Original article

Autoptic findings of sudden cardiac death (SCD) in patients with arrhythmogenic ventricular cardiomiopathy (AVC) from left ventricle and biventricular involvement

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A B S T R A C T

Objectives: To evaluate autoptic histopathological findings of arrhythmogenic ventricular cardiomyopathy (AVC) as major cause of sudden cardiac death (SCD) in young adults.
Background: According to Heart Rhythm Society (HRS)'s international consensus, histological criteria for AVC diagnosis include a progressive myocardial atrophy of the right ventricle characterized by a transmural fatty or fibrofatty replacement in a segmental or diffuse pattern (residual myocytes <60 % *vs* 60–75 % by morphometric analysis) explaining the electrical instability with increased risk of SCD. However, there is increasing evidence for atypical patterns of localizations and percentage of fibrofatty replacement suggesting the need to update histopathological features of AVC.
Methods: Histology examination of ventricles, atria, and septum was performed on 10 autopsy of SCD due to AVC. Staining with hematoxylin-eosin and PicroSirius Red/Fast Green were performed on the heart samples to identify specific fibrofatty patterns.
Results: Our analysis showed that: 1) myocardial replacement by a diffuse segmental fatty or fibro-fatty tissue characterized right and left ventricles as well as atrial walls; 2) the degree of fibrofatty tissue replacement was less than 40 % both in left ventricle (n = 4, 40 %) and biventricular (n = 6, 60 %) localization; 3) perivascular fibrosis, inflammatory infiltrate, areas of hypertrophy and/or areas of coagulative necrosis as signs of hypoxic damage in the first stage.
Conclusions: We confirmed prior evidence for fibrofatty replacement both in biventricular and septal localizations. Importantly, we observed a less degree (<40 %) of fibrofatty replacement as compared to current guidelines. This supports the need to further explore the histological patterns of fibrofatty infiltration in a larger study population to improve the histological diagnostic criteria of AVC.

1. Introduction

The AVC is a rare disease with a genetic transmission characterized by alterations of desmosomial proteins [1–7]. The AVC mainly affects the right ventricle but, in some cases, the left ventricle or both ventricles involvement is described [8,9]. In fact, the progressive replacement of right ventricular myocardium by fibrous tissue is a pathological hallmark of the disease, but fibro-fatty infiltration with a structural disarrangement of the full-thickness wall of the right and left ventricle are

described [8,9]. The myocardial structural alterations predispose to the arrhythmias and SCD especially in young people and athletes. According to Heart Rhythm Society (HRS)'s international consensus [8], the AVC diagnosis is based on major/minor criteria, including clinical aspects, family history, imaging and histological evaluation of fibrotic right ventricle replacement in percentage. The studies attempted to quantify the presence of adipose and fibrous tissue in the myocardial wall based on values of ventricular infiltration of at least 3% of adipose tissue and over 40 % of fibrous tissue [10,11]. The international guidelines and the

Table 1
Documental data.

Patients	Age	Sex	Time/cause of death	ECG depolarization/ conduction abnormalities	Tissue characterization wall
1	30	F	Hyperemesis at 5 W	RBBB I°	Fibro-fatty pattern with transmural fatty infiltration Biventricular and septal
2	27	M	Non-agonistic player (during a match)	–	Fibro-fatty pattern Biventricular and septal
3	18	M	Non-agonistic player (during a match)	–	Fibro-fatty pattern. Biventricular and septal. Cardiac conduction infiltrated by fibrosis
4	30	M	Non-agonistic player (during a match)	–	Fibro-fatty pattern restricted to left ventricle
5	16	M	Agonist water polo player (during a match)	–	Fibro-fatty pattern. Biventricular and septal. Cardiac conduction infiltrated by fibrosis
6	30	M	Non-agonist runner (at rest)	–	Fibro-fatty pattern restricted to left ventricle
7	30	M	Non-agonistic player (during a match)	–	Fibro-fatty pattern with transmural fatty infiltration. Biventricular and septal
8	45	M	Daily activity	–	Fatty pattern Biventricular and septal
9	28	M	Physical exertion	–	Fibro-fatty pattern restricted to left ventricle
10	34	F	Physical exertion	–	Fatty pattern restricted to left ventricle

Abbreviations: W: weeks; ECG: Echocardiography; RBBB: Right Bundle Branch Block.

task force criteria recommend the AVC diagnosis through the evidence of two main criteria, 1 major and 2 minor or 4 minor criteria of different categories. On the other hand, the diagnostic criteria show some limitations, due to the lack of sensitivity of many of the currently used criteria [8,10,11]. Relevantly, in AVC families with evidence of genetic mutations that cause disease, we might have mixed results which usually do not lead to a diagnosis of AVC in clinical practice. Therefore, there might be patients that do not match with AVC diagnostic criteria, but that might have a worse and fatal prognosis as SCD [10–12]. Here, we hypothesized that SCD often occurs in patients who are not screened for minor and/or major criteria of AVC. Therefore, we investigated SCD cases with AVC diagnosis confirmed by autopsy. It is important to consider that in differential diagnosis of AVC we have histologically considered other pathological conditions. Quite simply the main differential diagnoses to keep in mind are: lipomatous substitution, post-infarct fibrosis, and fibrosis that accompanies hypertrophy. According to many authors, lipomatous substitution is considered as an AVC entity; post-infarct fibrosis, and fibrosis that accompanies

hypertrophy have a well-defined localization in the area of the previous chronic damage, and appear more or less large with abrupt replacement of the damaged region while the characteristic of AVC is the passage between normal myofibre and fibrosis. Finally, the characteristic trans-parietal distribution of the fibrous-adipose tissue up to the sub-endocardial portion must orient to the exclusion of outcomes of other pathologies with different etiopathogenesis.

2. Materials and methods

Our group has a long-standing experience. Here, we revised 10 SCD autopsy obtained from a large series of autopsy carried out from 2010 to 2017, by evaluating histological findings in absence of previous clinical and instrumental diagnosis and without other systemic or specific cardiovascular comorbidities. Clinical and history data of the deceased were extracted from the forensic documentary data at the Legal Medicine Institute of the Federico II University, Naples (Italy). All autopsies were performed in accordance with the Recommendations on the Harmonization of Forensic Autopsy Rules of the Committee of Ministers of the Council of Europe (1999) and according to commonly accepted criteria for SCD, as already described in the literature [13–20].

Autopsy and histological examination

The autopsy did not show any pathological aspect in other organs or effusions. Sampling with histological examination was carried out for each organ, ventricles, atria, and septum including the conduction system for the heart. Sections (4 μm thick) from the original tissue samples fixed in 10 % neutral buffered formalin and embedded in paraffin blocks were stained with hematoxylin and eosin stain (H&E) to confirm the diagnosis. The further sections were stained with a PicroSirius Red/Fast Green for fibrosis evaluation.

3. Results

10 SCD cases (8 males and 2 females, age ranged from 16 to 45 years and average age of 28.8 ± 8.1) were identified with no previous history of other systemic or specific cardiovascular comorbidities; only in one case, we had a history of hyperemesis at 5 W of gestation and a previous history of a right bundle branch block (RBBB I°) event (Table 1). Besides the location of adipose/fibrous tissue in the right or left ventricles and the septum, seven fields at 400x magnification has been evaluated in five regions of the myocardium. We followed the basic anatomical-pathological criteria of observation at 400x introduced in 2010 as indicated in the specific texts of forensic pathology [21]. Obviously, this was the rule of our observation but with attention to the whole myocardium in the areas with more significant morphological aspects. The microscopic and digital morphometric analysis were performed. However, the microscopic observation showed a percentage of the fibrous tissue less than 40 %, while it was possible to identify different types of localization with the associated presence of adipose tissue (Table 1). Furthermore, it was highlighted a myocardial replacement by fibrous, prevalent fatty or fibro-fatty tissue. Also, we observed perivascular fibrosis, focal and mild-moderate perivascular inflammatory infiltrate, and areas of hypertrophy and/or areas of coagulation necrosis as signs of initial hypoxic damage. In 2 cases, we observed the replacement of the cardiac conduction system. The fatty or fibro-fatty tissue was restricted to the left ventricle in 4 cases and biventricular as well as septal in the 6 cases. The fatty pattern was observed in 2 cases and the fibro-fatty pattern was observed in 8 cases with transmural fatty infiltration in 2 patients (Table 1). The AVC histology has been confirmed by a morphometric analysis that showed a percentage reduction of myocytes with a fibrous replacement, as the mean of the evaluation of all the specimens drawn from different sites of left ventricle (4 patients), and both ventricle (6 patients) (Table 1). We found more evident myocardial adipose substitution, ranging from 8 % to 45 %, and irregular inflammatory infiltrates. We observed a different percentage of fibrous and adipose replacement of the ventricular and
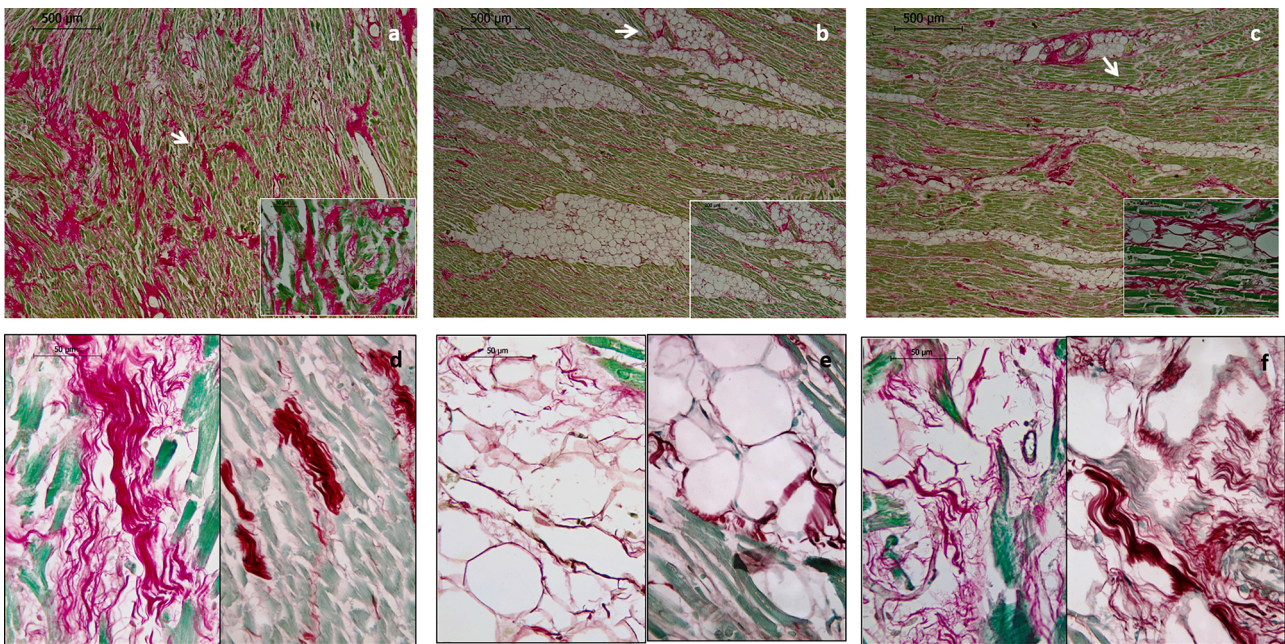
Fig. 1. Fibrosis evaluation (PicroSirius Red/Fast Green).
Collagen fibers appeared red, while the non-collagen proteins were green. **a.** Prevalent fibrous pattern; **b.** Prevalent fatty pattern; **c.** Fibro-fatty pattern; **d, e, f:** different cases of prevalent fibrous, fatty fibro-fatty patterns, respectively. The white arrow indicates fibrous tissue (a), fatty tissue (b), and fibrous-fatty tissue (c). (scale bar on the top left).
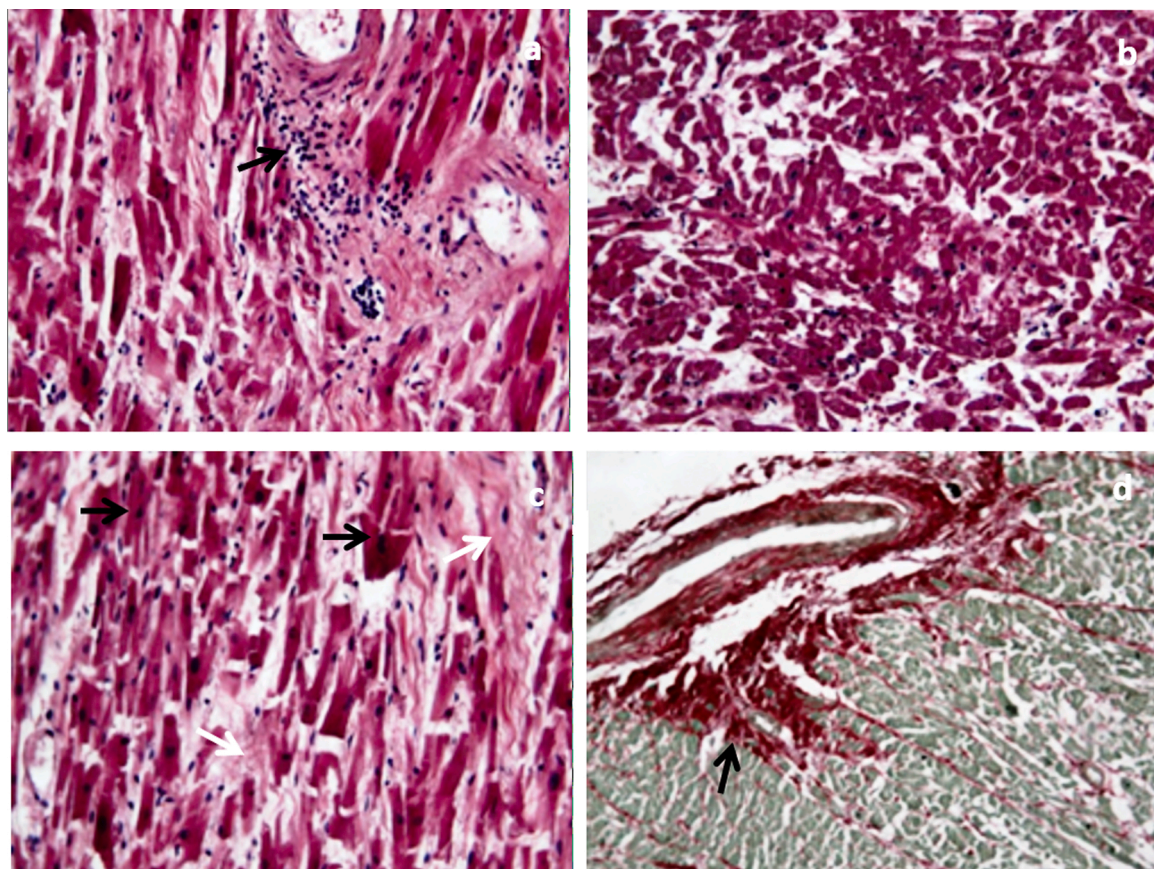

Fig. 2. Sections of right ventricle-septum-left ventricle.
a. The black arrow indicates the predominantly perivascular inflammatory infiltrate (H&E stain 400x); **b.** The associated coagulative necrosis is typical of the initial stages of hypoxic damage; **c.** The fragmentation with thickening and centralization of the cardiomyocytes nuclei (black arrow), and the fibrosis (white arrow) are characteristics of hypertrophic modifications (H&E stain 400x); **d.** Perivascular fibrosis (black arrow) (Sirius Red/Fast Green - stain 10x).

septal walls with different dislocation and with myocardial fibers fragmentation (Fig. 1). Moreover, it was observed an extensive adipose substitution of the myocardium in the outer third of the wall with features of transition between the muscle fibers and the adipose tissue, the middle or inner part of the wall (Fig. 1). The adipose zone spread sometimes in large sheets parallel to the muscle fibers. Moderately loose fibrosis was associated, both in the adipose substituting zone and in the remaining myocardium. Besides, the presence of predominantly mild and focal perivascular lymphoid infiltrations (Fig. 2a), areas of coagulative necrosis as initial hypoxic damage (Fig. 2b) as well as aspects of myocardial hypertrophy with fragmentation, anisonucleosis, and fibrosis (Fig. 2c), and also aspects of perivascular fibrosis were observed (Fig. 2d). We defined a fibro-fatty pattern and fatty pattern, indicating the evidence of fibro-fatty replacement vs. isolated fatty replacement of cardiac wall (Table 1) (Fig. 1). In detail, we had the majority of patients with fibro-fatty pattern (8 patients; 5 with biventricular location vs. 3 with left ventricle location) in comparison to only 2 patients with fatty pattern (1 with biventricular location vs. 1 with left ventricle location) (Table 1) (Fig. 1).

4. Discussion

Patients with borderline AVC have 1 major and 1 minor criterion, or 3 minors of different categories, and they have undefined diseases for minor abnormalities but are at risk requiring regular follow-up [8]. Conversely, the criteria for structural anomalies are based on a quantitative measure of ventricular volumes and functionality by using both magnetic resonance and echocardiography. To date, the imaging might have an inter-observers variability, which limits their clinical application [22–25]. In fact, guidelines criteria for diagnosis and management of patients suggest other exams for AVC diagnosis, such as the ECG. Indeed, the ECG abnormalities are relevant for the AVC diagnosis and they are reported until 98 % of affected cases [8,26,27]. ECG abnormalities are focused on the abnormality of ventricular repolarization, as the inversion of the T-wave in the precordial right derivations (V1-V3) in the absence of RBBB I° which is reported between 54 % and 85 % of affected patients. The complete and the incomplete right bundle branch block are excluded since they are also commonly seen in normal subjects, while the inversion of the T-wave in the absence of complete right bundle branch block is a major criterion observed only in 4% of women and in 1% of normal men. Yet, the presence of a post-excitation epsilon wave, a distinct small-amplitude wave reflecting delayed right ventricular activation, is considered a major diagnostic criterion [8,26,27]. Conversely, in absence of any specific ECG abnormality and diagnostic ECG pattern we found that QRS interval duration might predict both left ventricular and biventricular location of AVC. Intriguingly, the QRS interval duration expresses the regional conduction delay and the loss of myocardium due to AVC. However, the QRS interval duration is a non-invasive indicator of activation delay that occurred in the diseased region of the right ventricle [27,28]. Alterations of QRS duration might be caused by the presence of a different percentage and localization of fibrous tissue with residual hypertrophic myocardium and/or atrophic remnant myocardium, added to the vacuolated cardiomyocytes caused by ischemic distress [8]. The cases examined here did not have any of the mentioned ECG abnormalities but only one case showed a previous RBBB I° (Table 1). Our study showed that in the AVC the right ventricle is affect, but the septum, left ventricle, or both ventricles may also be involved. Intriguingly, in AVC cases we do not observed a unique right ventricle location of fibro-fatty or fatty tissue. This might explain the absence of any characteristic diagnostic ECG abnormality as the RBBB the inversion of the T-wave, and the presence of a post-excitation epsilon wave, which are main signs of altered and delayed right ventricular activation. Moreover, a prevalent fibro-fatty could be another possible explanation of the loss of the typical ECG patterns in undiagnosed AVC patients [26–28]. Therefore, these morphological left ventricular and biventricular alterations might affect the QRS interval duration, and this

in turn causes alterations in action potentials genesis, duration, and propagation in an altered myocardial chamber [29]. Indeed, electrical and structural alterations of cardiac tissue could cause ventricular arrhythmias and SCD also in the absence of pump failure [29]. Taken together, these histological alterations might cause an irreversible substrate modification of the heart, with electrical and mechanical instability, resulting in higher arrhythmic burden and SCD [30,31].

5. Conclusions

SCD might be the first clinical manifestation for patients with undiagnosed AVC. In these patients, autopsy showed left ventricular/biventricular fibrosis and adipose tissue infiltration in the absence of other diagnostic criteria of AVC. Because these abnormalities are not integrated into existing diagnostic criteria, these patients never received a confirmed clinical diagnosis or treatment. The development of more precise risk of SCD, looking about the family history, new genetic tests and guided by tissue biopsy and/or surgical diagnosis, and including more clinical signs and imaging exams not present in the diagnostic guideline criteria, can be useful for patients in absence of typical AVC diagnostic criteria.

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