inflammatory cardiomyopathy, and idiopathic cardiomyopathy.

Two diagnostic classifications of ACM were used. First, patients were classified according to TFC without EMB. Major (2 points) and minor (1 point) criteria were summed and, if the combined score was ≥4, patients were labeled as “definite ACM.” Otherwise, if the combined score was 2 or 3, patients were considered “at risk” for ACM. In particular, patients with a score of 2 were considered with a “possible” diagnosis, while patients with 3 had a “borderline” diagnosis. If the score was <2, the patient was not included in the study.

Second, we reevaluated TFC, taking into account EMB results in each subject. After EMB, patients were reclassified accordingly, and the reclassification rate was calculated.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics 23. Continuous variables are reported as mean±SD for normally distributed variables, and as median (first to third quartile) for nonnormally distributed variables. Categorical variables are reported as counts and percentage. Comparisons between groups were undertaken with parametric (Student’s t test) or nonparametric tests (Mann-Whitney U-test), as appropriate. The comparisons between categorical variables were performed with the χ² test and the Fisher exact test, as indicated. Using the final diagnosis as a reference, the diagnostic performance of each TFC was evaluated with regard to sensitivity, specificity, and area under the curve. To estimate the relative weights of each different TFC component, logistic regression was used. The diagnostic and classification contribution of EMB was evaluated by assessing the reclassification improvement. Two-tailed P values <0.05 were considered statistically significant.

Figure 1. Endomyocardial biopsy decisional algorithm.

ACM indicates arrhythmogenic cardiomyopathy; CMR cardiac magnetic resonance; ECHO, echocardiogram; EMB, endomyocardial biopsy; and TFC, Task Force Criteria.
RESULTS

Patient Population

A total of 104 patients with suspected ACM were included in our study. Mean age was 43.8±13.9 years, and 70% were men. Patients were referred for ECG abnormalities (15%), family screening (9%), arrhythmias (59%), syncope (12%), and heart failure (5%); see Table 1 and Table S1 for details. Eighty-five (82%) patients were referred for suspected ARVC, while the remaining 19 (18%) had suspected ALVC. CMR was performed in 102 patients (98%). Sixty-four patients (62%) underwent genetic testing. A pathognomonic variant was found in 25 patients (39%) in the overall population and in 49% of the patients who reached a definite diagnosis of ARVC. The most common genetic mutations were: PKP2 in 10 patients, DSG2 in 4 patients, and DSP in 6 patients. A detailed report of noninvasive evaluation is displayed in Table S2.

Endomyocardial Biopsy

All patients underwent EVM-guided EMB, which yielded a diagnostic finding in 92 patients (89%). In the remainder of the cohort, the histologic sample was inadequate or not evaluable. A mean of 3.8±1.0 specimens was sampled from each patient. The right ventricle was targeted in 85 (81%) of cases, the left ventricle in 12 (11%), and both ventricles in the remaining 7 (6%). After excluding ALVC patients, 40 (47%) patients presented a histologic pattern diagnostic for ACM. More specifically, 26 (31%) fulfilled a major tissue characterization criterion, while the remaining 14 (16%) fulfilled a minor criterion. Detailed results of the EMB evaluation are reported in Table S3.

Additionally, in 4 patients, EMB excluded ACM, and histologic analyses were suggestive for idiopathic dilated cardiomyopathy. At the end of the diagnostic workout, none of these 4 patients received a definite ACM diagnosis. Notably, only 2 complications were noted (2.2%), both related to vascular access, and both managed conservatively. No cardiac tamponade was observed.

Diagnosis and Task Force Criteria performance

After the initial evaluation, before EMB, 46 (54%) patients were considered at risk (24 [28%] with a possible diagnosis, 22 [26%] with a borderline diagnosis), and 39 (46%) had a definite diagnosis of ACM.

As shown in Figure 2 and Table S4, 20 (43%) patients considered at risk after noninvasive evaluation (12 from the “possible” group and 8 from the “borderline” group) received a definite diagnosis of ACM only after taking EMB into account. In the end, 59 patients received a definite diagnosis of ARVC (34% of these only after EMB). Even in patients who did not reach a definitive diagnosis, 4 were upgraded from possible to borderline. When evaluating the diagnostic performance of each individual TFC component, EMB appeared to be the better performing exam with respect to the final diagnosis of definite ACM (β, 2.2; area under the curve, 0.73; P<0.05 for both) as reported in Figure 3 and Table 2. The reclassification after EMB was of 28%. TFC score increased from 3.5±1.3 per patient to 4.3±1.4 (P<0.001). Table 3 shows how the different components of the TFC contributed to the final diagnosis. As showed by CMR results and confirmed by histologic analysis, 19 patients with suspected ARVC had biventricular...
involvement (13 with a definite diagnosis and 6 with borderline). A representative example of biventricular ACM is reported in Figure S1. Among the 25 patients with pathogenic mutations, 20 already fulfilled the diagnosis of ARVC, even before performing EMB. Among the 5 without a certain diagnosis, hence genetic carries without phenotypic manifestations, 3 were upgraded from borderline to definite, 1 from possible to borderline, and the last one remained unchanged as possible. Concerning the concordance between EMB and CMR, late gadolinium enhancement was present in 76% of patients with final ARVC definite diagnosis. In addition, the right ventricle was dilated in 69% of the patients, while a reduced ejection fraction of the right ventricle was observed in 46%. Late gadolinium enhancement was present in the absence of dilatation or dysfunction in 2 patients, while an aneurysm or bulging was present in 33%. Overall, all patients with positive EMB had at least a minor criterion by noninvasive imaging evaluation, and 6 patients had a major criterion. Table S5 represents the other positive TFC in patients at risk for ARVC, in whom EMB served as crucial test to reach a definite diagnosis of ARVC.

**Patients With Suspected ALVC**

Nineteen patients were referred for suspected ALVC. As reported in Table 1 and Table S1, patients with ALVC had nonsignificantly different age compared with patients with ARVC, and the percentage of men was also similar. This subset of patients was more frequently referred for heart failure evaluation (both acute and chronic), and the arrhythmic burden at presentation was nonsignificantly different. Right ventricular function and dimensions, at CMR evaluation, were normal in all patients; conversely, left ventricular late gadolinium enhancement was present in 95% of patients. A pathognomonic genetic
variant was present in 3 patients out of the 12 in whom genetic screening was carried out.

If classical TFC without EMB were to be applied in this patient population, only 3 (16%) would have been diagnosed with definite ALVC. The reclassification improvement after EMB was of 68%. TFC score increased from 2.8±1.0 per patient to 3.7±1.3 (P=0.04).

Table 4 shows how the different components of the TFC contributed for the final diagnosis. Remarkably, EMB was a significant component (being a major or minor criterion) to the final diagnosis in 86% of definite ALVC.

**DISCUSSION**

In the present study, we evaluated the role of EMB in patients with suspected ACM. The main findings are as follows: (1) EMB, which was always performed under the guidance of EVM, yielded optimal diagnostic performance with a negligible complication rate; and (2) EMB allowed reaching a definite diagnosis of ARVC in 34% of patients considered at risk for ARVC at noninvasive evaluation.

**EMB in ACM: The Missing Piece of the Puzzle?**

TFC encompass structural, histologic, electrocardiographic, arrhythmic, and familial features, which help the clinician in establishing a diagnosis of ARVC. Since 2010, the role of EMB has progressively declined because of its low sensitivity and inherent risks, especially out of fear of myocardial perforation. This trend...
was exasperated to the point that some authors stated that “the use of EMB may no longer be justifiable. …” As for guidelines recommendations, the American Heart Association/American College of Cardiology Foundation/European Society of Cardiology Scientific Statement confers only a class IIIB recommendation (level of evidence C) for EMB in patients with suspected ARVC.5 Indeed, looking at 2 recent large registries describing ARVC, we can note that the percentage of EMB being performed is 7% and 14% for 407 and 140 patients, respectively.14–17

However, imaging evaluation is far from being specific for ARVC. Indeed, Bomma et al18 reported that up to 73% of presumed patients with ARVC were misdiagnosed, based on CMR misinterpretation. Moreover, the agreement between echocardiography and CMR is low, thus reducing the degree of confidence in the results.19 As for the overall performance of 2010 TFC, this was recently analyzed in a paper by Bosman et al.14 They found that TFC have both a sensitivity and a specificity of 92%, with 11% false negatives and 14% false positives. In their study, TFC were compared with the opinion of 3 experts. Only 28 of 407 patients underwent EMB and, surprisingly, EMB fulfilled a major criterion for ARVC in just 1 patient. A wider usage of EMB in their study might have reduced the need for expert opinion, thus making the clinical judgment more objective. Additionally, Bosman et al tried to evaluate the relative weight of individual components of TFC. However, they did not include EMB because of the relatively low number of data. We performed a similar analysis, which is reported in Figure 3 and Table 2. Our analysis is limited by selection bias, having included mostly patients with dubious diagnosis. However, in our subset of patients, these results reinforce the concept that EMB, compared with other components of the TFC, appears to be the better performing exam with respect to the final diagnosis of definite ACM.

Additionally, if differentiating patients with ACM from healthy subjects is important, it is equally important to correctly identify patients with sarcoidosis or chronic myocarditis mimicking ACM. Previous studies have demonstrated that noninvasive tests have poor diagnostic yield in this setting.6,7,19–21 The main reason is that TFC were assessed relative to healthy individuals, which explains the low specificity when facing other arrhythmogenic diseases. Thus, especially in nonfamiliar forms of ACM, EMB might be the only tool able to adequately differentiate ACM from other phenocopies, as previously reported in the literature. In our paper, we have not specifically addressed this issue, as we just aimed to evaluate the confirmatory role of EMB in the setting of 2010 TFC. Yet it is worth reiterating the fact that 12 patients had histologic signs of active inflammation and no major or minor criteria for ACM, while 4 had a histologic pattern of idiopathic dilated cardiomyopathy. These 16 patients are separately reported in Table S6. In summary, a comprehensive clinical and instrumental evaluation is required to correctly manage these patients, and EMB plays a pivotal role.

Finally, ARVC is a progressive disease. Arrhythmic manifestation and structural abnormalities become more and more pronounced following the natural course of the disease, making the diagnosis certain even without EMB during subsequent follow-up. One may thus question the utility of EMB, when a close follow-up may better clarify the diagnosis. However, we believe that the main goal in ARVC management is to anticipate diagnosis and risk stratification at an increasingly earlier stage of the disease, to prevent sudden death attributable to sustained ventricular tachycardia or advanced heart failure.

Old and New Biopsies: What Is the Diagnostic Yield
One of the main reasons leading clinicians to progressively abandon EMB in patients with suspected ARVC is the low presumed sensitivity.4 If we add the potential risk of serious complications being an invasive procedure, the reason for EMB being progressively abandoned in clinical practice becomes intuitive. It has to be noted that ARVC is a segmental disease, which often spares the septum, which is instead the region most frequently sampled during “old” fluoroscopy-guided EMB.4 Obviously, histopathologic findings at EMB may be diagnostic of ARVC if performed in the appropriate position. Hence, the problem is not whether EMB is useful, but whether we are able to correctly identify and sample the diseased tissue.

The first step in this direction was made by Corrado et al,22 who already demonstrated in 2005 that areas of fibro-fatty replacement in the right ventricle could be correctly detected by EVM among patients with ARVC. Following this path and adding electrophysiological tools to conventional EMB (ie, intracardiac echo, EVM, steerable catheters and long sheaths, transseptal approach for left ventricular EMB), Casella et al were able to significantly increase the diagnostic yield of EMB in the setting of different structural cardiomyopathies.5,12,23–26 However, a specific analysis of this “new EMB” in a large population of patients with ACM has never been conducted. Our paper demonstrates that in patients with suspected ACM, EMB has optimal diagnostic yield (89%), with a very low complication rate. In particular, in patients with confirmed ACM, EMB satisfied a diagnostic criterion in 52% of the population, and served as fundamental tool for reaching a definite
diagnosis in 44%. Notably, the intracardiac complication rate was zero, although the right ventricular free wall was also sampled. This result is largely attributed to the use of intracardiac echocardiography, which enables the operator to biopsy “safe spots” of myocardium, away from thinned aneurismal regions, while readily monitoring for complications.

**One Disease, Many Subtypes**

ACM is currently thought to represent a much wider spectrum of disease compared with just 10 years ago.

The first is the mixed pattern of ACM with superimposed myocarditis. Bowles et al demonstrated that some cases of ACM are associated with viral genome in the myocardium and inflammatory infiltrates. The actual classification of such patients is still debatable. However, emerging evidences support the notion that this pattern may represent an early stage or a “hot phase” of the disease, associated with ongoing myocyte death and reactive inflammation. These patients are at increased risk for sudden cardiac death attributable to ventricular fibrillation, as compared with the “stable phase,” which is associated with reentrant ventricular arrhythmias. After EMB, these patients should thus be followed more strictly, and potential preventive tools (eg, implantable cardioverter defibrillator) might be considered, according to clinical judgment. On the other hand, myocarditis (whether infective, toxic, or autoimmune) can mimic ARVC as a disease phenocopy. The sporadic nature of the disease, together with a negative genetic test and clinical follow-up, besides possible personal history or laboratory test in keeping with external triggers of inflammation, can help in differential diagnosis.

The second subtype is the left-dominant form of arrhythmogenic cardiomyopathy. No guideline currently reports criteria for ALVC. In our cohort, strictly adhering to the current TFC without considering EMB, only 3 of 19 patients reached a definite ALVC diagnosis, and the differential diagnosis with chronic myocarditis and idiopathic cardiomyopathies was always challenging. EMB is pivotal in this setting, as an appropriate diagnosis poses significant clinical implications on the management of the patient and its relatives. A revised version of the current TFC as well as precise histologic criteria for left dominant forms are urgently needed to better identify and diagnose patients with ALVC.

**ARVC and Genetic**

ARVC is often a familial disease, and 60% of patients usually carry a causative genetic variant. The high genetic heterogeneity encompasses both desmosomal and non-desmosomal genes. In particular, while ARVC is mainly linked to PKP2 mutations, left ventricular forms are mainly associated with PLN, DSP, DSC2, and DSG2 pathogenic variants. However, the value of genetics in diagnostic criteria is hampered by different limitations, such as the difficult interpretation of variant pathogenicity, the incomplete penetrance, the phenotypic and genetic overlapping with other cardiomyopathies, and the technological limits of the current molecular diagnosis methods.

In particular, the incomplete penetrance and the large variability of clinical manifestations, renders ARVC diagnosis difficult. Indeed, the presence of a putative genetic mutation does not make the patient affected by ARVC. Given the fact that gross structural abnormalities, visible at imaging, are associated with a later stage of the disease, EMB may represent one of the few tools in our hands to adequately identify subclinical ARVC from asymptomatic mutation carriers.

Additionally, some limits of the classical genetic classifications were recently showed by Costa et al, who proved how, according to the new 2015 American College of Medical Genetics and Genomics Criteria, of the genetic mutation considered as putative mutations needed to be reclassified. This led to a downgrade in the diagnosis of 10% of the patients. This knowledge makes the information of papers referring to patients classified before 2015 less accurate, and possibly overemphasizing the effect of the genetic component. Additionally, the genetic panel used in patients with ARVC are under continuous evolution. This is an inherent limitation of all the retrospective studies, being patients tested in 2010, quite different from patients tested in 2019.

Since the identification of a mutation is regarded as a major criterion, it may contribute up to 50% to the diagnosis of ARVC. This is particularly true for left-dominant forms, for which the other diagnostic criteria are less specific. Therefore, a team of experts is needed for genetic data interpretation, and its weight in the diagnostic criteria is still matter of debate.

**Limitations**

It is not possible to define the number of patients in whom the conventional EMB would have been diagnostic, compared with EVM-guided EMB. Nonetheless, the overall percentage of positive EMB for ARVC in our study appears higher than the previously reported data with conventional biopsy.

The number of patients referred for arrhythmias represents more than half of patients evaluated in the study. This reflects the fact that the study was performed by the arrhythmology unit of our center. Thus, a selection bias may be present.

It is worth mentioning that the 2010 TFC specify that EMB has to be taken from the right ventricular free wall myocardium. When analyzing TFC score for EMB in suspected ALVC, specimens taken from the left ventricle were considered. In such cases, minor or major criteria were assigned, as if this specimen was taken...
from the right ventricular free wall. We recognize that this may be a “free” interpretation of the 2010 TFC. Yet we believe that in the absence of standardized criteria for ALVC, this represents the best management strategy in this subset of patients.

Genetic analysis certainly represents a fundamental cornerstone for ARVC diagnosis. We recognize that the low availability of information regarding genetic testing (62% of patients genotyped) is a major limitation of our work. On the other hand, the literature regarding ARVC diagnosis comprises many works that could not provide a full clinical, radiologic, histologic, and genetic evaluations of patients. This is inherent to the retrospective nature of these studies and the different availability provided by different centers. Specifically, EMB evaluation was missing in the vast majority of the cohorts published in recent years or present in <15% of the evaluated patients.

Another potential limitation is the absence of screening for filamin C, which, despite being rarely associated with ARVC, is nowadays usually included in genetic screening panels.

One final limitation is the absence of follow-up, which may have possible implications on the classification of some patients. However, we believe that in this cohort, what is important is not only to reach a diagnosis, but also to reach it as early as possible, to adopt all possible preventive measures.

CONCLUSIONS

This study confirms the high diagnostic efficacy and safety of EVM-guided EMB in patients with ARVC. It reinforces the concept that EMB is still a useful (yet unfortunately underused) tool, allowing upgrading of the diagnostic status of one-third of patients with a suspect ARVC. EMB acquires an even greater importance in patients without a genetic diagnosis, in whom the exclusion of phenocopies is essential, and for which noninvasive procedures do not allow definite results. Our study reinforces the idea that the relative weight of each individual TFC may not be as equal as currently assumed. In patients with suspected left-dominant disease, conventional TFC performed poorly. EMB may be of help, although specific criteria are currently lacking.

ARTICLE INFORMATION

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Supplemental Material
Supplemental Methods

Endomyocardial Biopsy, Protocol:

All procedures were guided by endocavitary EVM acquired with the CARTO system (Biosense Webster) and intra-cardiac echography (ICE) as previously described. Operators decided whether to map RV, LV or both based on the disease anatomical distribution, as shown by CMR and the presumed origin of ventricular arrhythmias, as assessed by 12-lead ECG. At least 150 mapping points were collected with an irrigated ablation catheter. A contact force of $\geq 5$ g was considered adequate. The voltage maps were edited setting the point density at 5 mm, manually eliminating intracavitary points, and re-confirming low voltage area by acquiring further points by the ablation catheter. Whenever low voltages regions were identified, a second mapping was performed in these areas to confirm this finding. Normal references value for identifying normal endocardial bipolar voltage was defined as $>1.5$ mV and normal unipolar voltages were defined as $>5.5$ mV in RV and $>8.3$ mV in the LV. At the end of the mapping phase, a merge of EVM and ICE-3D mapping was performed in order to check the completeness of EVM and the correlation between low-voltage areas and dyskinetic and/or fibrotic areas. The bioptome catheter was visualized into the CARTO system in order to correctly position the bioptome where the diseased myocardium was identified. In this way low voltage regions were identified. The bioptome (Bipal, Biosense Webster) was introduced through the right femoral vein to target regions of altered potentials with the help of a steerable sheath (Agilis NxT, St. Jude Medical). In case of left ventricular (LV) EMB, a transseptal approach was used. Per procedure, 3 to 6 samples were obtained. When no abnormal EVM voltages were encountered, bioptic samples were retrieved from regions of interest, as identified by CMR and/or ICE.(12)

All EMB samples were referred to the Cardiovascular Pathology Core Lab at the University Hospital in Padua. Histo-morphometric analysis is routinely applied to provide the final pathology report in patients with a suspicion of ARVC. More in detail, histological examination was performed on hematoxylin– eosin- and Heidenhain trichrome-stained slides to ascertain fibrous or fibrofatty replacement on right ventricle EMB. According to the updated 2010 diagnostic criteria, the histomorphometry quantification of fibrous or fibrofatty
replacement with <60% residual myocardium in at least one EMB sample is a major criterion, and 60%–75% residual myocardium is a minor criterion for ARVC.(2)

Cardiac Magnetic Resonance (CMR) protocol:

All CMR studies performed at our centers were performed with a 1.5-T unit (Discovery MR450, GE-Healthcare, Milwaukee, MN). All studies were carried out using dedicated cardiac software, phased-array surface receiver coils, and electrocardiogram triggering. Breath-hold steady-state free-precession cine imaging was performed in vertical and horizontal long-axis and in short-axis orientations. A stack of short-axis slices encompassing both ventricles from base to apex was used for biventricular volumes, mass and systolic function assessment. In addition, for ruling out ARVC, a set of axial long-axis views from diaphragm to the right ventricular outflow tract was acquired. The following acquisition parameters were applied: 30 phases, 10-25 views per segment, NEX 1, FOV 40 cm, a matrix of 224 x 224, a 60° flip angle, TR 3.6-4.2 and TE = TR/2. For detecting fat infiltration, the FSE/STIR method was used. Conventional breath hold T1 weighted fast spin echo images were acquired in the same short-axis views (8-mm slice thickness, no gap) and long-axis views with the following parameters: for FSE NEX 1, FOV 40 cm, matrix of 256x256, TR 1 RR interval and TE minimum (range 4.5-7.8 ms). A breath-hold short-TI inversion-recovery (STIR) spin-echo pulse sequence was used in the same short-axis and long-axis views with the following parameters: NEX 1, FOV 400 mm, TR 2 R-R intervals, TE 60 ms, TI 150 ms, matrix 256x256 and slice thickness 8 mm. A contrast-enhanced breath-hold segmented T1-weighted inversion-recovery gradient-echo sequence was used for myocardial fibrosis detection using the LGE technique. LGE-imaging was performed 10-20 minutes after administration of an intravenous bolus of 0.1 mmol/kg gadolinium-BOPTA (Multihance, Bracco, Milan, Italy). Inversion time was individually adapted to null the signal of remote myocardium (usual range 220-300 ms). The following parameters were used: FOV: 380-420 mm, TR/TE 4.6/1.3 ms, α 20°, matrix 256x192, ST 8 mm and no interslice gap.

CMR analysis
All exams were centrally analyzed at our center. CMR datasets were transferred to a dedicated workstation and analyzed with a cardiac software (cvi42, Circle Cardiovascular Imaging, Calgary, Canada) by two expert readers blinded to patient clinical history and data. For any disagreement on data analysis between the two readers, consensus agreement was achieved involving a third expert reader. On the stack of cine short-axis images, epicardial and endocardial contours were outlined by manual contouring and the papillary muscles were included in LV myocardial mass. Left ventricular volumes, stroke volume and ejection fraction were also quantified using the stack of cine short-axis images. Left ventricular volume, stroke volume and mass were normalized to body surface area. Right ventricle abnormalities such as right ventricle dilation, reduction of right ventricle ejection fraction, abnormalities of free wall kinesis and right ventricle LGE were assessed.
Table S1. Imaging baseline characteristics.

| Echocardiography | Overall (n = 104) | Suspected ARVC (n =85) | Suspected ALVC (n =19) | P |
|------------------|-------------------|------------------------|------------------------|---|
| LVEF, %          | 57.8 (10.6)       | 56.4 (8.8)             | 49.4 (14.6)            | 0.001 |
| LVEDV, mL        | 66.8 (24.1)       | 63.4 (19.0)            | 81.8 (36.6)            | 0.004 |
| LV WMA           | 14 (13.5)         | 12 (14.1)              | 2 (10.5)               | NS |
| RV FAC           | 38.1 (9.3)        | 37.1 (9.1)             | 41.7 (7.1)             | 0.04 |
| TAPSE, mm        | 21.5 (4.2)        | 21.3 (4.2)             | 22.9 (3.7)             | NS |
| RVOT PLAX, mm    | 27.8 (4.3)        | 28.1 (4.4)             | 26.7 (3.7)             | NS |
| RVOT PSAX, mm    | 26.0 (3.4)        | 26.3 (3.4)             | 24.9 (3.4)             | NS |
| RV WMA (%)       | 25 (24)           | 25 (34.1)              | 0 (0)                  | 0.003 |

| CMR               |                    |                        |                        |    |
|-------------------|---------------------|------------------------|------------------------|---|
| Available CMR     | 102 (96.2%)         | 81 (95.3)              | 19 (100)               | NS |
| LV EDVi, ml/m2    | 96.1 (28.0)         | 92.8 (26.0)            | 116.7 (32.1)           | 0.01 |
| LVEF, %           | 53.3 (9.2)          | 54.7 (8.5)             | 47.3 (9.8)             | 0.001 |
| LVEF <50%         | 28 (27.5)           | 17 (21.0)              | 11 (57.9)              | 0.002 |
| LV dilatation     | 26 (25.5)           | 17 (21.0)              | 9 (47.4)               | <0.001 |
| RV EDVi, ml/m2    | 103.3 (30.2)        | 107.1 (30.9)           | 81.7 (12.7)            | 0.009 |
| RVEF, %           | 48.5 (11.9)         | 45.9 (11.3)            | 59.8 (6.9)             | <0.001 |
| RVEF <40%         | 37 (36.2)           | 37 (45.7)              | 0 (0)                  | <0.001 |
| RV dilatation     | 45 (44.0)           | 45 (55.5)              | 0 (0)                  | <0.001 |
| WMA               | 72 (70.5)           | 63 (74.1)              | 9 (47.4)               | 0.02 |
| LGE               | 75 (73.5)           | 57 (67.1)              | 18 (94.7)              | 0.02 |
| Biventricular involvement | 19 (18.3) | 19 (22.3) | // | // |

CMR cardiac magnetic resonance, EF ejection fraction, EDV end-diastolic volume, FAC fractional area change, LV left ventricle, RV right ventricle, RVOT right ventricular outflow tract, PLAX parasternal long axis, PSAX parasternal short axis, TAPSE tricuspid annular plane systolic excursion, WMA wall motion abnormalities.
| Non-invasive Diagnostic Evaluation | Possible N=24 | Borderline N=22 | Definite N=39 | Total N=85 |
|-----------------------------------|--------------|----------------|--------------|------------|
| Echocardiography                  | 24 (100%)    | 22 (100%)      | 39 (100%)    | 85 (100%)  |
| Cardiac Magnetic Resonance        | 24 (100%)    | 22 (100%)      | 35 (90%)     | 81 (95%)   |
| ECG                               | 24 (100%)    | 22 (100%)      | 39 (100%)    | 85 (100%)  |
| HOLTER ECG                        | 24 (100%)    | 22 (100%)      | 39 (100%)    | 85 (100%)  |
| Electrophysiological Study (EPS)  | 20 (83%)     | 19 (86%)       | 38 (97%)     | 77 (90%)   |
| Positive EPS                      | 1 (4%)       | 6 (27%)        | 9 (23%)      | 16 (19%)   |
| Complete Familiar Pedigree        | 24 (100%)    | 22 (100%)      | 39 (100%)    | 85 (100%)  |
| Genetic Analysis                  | 11 (46%)     | 13 (59%)       | 25 (64%)     | 49 (58%)   |
Table S3. Detailed results of histo-morphometric quantification on EMB samples.

| Site | N. fragments | N. fragments with fibrosis/fibroadiposis | % myocardium | % fibrosis/fibroadiposis |
|------|--------------|----------------------------------------|--------------|--------------------------|
| RV   | 4            | 2                                      | 93,77        | 6,23                     |
| LV   | 5            | 4                                      | 44,84        | 55,16                    |
| RV   | 6            | 2                                      | 46,07        | 53,93                    |
| RV   | 4            | 1                                      | 95,81        | 4,19                     |
| RV   | 1            | 1                                      | 14,79        | 85,21                    |
| RV   | 3            | 2                                      | 78,76        | 21,24                    |
| RV   | 4            | 4                                      | 14,79        | 85,21                    |
| RV   | 4            | 4                                      | 79,12        | 20,88                    |
| RV   | 3            | 2                                      | 98,45        | 1,55                     |
| RV   | 3            | 3                                      | 74,76        | 25,24                    |
| RV   | 3            | 3                                      | 29,30        | 70,70                    |
| RV   | 5            | 3                                      | 12,33        | 87,67                    |
| RV   | 3            | 1                                      | 83,86        | 16,14                    |
| LV   | 3            | 3                                      | 48,74        | 51,26                    |
| RV   | 5            | 3                                      | 58,16        | 41,84                    |
| LV   | 5            | 3                                      | 47,95        | 52,05                    |
| RV   | 5            | 1                                      | 90,54        | 9,46                     |
| RV   | 5            | 1                                      | 90,65        | 9,35                     |
| RV   | 4            | 3                                      | 14,70        | 85,30                    |
| RV   | 4            | 4                                      | 28,94        | 71,06                    |
| LV   | 4            | 1                                      | 86,78        | 13,22                    |
| BIV  | 3            | 2                                      | 43,06        | 56,94                    |
| RV   | 2            | 2                                      | 55,67        | 42,33                    |
| RV   | 3            | 3                                      | 57,63        | 42,37                    |
| BIV  | 3+2          | 3                                      | 50,45        | 49,55                    |
| BIV  | 3            | 0                                      | 94,95        | 5,05                     |
| RV   | 3            | 1                                      | 58,28        | 41,72                    |
| BIV  | 2+1          | 2                                      | 62,03        | 37,97                    |
| RV   | 1            | 0                                      |              |                          |
| RV   | 5            | 2                                      | 65,89        | 34,11                    |
| RV   | 3            | 3                                      | 32,34        | 67,66                    |
| LV   | 5            | 4                                      | 69,34        | 30,66                    |
| LV   | 4            | 1                                      | 52,03        | 47,97                    |
| LV   | 7            | 5                                      | 81,2         | 18,8                     |
| BIV  | 5            | 3                                      | 47,2         | 52,8                     |
| LV   | 2            | 2                                      | 45,7         | 54,3                     |
| LV   | 8            | 3                                      | 85,4         | 14,6                     |
|   |   |   |   |   |
|---|---|---|---|---|
| LV | 3 | 1 | 50.7 | 49.3 |
| LV | 6 | 4 | 51.6 | 48.4 |
| LV | 4 | 1 | 90.6 | 9.4  |
| LV | 3 | 2 | 54.6 | 45.4 |
| BIV| 8 | 8 | 84.1 | 15.9 |
Table S4. Diagnosis: cross-tabulation before and after endomyocardial biopsy (EMB).

| TFC (EMB+) | Definite | Borderline | Possible |
|------------|----------|------------|----------|
| TFC (EMB-) | 39       | 0          | 0        |
| %          | 46%      | 0%         | 0%       |
| Borderline | 12       | 10         | 0        |
| %          | 14%      | 12%        | 0%       |
| Possible   | 8        | 4          | 12       |
| %          | 9%       | 5%         | 14%      |
| Total      | 59       | 14         | 12       |
| Total %    | 69%      | 16%        | 14%      |

Total % 100%
Table S5. Task Force Criteria (TFC) which were positive in patients “at risk” for ARVC, who reached a definite diagnosis after EMB.

|       | TFC 1 - Structural Abnormalities | TFC 3 - Repolarization | TFC 4 - Depolarization | TFC 5 - Arrhythmias | TFC 6 - Family History |
|-------|----------------------------------|-------------------------|-------------------------|---------------------|-------------------------|
| minor | 16                               | 0                       | 2                       | 14                  | 2                       |
| MAJOR | 2                                | 2                       | 1                       | 3                   | 1                       |
Table S6. Patients with no major/minor criteria for ACM, but with other pathological findings at EMB.

|   | B D | No Family history | 0 |   |   |
|---|-----|-------------------|---|---|---|
| 1 | 49 yrs. | Normal ECG | 0 | Male | SVT LBBB, superior axis |
|   |   | CMR Minor Criteria | 1 | Palpitations | TFC criteria = |
|   |   |   |   |   | EMB: Myocarditis |

The patient was known for SVT with previous ICD implant and ablation. CMR showed mildly dilated right ventricle with an area of akinesia, LGE and fatty infiltration. EVM identified a region of low potential corresponding to LGE. EMB was taken from that site not reaching criteria for ARVC but showing inflammatory infiltrates.

|   | B M | No Family history | 0 |   |   |
|---|-----|-------------------|---|---|---|
| 2 | 52 yrs. | Normal ECG | 0 | Male | PVC LBBB, sup axis |
|   |   | CMR Minor Criteria | 1 | Heart Failure | TFC criteria = |
|   |   |   |   |   | EMB: dilated cardiomyopathy |

A new diagnosis of HF with reduced ejection fraction was made. CMR showed large areas in the septum and posterior wall with fibro-fatty replacement. In the suspicion of ALVC, EMB was performed in areas of pathological tissue at EVM, corresponding to LGE.

|   | C G | Family history: minor | 1 |   |   |
|---|-----|-----------------------|---|---|---|
| 3 | 32 yrs. | ECG: minor | 1 | Male | Arrhythmias: none |
|   |   | CMR Major Criteria | 2 | Heart Failure | TFC criteria = |
|   |   |   |   |   | EMB: Myocarditis |

First degree relative died at 20 yrs. with suspected ARVC. Negative T waves V3-V6. RVEF 40% with area of dyskinesia and LGE infero-lateral. EMB from that site, area of pathological EVM, revealed active myocarditis.

|   | C P | No Family history | 0 |   |   |
|---|-----|-------------------|---|---|---|
| 4 | 53 yrs. | Normal ECG | 0 | Male | PVC > 500 |
|   |   | CMR Minor Criteria | 1 | Palpitations | TFC criteria = |
|   |   |   |   |   | EMB: Myocarditis |

At CMR, apical dyskinesia of the right ventricle with mildly increased volumes. Frequent PVC.

|   | C M | No Family history | 0 |   |   |
|---|-----|-------------------|---|---|---|
| 5 | 66 yrs. | Normal ECG | 0 | Male | SVT LBBB, superior axis |
|   |   | CMR Minor Criteria | 1 | Syncope | TFC criteria = |
|   |   |   |   |   | EMB: Myocarditis |

Patient known for recurrent syncope was admitted to the ED with SVT. Subsequent CMR showed reduced LVEF with areas of akinesia and fibro-fatty infiltrates in the LV. EVM identified low potentials in the areas of LGE and EMB was performed in the suspicion of ALVC but revealed inflammatory infiltrates.

|   | C M | No Family history | 0 |   |   |
|---|-----|-------------------|---|---|---|
| 6 | 49 yrs. | Neg. T wave V1-V3 | 1 | female | No arrhythmias |
|   |   | CMR major criteria | 2 | ECG alterations | TFC criteria = |
|   |   |   |   |   | EMB: dilated cardiomyopathy |

The patient was evaluated for suspected Brugada. Both echo and CMR found a dilated RV with focal area of wall motion abnormality. EMB was suggestive of DCM.

|   | F G | Family history for SCD | 0 |   |   |
| Age | Gender | ECG Abnormalities | TFC Criteria | EMB | Diagnosis |
|-----|--------|-------------------|--------------|-----|-----------|
| 25 yrs. | Female | Negative T wave V1- V3 | 2 | | Frequent PVC are incidentally diagnosed. At CMR RV is mildly dilated with segmental akinesia and LGE. EMB is diagnostic for acute myocarditis |
| | | Frequent PVC | 1 | | |
| | Sport Evaluation | TFC criteria = 1 | | | |
| | | EMB: Acute Myocarditis | | | |
| 8 | P M | No Family history | 0 | | Recurrent syncope. Evidence of frequent PVC and TVNS. RV dysfunction with areas of akinesia and LGE confirmed at EVM. EMB confirms myocarditis. |
| | 51 yrs. | Normal ECG | 0 | | |
| | Male | TVNS | 1 | | |
| | | CMR Minor Criteria | 1 | | |
| | Syncope | TFC criteria = 2 | | | |
| | | EMB: Lymphocytic Myocarditis | | | |
| 9 | S A | No Family history | 0 | | Frequent PVC and evidence at CMR of dilated RV with LGE and fatty infiltration. EMB shows chronic myocarditis. |
| | 65 yrs. | Normal ECG | 0 | | |
| | Male | Frequent PVC | 1 | | |
| | | CMR Minor Criteria | 1 | | |
| | Sport Evaluation | TFC criteria = 2 | | | |
| | | EMB: Chronic Myocarditis | | | |
| 10 | S N | Family history for SCD | 1 | | Positive family history for SCD. During sport evaluation, evidence of Negative T wave V1-V3. At CMR evidence of fibro-fatty infiltration into the LV, mildly dilated with an area of hypo-kinesia. At EVM guided EMB evidence of lymphocytic myocarditis. |
| | 28 yrs. | Negative T waves V1- V3 | 1 | | |
| | Male | No arrhythmias | 0 | | |
| | | CMR Minor Criteria | 1 | | |
| | Sport Evaluation | TFC criteria = 3 | | | |
| | | EMB: Lymphocytic Myocarditis | | | |
| 11 | S M | No Family history | 0 | | The patient was found to have SVT and ECG abnormalities. At CMR both ventricles were dilated with areas of wall motion abnormalities and LGE. In lights of a recent infective disorders, EMB was performed to rule out myocarditis. |
| | 43 yrs. | Negative T waves | 1 | | |
| | Male | SVT LBBB, superior axis | 2 | | |
| | | CMR Major Criteria | 2 | | |
| | Syncope | TFC criteria = 5 | | | |
| | | EMB: Chronic active Myocarditis | | | |
| 12 | G G | No Family history | 0 | | ECG abnormalities and frequent PVC. At CMR evidence of mildly dilated LV with focal area of fatty substitution and LGE. At EMB evidence active inflammation and parvovirus B19. Subsequent genetic evaluation was positive for DSP (Major criteria) |
| | 21 yrs. | Negative T wave V1- V3 | 2 | | |
| | Female | Frequent PVC | 1 | | |
| | | CMR Minor Criteria | 0 | | |
| | ECG abnormalities | TFC criteria = 4 | | | |
| | | EMB: Myocarditis | | | |
| 13 | M M | No Family history | 0 | | |
| Age | Family History | ECG Abnormalities | CMR Major Criteria | EMB: Myocarditis | CMR Minor Criteria | EMB: Dilated Cardiomyopathy | EMB: Eosinophilic Myocarditis | EMB: Idiopathic DCM |
|-----|----------------|-------------------|--------------------|----------------|-------------------|-------------------------|---------------------------|------------------|
| 53 yrs. | male | Negative T wave V1-V3 | 2 | |
| | | TVNS | 1 | |
| | | CMR Major Criteria | 2 | |
| | | ECG abnormalities | TFC criteria = 5 | |
| | | EMB: Myocarditis |  |
| | | ECG abnormalities and TVNS. At CMR evidence biventricular dilation and dysfunction with areas of fibro-fatty infiltration. EMB was negative for fibro-fatty infiltration. However, evidence of active inflammation without viral infection was detected. |
| 14 yrs. | male | No Family history | 0 | |
| | | Negative T wave V4-V6 | 1 | |
| | | Frequent PVC | 1 | |
| | | CMR Minor Criteria | 1 | |
| | | ECG abnormalities | TFC criteria = 3 | |
| | | EMB: Myocarditis |  |
| | | ECG abnormalities and frequent PVC started the investigation. CMR showed biventricular dysfunction, with normal dimension, absence of LGE and dyskinesia of the RV free wall. EVM was negative. EMB on the septum showed idiopathic DCM |
| 15 yrs. | male | No Family history | 0 | |
| | | Normal ECG | 0 | |
| | | Frequent PVC | 1 | |
| | | CMR Minor Criteria | 1 | |
| | | Sport Evaluation | TFC criteria = 2 | |
| | | EMB: Myocarditis |  |
| | | During sport evaluation evidence of frequent PVC. CMR showed a mildly dilated, RV with a focal area of dyskinesia. LGE and EVM are concordant in a region close to the RVOT. EMB performed there shows eosinophilic myocarditis. |
| 16 yrs. | male | No Family history | 0 | |
| | | Negative T waves | 1 | |
| | | NSVT | 1 | |
| | | CMR Major Criteria | 0 | |
| | | NSVT | TFC criteria = 2 | |
| | | EMB: Dilated Cardiomyopathy |  |
| | | Evidence of NSVT and ECG abnormalities. At CMR dilation and dysfunction of the LV with an area of fibro-fatty replacement and dyskinesia. To investigate left dominant ACM, EMB was performed and showed DCM |

ACM arrhythmogenic cardiomyopathy, ALVC arrhythmogenic left dominant cardiomyopathy, ALVC arrhythmogenic right ventricular cardiomyopathy, CMR cardiac magnetic resonance, DCM dilated cardiomyopathy, ED emergency department, EF ejection fraction, EDV end-diastolic volume, EMB endomyocardial biopsy, EVM electroanatomic voltage mapping, HF heart failure, ICD implantable cardioverter defibrillator, LBBB left bundle branch block, LGE late gadolinium enhancement, LV left ventricle, NSVT non-sustained ventricular tachycardia, PVC premature ventricular complex, RBBB right bundle branch block, RV right ventricle, SCD sudden cardiac death, SVT sustained ventricular tachycardia, TFC task force criteria, WMA wall motion abnormalities
Figure S1. Representative case of biventricular arrhythmogenic cardiomyopathy (ACM).

Panel A: late gadolinium enhancement (LGE) and fibro-fatty infiltration into the right ventricular (RV) free-wall. Panel B: LGE infiltration in the infero-basal portion of the interventricular septum of the left ventricle (LV). Panel C and D: RV endomyocardial biopsy (EMB) with focal fibro-fatty substitution of cardiomyocytes (Heidenhain trichrome, C panoramic view – scale bar 200 micron, D, x100 – scale bar 100 micron). Panel E and F: LV EMB with endocardial fibrosis and myocytes changes with abnormal nuclei in proximity to an area of replacement-type fibrosis with few adipocytes (E, Heidenhain trichrome, panoramic view – scale bar 200 micron, F, Hematoxylin-Eosin, x200 – scale bar 50 micron).