THE ROLE OF EXTENSIVE DIAGNOSTIC WORK-UP IN YOUNG ATHLETES AND NON-ATHLETES WITH COMPLEX VENTRICULAR ARRHYTHMIAS

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Short Title: Concealed cardiomyopathies in athletes with ventricular arrhythmias

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Abstract

Background. Ventricular tachyarrhythmias (VA) represent the first cause of death in athletes. The difference between electroanatomic substrate in athletes and non-athletes with complex VA is unknown.

Objective. To compare the electroanatomic substrate of complex VA in athletes versus non-athletes.

Methods. We prospectively enrolled young athletes and non-athletes with VA. Patients underwent 2D echo, cardiac magnetic resonance (CMR), coronary angiography, 3D-electroanatomic mapping (3D-EAM) and 3D-EAM guided endomyocardial biopsy (EMB). Follow-up included 24h ECG Holter or ICD/loop recorder interrogation for VA recurrence.

Results. We enrolled 33 consecutive patients, 18 (56%) competitive athletes and 15 (44%) non-athletes. Left and right ventricular (LV and RV) findings by echo and CMR did not show structural disease. Nine (50%) athletes were asymptomatic compared to 1 (7%) non-athlete ($p<0.05$). Unifocal origin of VA was reported in 14 (93%) athletes and in 17 (94%) non-athletes. Athletes showed a larger RV unipolar than bipolar scar (18 ± 17 cm^2 versus 3 ± 3.8 cm^2, $p= 0.04$). Diagnostic yield of EMB was 50% in athletes and 40% in non-athletes. Among athletes, the final diagnosis was myocarditis in 2 cases, arrhythmogenic ventricular right cardiomyopathy and focal replacement fibrosis in one case each. Among non-athletes, EMB revealed focal replacement fibrosis in 4 cases. At median follow-up of 18.7 months, Kaplan Meyer curves showed lower VA recurrence in detrained athletes than non-athletes (53% versus 6%, $p= 0.02$).

Conclusions. Our data showed the need for an extensive diagnostic work-up in apparently healthy young patients with complex VA in order to characterize concealed cardiomyopathies.

INTRODUCTION

The annual incidence of sudden cardiac death in young athletes has been estimated at 0.7–3.0 per 100.000 [1,2].

The most common cause of death in athletes is sustained ventricular arrhythmias (VA), representing the initial clinical manifestation of concealed cardiomyopathies [1-4].

In order to diagnose proarrhythmic substrates, the ‘‘Italian model’’ of pre-participation screening in athletes, currently recommended by the ESC [5], is based on resting 12-lead ECG and consequent evaluation in the case of premature ventricular contractions. However, subclinical pathological substrates could remain undetected by currently available non-invasive diagnostic techniques [6,7].

The first aim of our study was to evaluate the contribution of an extensive diagnostic work-up in young patients with complex VA, including non-invasive imaging (echocardiography and cardiac magnetic resonance), and invasive three-dimensional electroanatomical mapping (3D-EAM), followed by endomyocardial biopsy (EMB) and genetic testing when clinically indicated.

The second aim was to understand clinical predictors of complex VA recurrence at follow-up.

METHODS

Study design and patient population

In this prospective single-center observational cohort study, we consecutively enrolled two groups (non-athletes versus competitive athletes) of patients with age ≤40 years with complex VA as common clinical presentation, from 2013 to 2018. Complex VA were defined as:

1. High burden of premature ventricular contractions (≥25% of total beats in 24h ECG Holter) or non-sustained ventricular tachycardia during 24h ECG Holter or maximal exercise stress test;
2. Sustained ventricular tachycardia or ventricular fibrillation.

Exclusion criteria were:

1. Coronary heart disease (excluded by normal coronary angiography/coronary computed

tomography-angiography or maximal stress test negative for ischemia);

2. Valvular heart disease;
3. Dilated cardiomyopathy [8];
4. Heart failure;
5. Hypertrophic cardiomyopathy

The athlete group, according to the definition of the American Heart Association [9], underwent pre-participation screening at our Sports Medicine Department. This referral center evaluated the indication for temporary disqualification related to VA, as indicated before [5].

All 18 athletes were admitted to our Arrhythmia Unit and studied by our diagnostic work-up after median time of 3 months (IQR 2-4) of temporary detraining related to disqualification.

A thorough medical history and physical examination were obtained on admission to our Arrhythmia Unit in all patients. Our diagnostic work-up included: 2D-transthoracic echocardiography, CMR, and 3D-EAM. Endocardial bipolar and unipolar voltage mapping was performed by CARTO 3, according to the exit site of clinical VA or premature ventricular contractions and/or CMR abnormalities. Endomyocardial biopsy was guided by low bipolar voltage areas.

Informed consent was obtained from each patient; the study protocol was approved by our institutional review board and ethics committee.

Echocardiography

All echocardiographic images were acquired using a commercially available ultrasound system (Toshiba) with a 1.5–4 MHz phased array transducer.

All echocardiographic data were analyzed by a single experienced researcher according to current recommendations [10].

Cardiac magnetic resonance imaging

Cardiac magnetic resonance was performed according to the current guidelines [11] using an Ingenia 1.5 T MR scanner (Philips Healthcare, Best, The Netherlands). Dynamic contiguous short-axis cine loops from the base to the apex were acquired using a steady-state free precession (SSFP) sequence. Thirty phases for each cardiac cycle were acquired using retrospective gating and expiratory breath-hold. Long-axis (2 chamber and 4 chamber) SSFP cine loops were also acquired. The T2-weighted images were obtained with a triple inversion-recovery-prepared turbo spin-echo sequence in the short axis. Late-gadolinium enhancement images were acquired 10 min after the iv administration of 0.1 mmol/kg of Gadobutrol (Gadovist; Bayer Schering, Berlin, Germany). All acquired images were transferred to a workstation and assessed by a single expert reader.

3D-Electroanatomic mapping: CARTO

A 3.5 mm distal tip irrigated catheter (Navistar Thermocool or Smarttouch, Biosense Webster, Diamond Bar, CA) was used as the mapping catheter. For both ventricles a value of > 1.5 mV defined normal endocardial bipolar electrogram, a value < 0.5 mV defined endocardial "bipolar scar". We evaluated bipolar scar localization and area measurement (cm^2, % of total mapped area) [12].

A value < 8 mV defined left ventricle (LV) endocardial "unipolar scar" and a value < 5 mV defined right ventricle (RV) endocardial unipolar scar. We evaluated unipolar scar localization and area measurement (cm^2, % of total mapped area) [13].

The endocardial unipolar electrogram recording may provide a valuable clue suggesting the presence of an epicardial electroanatomic scar, as previously reported [13].

Endomyocardial biopsy

Endomyocardial biopsy was performed according to the 2007 ESC Guidelines [14].

Left ventricular or biventricular EMB was preferred through the right femoral vein and femoral

artery for access to the RV and LV respectively [15].

Endomyocardial biopsy was guided by low voltage areas detected by 3D-EAM, as described elsewhere[25] and the local site of EMB as well as possible early complications were monitored by intracardiac echocardiography. Samples for histology and immunohistochemical analysis were promptly fixed in 10% formalin or snap frozen in liquid nitrogen depending on the antibody that was going to be used [15].

Serial sections from paraffin-embedded biopsy samples were stained with hematoxylin-eosin and Azan-Mallory trichrome stain and evaluated for the presence and extent of inflammatory infiltrates and/or myocyte damage, and the type of myocyte damage (necrosis, myocytolysis, apoptosis); interstitial and/or focal replacement fibrosis was also analyzed.

The following antibodies were used for the characterization of the inflammatory infiltrates: anti-CD45, CD45RO, CD3, CD4, CD8, CD20 and CD68/PGM1. Histological analysis was performed blindly by two pathologists.

The diagnosis of myocarditis was made according to Caforio et al.[8], the diagnosis of ARVC was made according to modified criteria by Marcus et al.[16].

Genetic testing

Genetic testing for hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, Brugada syndrome and Long QT syndrome was performed by Next Generation sequencing technology. Variant interpretation was based on the American College of Medical Genetics guidelines, as: “Pathogenic”, “Likely Pathogenic”, “Variant of Unknown Significance” (VUS), “Likely Benign” and “Benign” [17]. Genetic testing was performed in cases of sustained VA and/or family history of sudden death.

Follow-up

Follow-up included evaluation of complex VA by 24h ECG Holter monitoring or by

ICD/loop recorder interrogation, performed every 6 months from discharge; VA recurrence indicated as premature ventricular contractions ≥25% at 24h ECG Holter, non-sustained ventricular tachycardia, sustained ventricular tachycardia or ventricular fibrillation were the composite endpoint of our study. In particular, all competitive athletes were detrained after discharge.

Statistical analyses

Continuous data were expressed as mean ± standard deviation, as appropriate, for all of the variables collected in the entire population or specific subgroups. The normally distributed continuous variables were presented as the mean values and standard deviation and were compared using a Student t test. Frequencies were compared using the chi-square test.

In order to assess the accuracy of 3D-EAM compared to CMR in scar detection and to measure the agreement by the two imaging tools, we calculated Cohen Kappa Coefficients with 95% coefficient intervals [18].

To determine whether baseline variables were independently associated with VA at follow-up, a Cox proportional-hazards regression model was applied. The results of the Cox regression analysis are represented with the Hazard ratio (HR) and 95% confidence intervals (CI).

The event-free survival curve was plotted using the Kaplan–Meier method with the statistical significance examined by the log-rank test.

To determine whether detraining was associated with a decrease in arrhythmic events at follow-up, a Mc Nemar test was applied.

The level of statistical significance was set at a 2-tailed alpha level <0.05. All statistical analyses were performed with SPSS® version 20.0 software (© Copyright IBM Corporation 1994, 2017).

RESULTS

Clinical presentation

We consecutively enrolled 33 young patients, with a mean age of 27 years: 18 (56%) were competitive athletes from our Sports Medicine Unit while 15 (44%) were non-athletes from our Arrhythmology Unit (Supplementary table 1). Among the athletes, 13 patients (72%) played mixed and 5 (28%) endurance sports. On admission, athletes were younger (23 vs 32 years old, p= 0.003) and more frequently asymptomatic than non-athletes (50% vs 6% p=0.009, Supplementary table 1). Baseline ECG findings were reported in Supplementary table 1: the mean heart rate was 75 ±15 bpm, mean PR 157 ±20 msec, mean QTc was 400 ±30 msec; 5 patients presented with complete RBBB, 1 patient with LBBB; we did not find Brugada type 1/2 pattern or T wave inversion.

Athletes presented more frequently with non sustained ventricular tachycardia and premature ventricular contractions ≥25% by 24h ECG Holter than non-athletes (88% vs 66%, p=0.043; Supplementary table 1). In particular, 9 athletes presented with PVC>25%, 7 with non sustained ventricular tachycardia and 2 with sustained ventricular tachyarrhythmias. Seven non-athletes presented with PVC>25%, 3 with non sustained ventricular tachycardia and 5 with sustained ventricular tachyarrythmias. VA exits at baseline ECG in the two groups of patients are reported in figure 1 (panel 1A and 1B). Unifocal origin of VA was reported in 14 (93%) athletes and in 17 (94%) non-athletes.

Imaging findings

Athletes and non-athletes did not differ in echocardiographic and CMR findings (Supplementary table 2).

At transthoracic echo, cardiac contractility and volumes were normal in both groups. There was no evidence of significant hypertrophy in both groups. CMR was performed in 30 patients (91%), 17 athletes and 13 non-athletes, and showed normal cardiac volumes and function in both groups, with

late gadolinium enhancement in 5 athletes and in 4 non-athletes, with no difference between right and left ventricle. In particular, subepicardial and transmural pattern were described in 5 and 4 patients respectively, without evidence of subendocardial pattern.

3D-EAM findings

Twenty-seven patients (82%) underwent biventricular 3D-EAM, 3 patients (9%) underwent right ventricular 3D-EAM and 3 patients (9%) left ventricular 3D-EAM as indicated by VA exit.

Right ventricular endocardial 3D-EAM was performed in 30 patients out of 33 (91%) (2 athletes and 1 non-athlete did not undergo 3D-EAM): unipolar and bipolar scar areas were found in 14 (46%) out of 30 patients (8 athletes and 6 non-athletes).

We observed that mean right ventricular mapped volume was significantly larger in athletes than in non-athletes (p= 0.03, Supplementary table 3). Mean right ventricular "bipolar and unipolar scar" areas were similar in both groups. In athletes, right ventricular "unipolar scar" areas were significantly larger than "bipolar scar" areas (p=0.04), while in non-athletes, right ventricular "unipolar and bipolar scar" areas were similar (Supplementary table 3).

Left ventricular endocardial 3D-EAM was performed in 30 patients out of 33 (91%) (2 athletes and 1 non athletes did not undergo 3D-EAM); "unipolar and bipolar scar" areas were found in 6 (20%) out of 30 patients (3 athletes and 3 non athletes). Mean left ventricular "bipolar and unipolar scar" areas were similar in both groups.

The most frequent site of "bipolar and unipolar scar" was the right ventricular outflow tract (13 patients out of 30, 43% of mapped right ventricles, 6 athletes and 7 non-athletes).

We found 9 patients with late gadolinium enhancement detected by CMR compared to 20 patients with scar detected by 3D-EAM, with low inter-reliability between the two imaging tools, (Cohen's Kappa = -0.09).

Histological findings

In our population, 18 patients (54%) underwent EMB by 3D-EAM-detected scar (8 athletes and 10 non-athletes) without complications. At least 3 diagnostic fragments were analyzed from each

EMB. In athletes, 3 EMB were classified as normal myocardium, 2 were classified as "myocarditis", 1 as arrhythmogenic right ventricular cardiomyopathy, 1 as myocardial focal replacement fibrosis (or microscarring), 1 was reported as non-diagnostic for the suboptimal amount of myocardial tissue (fig 2A). Consequently, the diagnostic yield of 3D-EAM-guided EMB in athletes was 50% (4 diagnostic EMB out of 8 biopsied athletes).

In non-athletes, 4 EMB were classified as normal myocardium, 4 as myocardial focal replacement fibrosis, and 2 were reported as suboptimal for diagnostic purposes (fig 2B). Consequently, the diagnostic yield of 3D-EAM-guided EMB in non-athletes was 40% (4 diagnostic EMB out of 10 biopsied non-athletes). Histological patterns of myocarditis and myocardial focal replacement fibrosis are shown in figure 3.

With regard to myocardial focal replacement fibrosis, in the non-athletes group this histological pattern was found in 2 patients with autoimmune disease and microvasculopathy, in 1 patient with inflammatory cardiomyopathy and in 1 patient with evidence of left ventricular late gadolinium enhancement, without history of previous cardiomyopathies. In the athletes group, the single patient with a histological pattern of myocardial focal replacement fibrosis presented with evidence of left ventricular scar, by 3D-EAM without history of previous cardiomyopathies.

Gene variants

Targeted NGS was performed in 15 patients of the cohort including 9 (60%) athletes and 6 (40%) non-athletes, based on the presence of sustained VA and/or family history of sudden death.

Three heterozygous variants were identified in 2 patients (13%), both athletes. In particular, one patient, with suspicion of AVRC, carried a frameshift pathogenic variant c.1707_170 8insAC (p.Met571QfsX8), in the *DSP* gene. The other patient, who had been referred with suspicion of AVRC not confirmed by our clinical pathway, carried two missense variants, both classified as VUS, one in DSP, p .Cys1805Phe and the other in SCN5A, p.Arg693Cys, as shown in Figure 4.

These results were validated by direct sequencing.

Follow-up

At discharge, 8 devices (6 ICD and 2 loop recorders) were implanted as indicated by guidelines [19]. All athletes were discharged with a recommendation of detraining and only 1 was started on antiarrhythmic therapy (β-blockers) while, among non-athletes, 15 patients were discharged on antiarrhythmic therapy (14 β-blockers, 1 IC class).

After a median follow-up of 18.7 months (range 1-51 months), a VA composite outcome occurred in 1 athlete (1 non sustained ventricular tachycardia) and in 8 non-athletes (2 ventricular fibrillation and 6 non sustained ventricular tachycardia), with a higher persistence of complex VA in non-athletes than in detrained athletes (respectively 53% versus 6%, p=0.02, figure 5). Particularly in the athletes group, after detraining from discharge, non sustained ventricular tachycardia dropped from 7 (38%) at baseline to 1 (6%) at follow-up. At Cox univariate analysis, we did not find statistically significant predictors of VA in our population (Supplementary table 4).

DISCUSSION

This is the first study on extensive diagnostic work-up in a series of consecutive young patients with complex VA as first common clinical presentation, including athletes and non-athletes. In our study, athletes presented more frequently with non sustained ventricular tachycardia and premature ventricular contractions >25% in 24h compared to non-athletes (p= 0.04). Moreover, athletes with complex VA were younger (p= 0.003) and more frequently asymptomatic (p= 0.009) compared to non-athletes. This different clinical presentation could be related to an early and effective ECG screening in athletes by Sport Medicine Department, as described in the literature [5, 20].

Figure 4 summarizes the results of our extensive diagnostic work-up in the two groups. We started with the first-level diagnostic tools as echo and CMR. Our young population showed structurally normal heart in terms of cardiac non invasive imaging, with only 30% of patients showing late

gadolinium enhancement, with no difference between athletes and non-athetes. Further evaluation with 3D-EAM, as second level approach, allowed us to identify abnormal low voltage areas in 60% of our population (mainly localized in the right ventricle), with no difference between athletes and non-athletes. Among patients showing abnormal low voltage areas, 3D-EAM-guided EMB documented well-characterized histopathological diagnosis in 50% of athletes and in 40% of non-athletes. In particular, among competitive athletes, the final diagnosis based on our extensive diagnostic work-up has been: 2 myocarditis, 1 myocardial focal replacement fibrosis and 1 arrhythmogenic right ventricular cardiomyopathy. Among non-athletes, our diagnostic work-up identified 4 cases of myocardial focal replacement fibrosis. As take-home message, this study defined a clear pathological substrate in almost half of our apparently healthy young population with VA.

In this regard, in a selected series of 13 athletes with VA, Dello Russo et al. reported normal heart structure by non-invasive imaging but histological diagnosis of concealed cardiomyopathies in whole population (7 myocarditis, 5 arrhythmogenic right ventricular cardiomyopathy, 1 contraction band myocardial necrosis) by 3D-EAM-guided EMB [21]. We have extended this diagnostic work-up to competitive athletes and non-athletes with a common arrhythmic phenotype (complex VA) and a structurally normal heart.

In keeping with previous results, in our study 3D-EAM-guided EMB was safe and effective, providing a total diagnostic yield of 50% in athletes and 40% in non-athletes, including diagnosis of pathological substrates [6, 21].

It is noteworthy that in all non-athletes, the histological substrate was myocardial focal replacement fibrosis, whereas athletes presented more heterogeneous histological patterns. In particular, myocarditis is considered the main acquired cause of sudden cardiac death in athletes and, as previously reported in the literature, high-intensity training may exacerbate heart damage when an ongoing myocarditis is present [2, 3]. On the other hand, the higher prevalence of myocardial focal replacement fibrosis in the non-athlete group could represent a multistep structural

microscarring and consequent electrophysiological remodeling after repeated cardiac injury [22]. In this group, we could not formulate on etiopathological hypothesis for this injury.

Analyzing in detail the different diagnostic tools in our study, 3D-EAM was found more reliable in detecting subtle cardiac arrhythmogenic substrates compared to CMR (kappa Cohen <0.01).

Moreover, endocardial and transmural scars were respectively detected in 33% and 50% of patients, mainly localized in the right ventricular outflow tract, with no difference between the two groups (Supplementary table 3). In keeping with our results, in a recent study by Haissaguerre et al., the highest prevalence of electroanatomic abnormalities was localized by 3D-EAM in the right ventricle despite normal cardiac imaging in 46% of young patients who survived ventricular fibrillation [24]. Endo-epicardial mapping of this group of 24 young survivors with normal CMR suggested that structural alterations involved a part of the right ventricle [24]. In particular, only in our athlete group, the right ventricle transmural scar area was significantly larger than the endocardial scar area; this electroanatomic finding could be related to transmural right ventricle remodeling, due to intermittent pressure and volume overload during training [2].

Regarding genetic findings, a small proportion of patients (2%) of patients carried potentially pathogenic variants in genes involved in structural and/or electrophysiological cardiac abnormalities. Both carriers of these variants, two in the *DSP* and one in the *SCN5A* gene, were athletes. Our results confirm the utility of genetic testing in patients with a highly suggestive clinical phenotype. .

At median follow-up of 18.7 months, non-athletes showed a higher persistence of complex VA than in detrained athletes (respectively 53% versus 6%, p=0.02). In particular, in athletes detrained from discharge, complex VA dropped from 38% at baseline to 7% at follow-up [25]. This result could be the consequence of reduced exercise-related arrhythmogenic triggers in patients with concealed cardiomyopathies [25]. Consequently, our diagnostic work-up could be a useful tool not only to better stratify ventricular arrhythmic risk, but also to avoid vigorous training and

competition in young athletes [26].

This study is subject to several limitations. We enrolled a relatively small number of patients in the two groups. The non-athletes group presented with higher prevalence of symptoms and VT/VF on admission than athletes, with consequent different antiarrhythmic therapy at discharge. These baseline differences could be related to an early screening in athletes. Another limitation is the absence of CMR data in 9% of our population (only 91% of patients performed CMR); moreover, a T1 mapping sequence was not available, with consequent lack of evaluation of replacement fibrosis in these patients [27]. Only eight patients were implanted with devices, which allowed continuous monitoring of VA, while in the remaining patients this information was obtained by 24h-ECG Holter, although this was also the case in several previous studies in this setting [28]. Finally, real-time PCR for virus was not included in in our analysis.

CONCLUSIONS

Our data confirmed the diagnostic value of an extensive diagnostic work-up including CMR, 3D-EAM mapping, 3D-EAM-guided EMB and genetic testing in the characterization of the arrhythmogenic substrate in apparently healthy young athletes without structural cardiac abnormalities,normal resting ECG and complex VA. Future extension of this research including comprehensive CMR evaluation, device monitoring and RT-PCR for the whole population will be needed.

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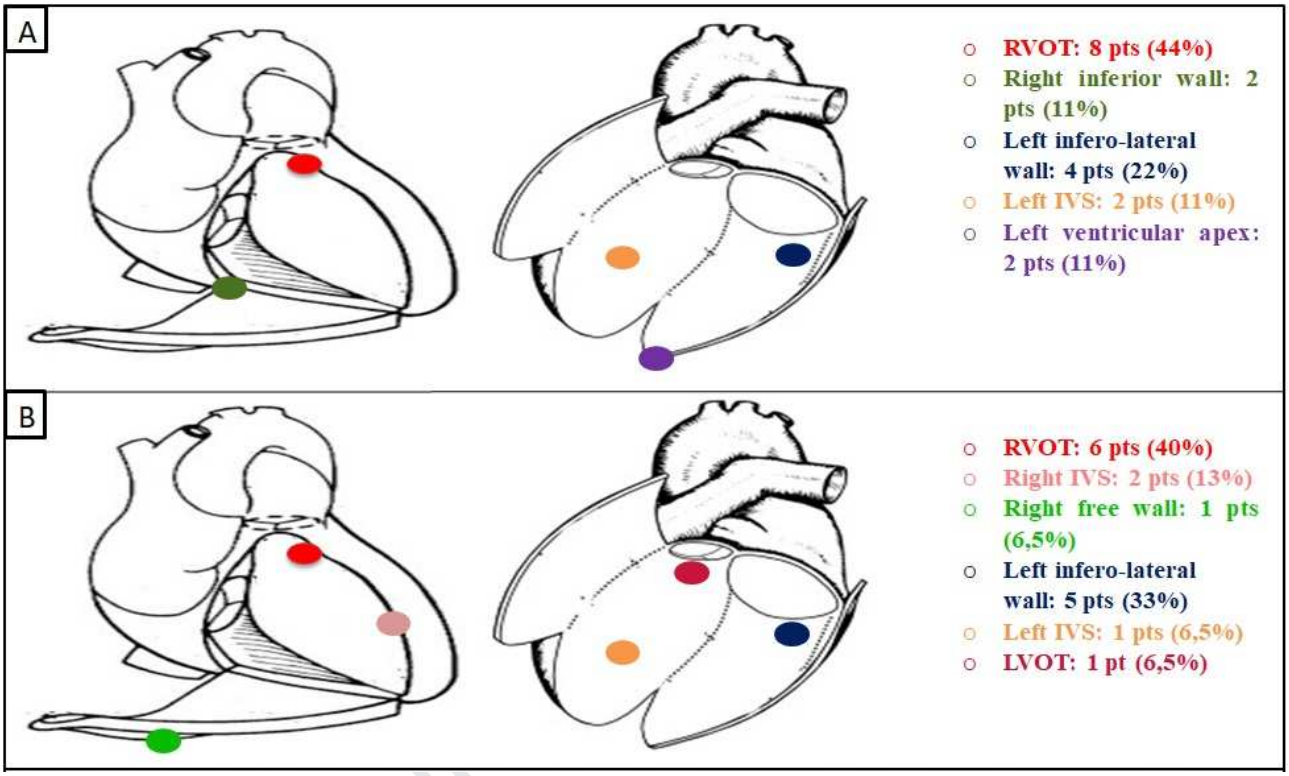
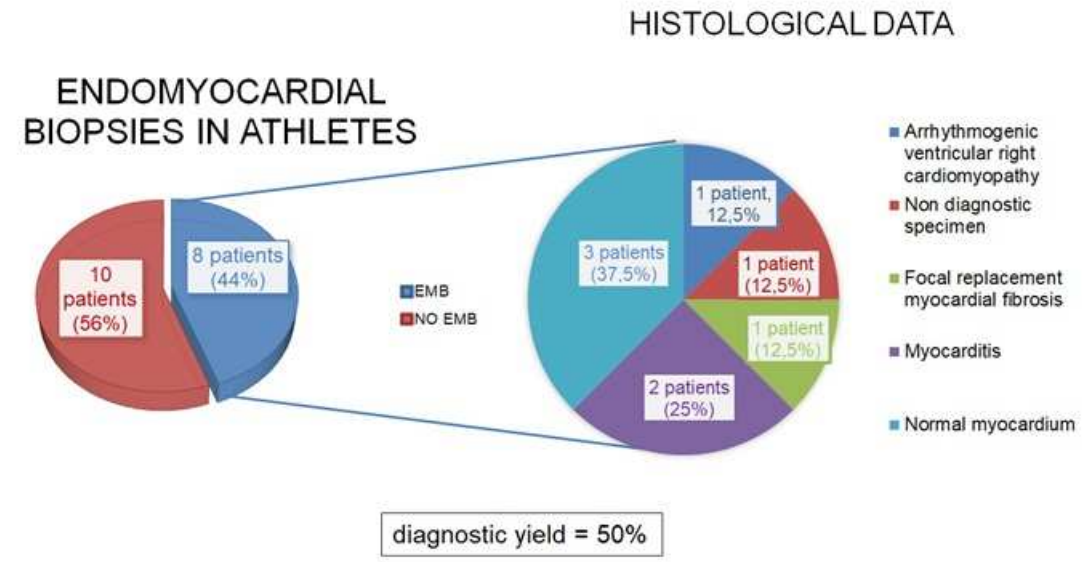
FIGURES

Figure 1. Ventricular arrhythmias exit at 12-lead ECG at baseline in athletes' group (panel A) and in non-athletes' group (panel B). *IVS: interventricular septum; LVOT: left ventricular outflow tract; pt: patient; pts: patients; RVOT: right ventricular outflow tract.*



476 **Figure 2** A. EMB findings in athletes; B. EMB findings in non-athletes



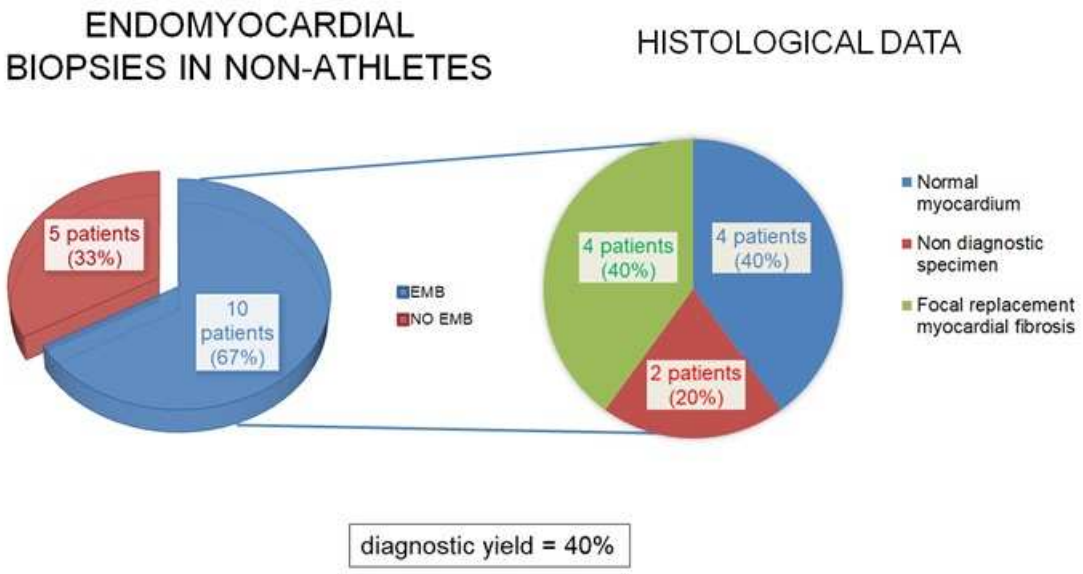


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Figure 3. Histological patterns of myocarditis and focal replacement fibrosis. A&B Endomyocardial biopsy performed in male non-athlete: focal replacement fibrosis in one sample (A: haematoxylin and eosin stain; B: Mallory trichrome stain). C&D Endomyocardial biopsy performed in female athlete: at low magnification morphology no evidence of alterations; with immunohistochemistry, foci of CD3+ lymphocytic infiltration suggest borderline myocarditis (C: haematoxylin and eosin stain; D: immunoperoxidase).

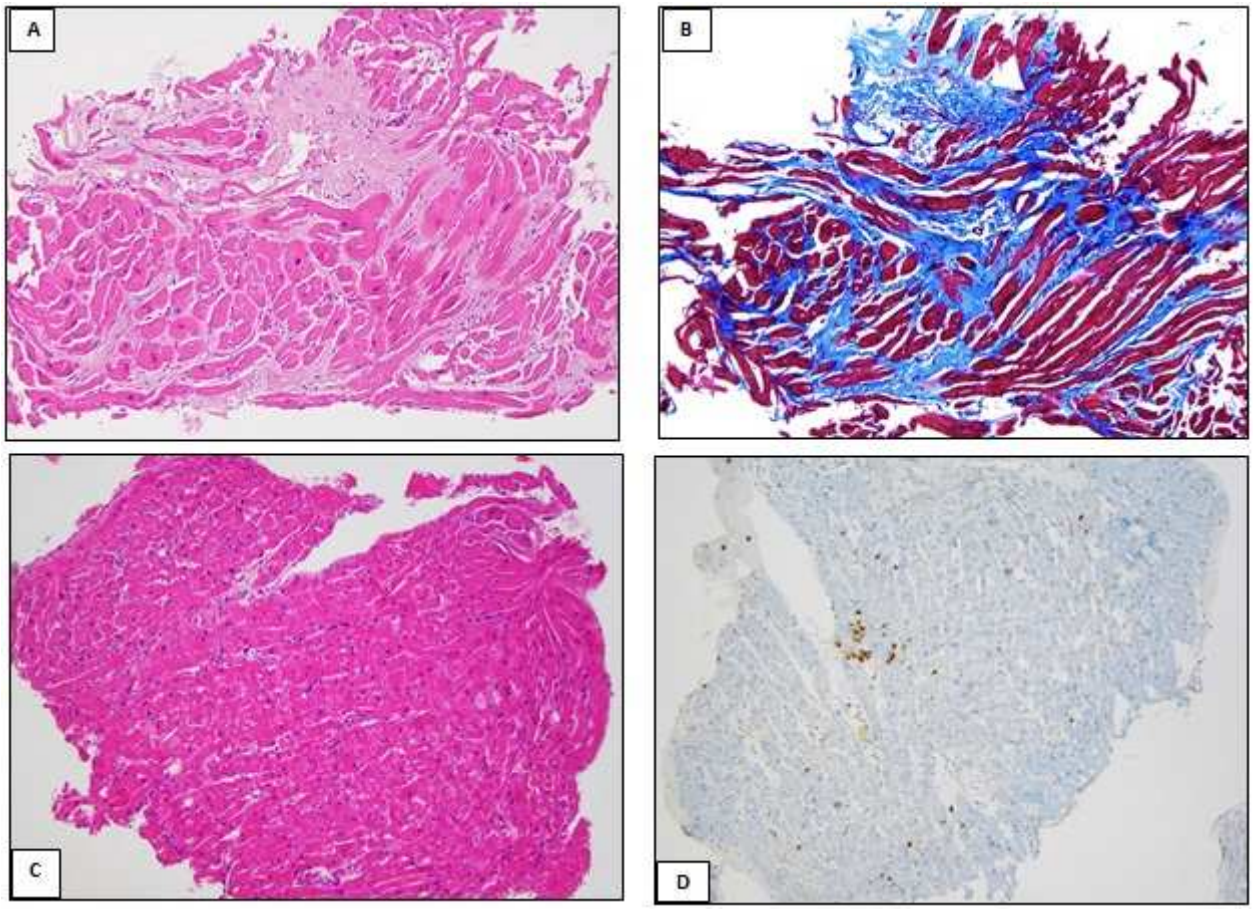


Figure 4. A. Diagnostic Work-up in athletes; B. Diagnostic Work-up in non-athletes

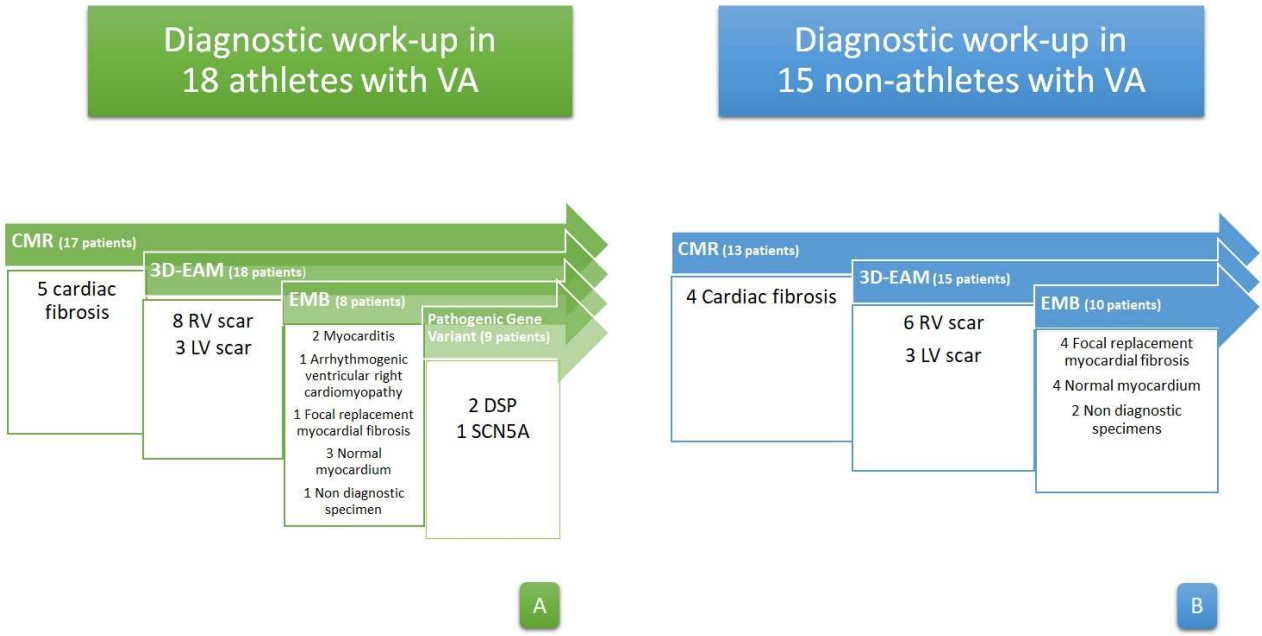
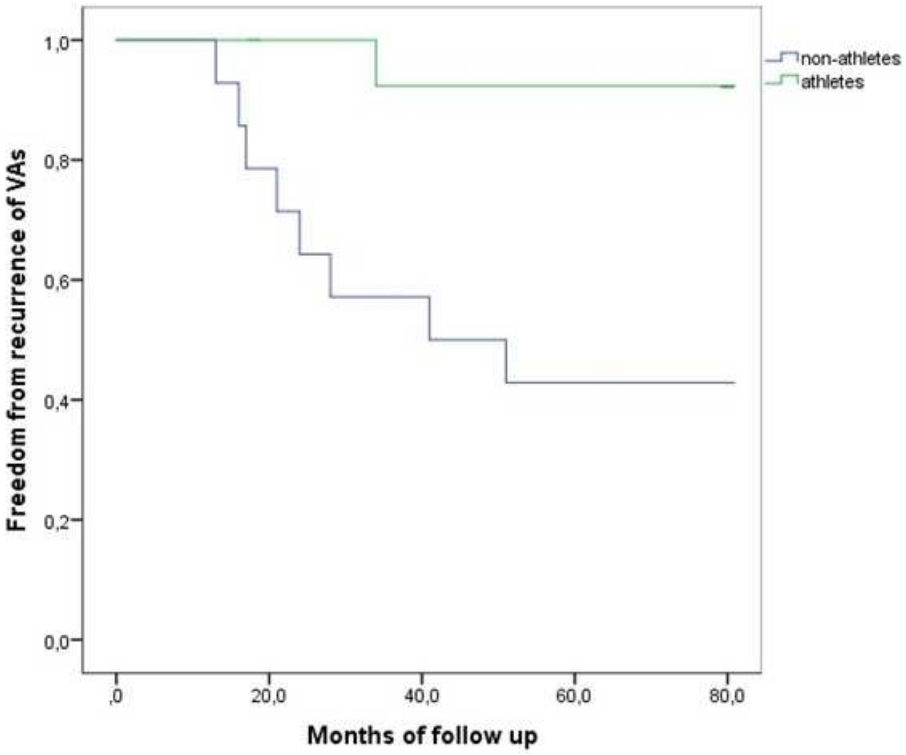


Figure 5. Kaplan Meier Log-survival curves (end-point: premature ventricular contractions >25% / non sustained ventricular tachycardia + sustained ventricular tachycardia / ventricular fibrillation)



VAs= ventricular arrhythmias

Number of patients at risk

Athletes	18	18	17	17	17
Non-athletes	15	12	9	7	7