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CLINICAL FEATURES AND FOLLOW-UP ANALYSIS OF PATIENTS AFFECTED WITH ARRHYTHMOGENIC LEFT VENTRICULAR CARDIOMYOPATHY

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Summary

ABSTRACT

Background. Arrhythmogenic cardiomyopathy (ACM) is a genetically determined myocardial disease characterized by myocyte necrosis and fibro-adipose replacement, leading to ventricular dysfunction and onset of life-threatening ventricular arrhythmias (LTA). ACM was initially considered to be a disease of the right ventricle (RV), with left ventricular (LV) involvement usually mild and mainly due to a disease progression toward a biventricular phenotype, in the last years mainly through cardiovascular magnetic resonance (CMR) studies, a left-dominant form of the disease was described (arrhythmogenic left ventricular cardiomyopathy: ALVC). However, this phenotype has not yet been fully characterized and specific risk stratification parameters are currently lacking.

Aims. To characterize the genetic background, the clinical features, and the outcome of an ALVC cohort of patients and to compare this phenotype with the right dominant (ARVC) and biventricular (BIV) forms. Moreover, we sought to investigate possible prognostic predictive factors for LTA in ALVC patients.

Methods. From the entire cohort of 544 patients (both probands and family members) followed at the Inherited Arrhythmogenic Cardiomyopathy Unit of the University of Padua, we selected 434 patients affected by ACM (80%). According to phenotype, the patients were divided into three groups: ARVC, ALVC, and BIV. Family and personal history, genetic analysis, instrumental findings (ECG, 24-h Holter ECG, and CMR), and follow-up data were compared.

Results. The study population was composed of 436 ACM patients, of whom 273 (63%) were male, and 284 (65%) were probands. According to the phenotype, the general population was divided into three groups: 184 ARVC patients (42%), 112 ALVC (26%), and 140 BIV (32%). In the ALVC cohort, 70 patients (63%) were male and 66 (59%) were probands. The median age at diagnosis was 38 years (IQR 26-49). According to the 2010 Task Force criteria, a definite diagnosis of ACM was obtained in 33 cases (30%), while by means of the 2020 Padua criteria, a significant increase in definite

diagnoses was observed (61 patients, 54%). Genetic analysis identified the presence of a pathogenic/likely pathogenic genetic variant in a causative gene in 61 patients (54%), with the most common disease genes being *Desmoplakin* (*DSP*, 39, 63%), followed by *Filamin-C* (11, 16%). The ECG was abnormal in 74 (66%) cases, mainly due to the presence of low QRS complex voltages in the peripheral leads (52, 46%) and T wave inversion in V4-V6 (22, 20%). A total of 106 patients (95%) underwent CMR. Late gadolinium enhancement distribution in LV consisted mainly of subepicardial stria (90%). A significant negative association was observed between LV ejection fraction and the extent of LV-LGE (β= -0.853, p=0,007). Overall, 18 patients (16%) had LTA episodes; in 9 (8%), this was the first clinical manifestation of the disease leading to ACM diagnosis. Five patients (5%) experienced heart failure (HF), and 2 (2%) underwent cardiac transplantation, while 2 (2%) died due to refractory HF. Four patients (4%) died suddenly, in all cases as the first manifestation of the disease. Finally, 11 patients (10%) presented at least one hot phase episode. According to results of the Cox regression with adaptive elastic-net penalty, proband status (HR 1.21), history of syncope (HR 2.53), and T wave inversion (TWI) in V4-V6 (HR 1.26) were associated with an increased arrhythmic burden. Comparison between the three phenotypes revealed that patients belonging to the ALVC group showed a significantly lower incidence of LTA (16%), compared to ARVC (30%) and BIV (37%), p=0.001. HF was more frequent in BIV forms (20%) than in ALVC (5%) and ARVC (1%), p<0.001. Similarly, the mortality rate was observed to be significantly higher in BIV patients (11%), compared to ALVC (2%) and ARVC (1%), as well as heart transplantation (0% in ARVC, 2% in ALVC and 10% BIV, p<0.001). No statistical differences between the three groups were observed regarding the incidence of hot phase episodes (p=0.121).

Conclusions. Differential diagnosis between ALVC and phenocopies can be challenging. Padua criteria seem to improve the diagnostic sensitivity, allowing a significant increase in definite diagnoses in ALVC patients. The genetic test showed the presence of a causative genetic variant in 54% of patients, mainly on the *DSP* gene. Proband status, history of syncope, and presence of TWI from V4-V6 were found to be associated with an increased risk of LTA in the ALVC population. The

comparison between the three phenotypes revealed that ALVC showed a lower incidence of LTA, HF, and death compared to BIV and ARVC groups.

INTRODUCTION

Arrhythmogenic Cardiomyopathy (ACM) is a genetically determined myocardial disease characterized by myocyte necrosis and fibrofatty replacement. The main clinical aspects are morphological alterations of ventricles and presence of ventricular arrhythmias, which can even lead to sudden cardiac death (1,2).

Historical notes and first clinical descriptions

The first historical description dates back to 1736 when Giovanni Maria Lancisi, in the book *De Motu Cordis et Aneurysmatibus*, described a large family with recurrence of heart failure and sudden death with presence on the autopsy of right ventricular aneurysms (3). In 1961 Dalla Volta et al. reported a series of patients who presented a dilatation of the right ventricle (RV) of non-ischemic origin and in whom cardiac catheterization demonstrated the presence of the so-called “auricularization” of the right ventricle pressure curve (4). In 1982 Marcus et al. reported the first detailed clinical description of a series of 24 adult patients affected with the disease showing recurrent episodes of ventricular tachycardia with left bundle branch block morphology, inverted T waves on the right precordial leads at electrocardiogram (ECG), and RV dilatation (5). Histology examination documented the presence of extensive substitution of the RV myocardium with fatty and fibrous tissue.

In 1988, Thiene et al. reported the detailed pathological features of the disease, which consist of necrosis of myocytes with fibro-fat replacement, and for the first time, ACM was identified as an important cause of sudden cardiac death (SCD) in young subjects, particularly athletes (6). In the same year, Nava et al. defined the hereditary trait of the disease, characterized by autosomal dominant transmission with variable penetrance (7).

Initially, the disease was thought to be a developmental defect present from birth. Hence the term 'arrhythmogenic right ventricular dysplasia' was used. However, the evidence of hereditary transmission and the increased clinical definition in later years led to a revision of the nomenclature, replacing the term dysplasia with cardiomyopathy (7).

Epidemiology

The ACM prevalence is difficult to estimate due to the frequent misdiagnoses, but it reasonably ranges from 1:1000 to 1:5000 (2,8). Usually, it becomes clinically overt in the second-fourth decade of life, and males result in being more frequently affected with respect to females (up to 3:1). Interestingly, increasing evidence suggests the presence of cases in pediatric patients (9,10). Moreover, ACM represents one of the most frequent causes of SCD in youth, especially in athletes. The overall mortality rate is variable depending on the study ranging from 0.08% per year (in a follow-up of 8.5 years) to 3.6% per year (in a follow-up of 4.6 years) (34). According to the 1988 study by Thiene et al., 20% of deaths in young people and athletes in the Veneto region are caused by a previous undiagnosed ACM (6). Further studies confirmed ACM as one of the leading causes of SCD in athletes of the Veneto region of Italy (14% of cases), and athletes with ACM were found to have 5.4 times higher risk of SCD (11). A similar prevalence was found in a British study and significantly lower in a US series (6%)(12,13).

Pathological findings

The ACM pathologic hallmark consists of ventricular myocardial atrophy followed by fibro-fatty tissue replacement; this process is progressive, starting from the epicardium and then extending to the endocardium, eventually becoming transmural(14,15) (Figure 1). Fatty infiltration that in original descriptions was one of the milestones of the disease is now not considered a sufficient morphologic hallmark of the disease, as replacement-type fibrosis and myocyte degenerative changes should always be identified (2,16). The progression of the pathologic process can lead to wall thinning and aneurysms, typically located at the RV's inferior, apical, and infundibular walls (the so-called "triangle of dysplasia").

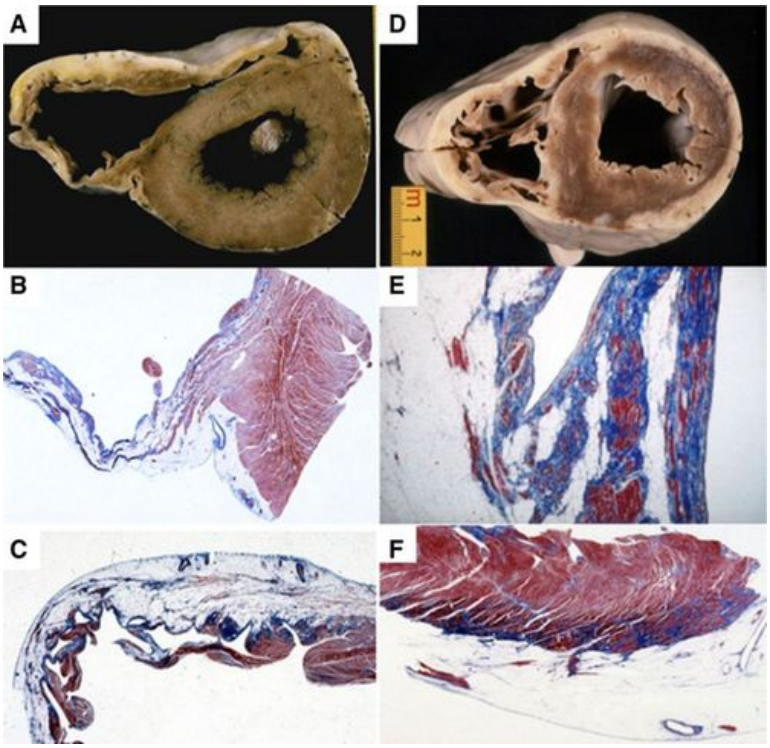

Figure 1. **Pathologic features of arrhythmogenic cardiomyopathy**. Classical right ventricular (RV) variant: A, Gross transverse section of the heart that shows anterior and posterior RV wall thinning because of myocardial atrophy and a subtricupid aneurysm. Full-thickness histology of the posterior (B) and anterior (C) RV-free wall shows fibrofatty tissue replacement. There is thinning and residual myocardium confined to the endocardial trabeculae (trichrome stain). Biventricular variant: D, Gross examination of a transverse section of the heart. Note the transmural RV free wall involvement as compared with the subepicardial midmural left ventricular (LV) free wall involvement. E, Histology of the RV free wall confirms the transmural myocardial atrophy with fibrofatty replacement. F, the histology of the LV free wall shows replacement-type fibrosis of the outer layer with preserved wall thickness (trichrome stain). Reproduced with permission from Corrado et al. Circulation Research. Arrhythmogenic Cardiomyopathy, Volume: 121, Issue: 7, Pages: 784-802.

Although the original description characterized the disease by an exclusive or at least predominant RV involvement, over the years, a left ventricular (LV) involvement has been described (17,18). Indeed, up to 76% of patients undergoing autopsy revealed LV involvement mainly localized in the subepicardial or mid-mural layer of the free wall (17). Subsequently, the improvement of imaging techniques, particularly Cardiac Magnetic Resonance (CMR) with contrast agent, has demonstrated that LV is frequently involved. For this reason, the current phenotypic classification of the disease considers the presence of three variants (19):

- "Right dominant" (also referred to as "Arrhythmogenic right ventricular cardiomyopathy: ARVC"), characterized by the predominant RV involvement, with no or minor LV abnormalities.
- "Left-dominant" (also referred to as "Arrhythmogenic left ventricular cardiomyopathy: ALVC") characterized by a predominant LV involvement, with no or minor RV abnormalities.
- "Biventricular" (BIV) with a similar involvement of the RV and LV.

This led over the last few years to use the broader term "Arrhythmogenic cardiomyopathy," which includes all these phenotypic expressions (1,20). However, the 2019 expert consensus statement endorsed by the Heart Rhythm Society (HRS) defined "Arrhythmogenic cardiomyopathy" as an arrhythmogenic disorder of the myocardium not secondary to ischemic, hypertensive, or valvular heart disease (21). Therefore, from the authors' perspective, ACM encompasses a broad spectrum of genetic, systemic, infectious, and inflammatory disorders (21). However, this definition is not universally accepted. In a recent paper, Corrado et al. argued that this definition appears inappropriate because ACM is a nosographically specific condition characterized by typical cardiomyopathic features (22).

Genetic background

Since the first descriptions, an inheritable disease pattern with a familial recurrence has been demonstrated (7). In addition, it became evident that in most cases, ACM was inherited through an autosomal dominant transmission with incomplete penetrance and variable expressivity(1).

In 1986 a disease variant named "Naxos syndrome" characterized by the association between ACM and palmoplantar keratoderma/woolly hair (cardio-cutaneous syndrome) was firstly described (23). Notably, Naxos disease is inherited in an autosomal recessive manner with complete penetrance. In

2000, a deletion in the desmosomal gene plakoglobin (*JUP*) was found as the underlying genetic cause of Naxos syndrome (24).

A second cardio-cutaneous syndrome, named Carvajal disease from the name of physician who first described the pathology, played a pivotal role in the genetic definition of ACM. It is typically characterized by woolly hair, striate palmoplantar keratoderma, and left-sided ventricular cardiomyopathy leading to SCD (25). As Naxos disease is transmitted in an autosomal recessive manner, genetic studies demonstrated that a genetic variant of Desmoplakin (*DSP*) gene was linked to this syndrome (26).

In 2002, Rampazzo et al. demonstrated that *DSP* genetic variants were also present in families with a classical form of ACM, with an autosomal dominant transmission (27). From that moment, causative variants in other genes encoding for the main components of the desmosome were found to be linked to ACM (28). Thus, it has become evident that abnormalities in desmosome structure have a crucial role in ACM pathogenesis. For this reason, ACM is now considered to be mainly a 'disease of the desmosome.'

Desmosomes are complex structures consisting of proteins responsible for cell adhesion and signaling. One crucial function is to tether adjacent cells mechanically by joining their intermediate filaments (IFs) to create a unified cytoskeletal network (1,2).

Genetic studies demonstrated that approximately 30–50% of ACM patients carry a pathogenic mutation in a desmosome gene (1).

The most frequent gene found in ACM patients is *PKP2* (19-46 %) followed by *DSP* (1-16 %), *DSG2* (2.5-10%), *DSC2* (1-8%) and *JUP* (1%). Moreover, approximately 10-25% of ACM patients carry compound mutations (29).

Plakophilin 2 (*PKP2*) is the most frequently mutated gene in ACM patients. The gene was firstly characterized in 1996 and was initially thought to be responsible for the mechanical stabilization of the cell. However, over the years, the PKP2 gene has proved to play a pleiotropic role for this protein, with functions ranging from the regulation of intracellular signaling to electrophysiological, the

trafficking regulation and the control of transcriptional processes (30). Studies sought to discover the relationship between defective *PKP2,* and presence of ventricular arrhythmias have shown that *PKP2* is required to maintain the integrity and formation of the gap junction. Moreover, recent studies demonstrate that *PKP2* and intercalar disk partner proteins can also translate information initiated at the site of cell-cell contact into intracellular signals that modulate electrical and transcriptional pathways fundamental to homeostasis (31,32). In particular, a loss of *PKP2* function results in altered sodium (33) and calcium (34) currents, with increased arrhythmic propensity. Through the study of mouse models, it was possible to identify a direct role for *PKP2* in the activation of Hippo and Wnt pathways, which are implicated in fibro-adipose substitution in ACM(35). Recently, Cerrone et al. studied the transcriptomic changes caused by resistance training in PKP2-deficient mice. They found that loss of *PKP2* caused a cardiac contractile deficit, decreased muscle mass, and increased functional/transcriptomic signatures of apoptosis, despite increased fractional shortening and calcium transient amplitude in individual myocytes (32). For the first time, it was reported an increase in sarcomere shortening resulting from the loss of *PKP2*, a seemingly paradoxical effect compared to the organ's reduced contractile function. Authors concluded that the *PKP2*-dependent mechanical dysfunction of the ventricles is not cell-based, but rather is consequent to a reduction in overall muscle mass and/or an impeded remodeling process that should occur following exercise (32). Although many *PKP2* gene variants have been associated with ACM over the years, the role of some of them as causative has been questioned. By analyzing the variants in genetic databases, it was established that most *PKP2* variants (77%) associated with ACM resulted in radical protein alterations (30,36). A recent multicenter study demonstrates the specificity of *PKP2* truncating variants for ARVC within the ACM disease spectrum (37). The majority of mutations have been identified in the C-terminal portion of the protein (38). Pathogenic variants in this gene are usually linked to the “classical” form of the disease with a predominant RV involvement (39).

Desmoplakin (*DSP*). *DSP* is the most abundant component of the desmosome, and it has a tripartite structure that includes a globular N-terminal plakin domain, a central alpha-helical rod domain, and

a C-terminal tail domain(40,41). The *DSP* gene, undergoes alternative splicing to produce three isoforms: a long (*DSP*-I), an intermediate (*DSP*-Ia), and a short (*DSP*-II) isoform(41). *DSP*-I is the predominant cardiac isoform; however, it is also present in the skin. *DSP*-II was thought to be restricted to the skin; however, *DSP*-II transcriptions have been found in the left atrium and ventricle, interventricular septum, left auricle, and apex of the heart, but at a much lower expression level than that of *DSP*-I (42). *DSP* binds IFs within the dense inner plaque, thereby tethering the IFs to the plasma membrane (43). The main IF protein in mature striated myocytes is desmin(42,44). Suppression of *DSP* expression in cardiac myocytes led to PKG release from desmosomes, their translocation to the nucleus, and a 2-fold reduction in canonical Wnt/b-catenin signaling. This led to elevated expression of adipogenic and fibrogenic genes in vitro and abnormal cardiac adipose tissue and fibrosis in vivo. A loss of *DSP* has been demonstrated to be capable of activating the Hippo pathway and also affecting Cx-43 expression(42,45,46).

Several variants involving this gene have been identified so far, often leading to the synthesis of a truncated protein at the N-terminal or C-terminal side. Interestingly, some evidence points out that these mutations can have different phenotypic expressions. If mutation involves the N-terminal portion, the resulting phenotype is a classic form of ACM with autosomal dominant transmission Campo (20). In contrast, mutations on the C-terminal end (the one that interacts with intermediate filaments) are usually expressed with predominant LV involvement (ALVC) (47,48). The presence of a homozygous mutation can be characterized phenotypically by biventricular ACM forms with almost exclusively fibrous infiltration associated with cutaneous involvement (Carvajal syndrome)(49). The different clinical ACM phenotypes linked to other protein domains of desmoplakin (N- or C-terminal) suggest the presence of distinct molecular mechanisms underlying the different disease variants. Thus, it has been speculated that the left-dominant variant may be secondary to an altered desmoplakin-desmin linkage, which compromises the integrity of the cytoskeleton in cardiomyocytes while an alteration in the relationship between desmoplakin and other components of desmosome would cause a classical disease phenotype(48). Recently, a study

compared clinical data of patients with truncating mutations in *DSP* and *PKP2* genes(50). The authors concluded that LV involvement was exclusively present in patients with *DSP* mutations, which also had a preserved systolic function of both ventricles compared to *PKP2* patients. At CMR, these patients frequently showed LGE on the LV, mainly located in lower and infero-septal segments. Of note, a frequent positive history of chest pain episodes in *DSP* patients was reported, both probands and family members; it has also been noted that acute episodes of myocardial damage can occur even in the presence of normal systolic function (50). Wang et al. evaluated a cohort of 91 patients harboring a pathogenic variant of *DSP* and they found a prevalence of myocardial injury of 20% and an association with a worse disease outcome (51). Finally, Bariani et al published a series of 73 patients with a pathogenic/likely pathogenic variant of *DSP*. The study demonstrated that the clinical phenotype is wide, with the majority of patients showing a LV involvement, and 22% of patients with RV abnormalities in keeping with a "classical" ACM form. Moreover, in ALVC forms, HF and significant ventricular arrhythmias seem to be less common compared to ARVC and BIV variants. Finally, females show more frequently a LV involvement and have a better outcome (52).

Desmoglein 2 (*DSG2*). DSG2 is part of the cadherins family. All these proteins have tripartite functional domains: (1) an extracellular, calcium-inducible, amino-terminal domain, essential for homophilic intercellular associations, with four domains (EC1 to EC4), followed by an extracellular anchoring (EA) domain; (2) a single transmembrane domain; and (3) a cytoplasmic domain that anchors the cytoskeleton, an essential process for cell adhesion(53). DSG2 was described as a causative gene of ACM for the first time in 2006, and it is now estimated to be present in 10% of forms (53,54). Phenotypically, a pathogenic variant in the *DSG2* gene is frequently found in patients with a BIV form of the disease. Nine different mutations of DSG2 gene have been reported so far. They are mainly located in the N-terminal region, responsible for a classical ACM phenotype, even if, in some cases, a phenotypic overlap with dilated cardiomyopathy (DCM) has been reported (55).

Desmocollin 2 (DSC-2): DSC-2 was first reported as an ACM causative gene in 2006 (56). Presence of DSC-2 mutations lead to premature truncation of desmocollin protein, with loss of its normal

function, and they are associated with right dominant forms. Both autosomal recessive and dominant transmission are reported (57).

Plakoglobin (*JUP*): deletion at the C-terminal end of JUP gene leads to the formation of a truncated protein. Homozygous mutations are associated with Naxos cardio-cutaneous syndrome, with autosomal recessive transmission, while heterozygous mutations are expressed with only cardiac involvement (58).

Although less frequently found, mutations in non-desmosomal genes have also been linked to ACM: Desmin (DES), Filamin C (FLNC), Transmembrane protein 43 (TMEM-43), Lamin A/C (LMNA), Phospholamban (PLN), α-T-catenin (CTNNA-3), Cadherin-2 (CDH2) and Transforming growth factor β3 (TGF-β3).

α-T-catenin (CTNNA3): this protein interacts with *PKP2* in the intercalated disc. This gene's mutation leads to a decreased binding capacity to desmosomal components, resulting in impaired intercellular adhesion function. This form is usually characterized by incomplete penetrance (59).

Cadherin-2 (CDH2): it is an integral glycoprotein that mediates cell adhesion in presence of calcium. The intracellular domain is connected to actin filaments by catenins. Recently, in a worldwide cohort of patients affected by ACM, previously negative at the genetic examination, mutations in CDH2 were detected in 1.2% of cases. Moreover, these patients show an increased risk of ventricular arrhythmias, while evolution toward heart failure is rare (60).

Laminin (LMNA): *LMNA* is one of the main constituents of the nuclear lamina. Besides its structural function, it plays a crucial role in the regulation of gene expression, intracellular signal transduction, and DNA replication. Pathogenic variants of this gene can lead to a broad spectrum of pathologies, including muscular dystrophies (e.g., Emery-Dreifuss), Hutchinson-Gilford Progeria Syndrome and cardiac manifestation (61). Among the latter, *LMNA* genetic variants have been first reported in patients with Dilated cardiomyopathy (DCM) with the frequent presence of conduction disturbances and a high degree of arrhythmic instability that does not correlate with LV systolic function. This

evidence led to the indication for ICD implantation in patients carrying a pathogenic variant of the *LMNA* gene and showing a value of LV-EF below 45% (21).

On the other hand, its putative role as an ACM-causing gene is still limited and debated (39,62). The first association of LMNA and ACM dates back to 2012, when Quarta et al. described four patients carrying a *LMNA* genetic variant who showed a cardiac phenotype in keeping with ACM (63). Common features of these patients were family history of cardiomyopathy and/or sudden death, T-wave inversion in precordial leads, and atrioventricular (AV) and/or intraventricular conduction delays at ECG, while a RV dilation/dysfunction was present in 3 out of 4 patients (63). Other publications in the following years reported similar findings (64,65).

Desmin (DES): it is an intermediate filament protein essential for the organization of the cytoskeleton and structural maintenance of cardiomyocytes. The clinical phenotypes associated with DES mutations are heterogeneous and range from isolated myopathies to different kinds of cardiomyopathies (54). In a study on the prevalence of DES variants in DCM, LMNA genetic variants accounted for up to 2% of disease manifestations (55). A meta-analysis of 159 patients with 40 different DES mutations reported in the literature indicated that up to 50% of carriers showed a cardiomyopathy, mostly DCM (17%), RCM (12%), HCM (6%) and less commonly ACM (1%)(66). It is interesting to underline that recently a mutation of this gene, compromising the binding between desmin and desmoplakin, has been described in ACM subjects with predominant and severe involvement of the LV (67).

Transmembrane protein 43 (TMEM-43): Transmembrane protein 43 is a 45-kDa putative membrane protein encoded by the TMEM43 gene (68). This gene contains a response element for the adipogenic transcription factor PPAR gamma, which may explain the fibrofatty replacement of the myocardium in ARVC. A TMEM43 mutation underlies one form of ACM identified in a founder population on the island of Newfoundland in Canada. Merner et al. reported TMEM43 variants responsible for a severe, full-penetrance phenotype of ACM associated with a high risk of SCD(69).

Filamin C (FLNC): Filamin C (FLNC) is an essential structural crosslinker of actin rods at the sarcomeric z-disc of both cardiac and skeletal muscle. Moreover, as Filamin A, FLNC can serve as a nodal point for sarcomeric mechano-transduction in different muscle cells (70). Filamin C was first reported to be associated with various forms of skeletal myopathy (71). Truncating FLNC mutations have been identified in DCM patients. Consistently with other genetic variants found in cardiomyopathy, FLNC variants found in human DCM do not come along with concomitant myofibrillar myopathy. In 2016 Ortiz-Genga identified 23 new truncating variants of FLNC in a DCM cohort. Moreover, FLNC-DCM phenotypes were found to show a marked LV-dilation and systolic dysfunction, a high degree of myocardial fibrosis and ECG abnormalities (T-Wave inversion and low voltage QRS) (72). Interestingly, Begay et al. described a phenotypic RV involvement in a series of FLNC truncated mutation carriers, thus indicating a potential phenotypic overlap between DCM and ACM in some FLNC mutation carriers (73). It is noteworthy that truncating FLNC variants have been also reported in patients affected with the "classical" form of ACM (74). Celeghin et al reported a series of ACM probands tested negative for mutations in ACM-related genes who underwent FLNC genetic screening, with detection of novel FLNC variants in 4% of cases. Clinical evaluation found that the most common ACM disease phenotype was the left-dominant one and that patients had a late disease onset (after 40 years). In addition, FLNC-associated cardiomyopathy was characterized by ECG abnormalities such as low QRS voltages and inferolateral/ lateral TWI, frequent and complex VAs, and extensive nonischemic LV LGE/myocardial fibrosis on CMR or postmortem analysis (75). Gigli et al recently analyzed a large series of FLNC variants carriers (76) and found an ALVC phenotype in 21% of cases, a DCM phenotype in 42% and a ARVC phenotype in 3% of cases.

Phospholamban (PLN): this is a transmembrane protein of the sarcoplasmic reticulum involved in calcium transport by inhibiting the activity of the SERCA2 (sarcoplasmic/endoplasmic reticulum calcium ATPase) pump. The activity of SERCA2a and its interaction with PLN determines the rate of relaxation and contraction of the cardiac myocyte (77). Among the pathogenic variants identified, R14del is the most common within the cohorts of patients affected by DCM and ACM, particularly

in Netherlands, where it reaches a prevalence of 10-15% in ACM patients (78). Clinical-instrumental findings of these patients are low QRS voltage at ECG, high frequency of malignant ventricular arrhythmias and end-stage heart failure(78,79). Van Rijsingen et al., through the study of a cohort of 403 patients carrying R14del mutation, observed that during a follow-up period of approximately 4 years, 19% of patients had a malignant ventricular arrhythmia and 11% showed an end-stage heart failure. In addition, the authors highlighted the role of left ventricular ejection fraction < 45% and the presence of non-sustained ventricular tachycardia on Holter ECG as independent predictors of malignant arrhythmias (79). These results were subsequently incorporated into the 2019 HRS ACM consensus document, providing an indication for ICD implantation in patients carrying pathogenic mutation on *PLN* and at least one of the two risk parameters highlighted in the previous publication (21). Recently, Verstraelen et al. proposed a risk score incorporating new clinical parameters such as premature ventricular contraction count/24 h, amount of negative T waves, and presence of low-voltage electrocardiogram (80).

In the last ten years, an increasing number of evidence and advances in molecular research have led to a changing in the etiology of ACM. From the initial idea of a monogenic disease, recent findings suggest rather a complex genetic condition, in which the phenotype is determined by the interaction of multiple genetic and environmental factors (1,2). The frequency of compound and digenic heterozygosity is reported to be 10-25% of cases depending on the study population. Noteworthy, genotype-phenotype correlation studies have shown that a higher mutational load correlates with an unfavorable clinical course, a higher risk of SCD and frequent biventricular involvement (81–83)

Clinical features and natural history

In ACM the presence of fibro-fatty tissue leads both to morphological ventricular abnormalities and circuits that constitute the anatomic basis of re-entry ventricular arrhythmias. The phenotypic aspects of ACM can variate in a considerable way, ranging from asymptomatic family members with mild forms of the disease to symptomatic patients who experienced life-threatening ventricular

arrhythmias or refractory heart failure (1). In affected families the presence of gene mutations carriers who do not show any signs of the disease (the so called “healthy carriers”) has been reported. The most common clinical presentation consists of arrhythmic symptoms such as palpitations, syncopal episodes or cardiac arrest; unfortunately, SCD can be the first clinical manifestation of the disease in previously asymptomatic individuals, especially in the young and in competitive athletes (84,85). The prognosis in ACM patients is related to the degree of electric instability and of ventricular muscle disease. Some external factors can worsen the disease progression, among them sports activity was the most investigated. James et al. first reported in humans that a history of intense exercise was more often associated with desmosomal gene mutation carriers developing the disease and patients with overt ACM suffering major ventricular arrhythmias (86). From then on, many other studies (87–93) confirmed that sports activity, especially if prolonged, promotes the development of ACM in genotype positive/phenotype negative patients, deteriorates ventricular function in patients with overt ACM, trigger ventricular arrhythmias, and increase the likelihood of ICD interventions. Indeed, the physical activity generates a mechanical stress at the level of a previously genetically impaired cell-cell adhesion, thus promoting myocyte death. The risk of VAs and mortality both in ACM patients and genotype-positive relatives can be reduced by lowering exercise (8,89,90). Thus, pre-clinical genetic testing among asymptomatic gene-carriers with the aim to educate to lifestyle changes can prevent the development of the disease in this category. Furthermore, identification of early stages of the disease through preparticipation screening and disqualification from competitive sports activity may prevent disease progression and fatal arrhythmias (94). Accordingly, both European and American guidelines recommend restriction from competitive sports activity for ACM probands and at-risk relatives as a measure aimed to reduce the risk of SCD (95,96). However, considering the general physical and mental health benefits related to exercise, ESC guidelines allow a maximum of 150 minutes of low-moderate intensity exercise per week in all affected and at-risk subjects (95).The overall mortality rate varies in literature, due to the different patients selection. In a study on 37 ACM families with a mean follow-up of 8,5 years, a mortality of 0.08% per year was found (8), while in a

series of 61 ACM patients with a mean follow-up of 4.6 years the mortality rate was estimated to be of 4% per year (97). This high variability is probably in relation to the different populations and reflects the wide spectrum of ACM clinical phenotype (2).

Diagnostic tools in ACM

Clinical diagnosis of ACM is multi-parametric, based on a combination of clinical and instrumental parameters. Unfortunately, no specific diagnostic test for the disease has been found so far and since the disease discovery scoring diagnostic systems have been developed. The first diagnostic criteria date back to 1994, when an international task force selected clinical and instrumental parameters to diagnose the original form of the disease, i.e., the right-dominant phenotype. At that time, the presence of a family history of ACM, ECG typical features, presence of ventricular arrhythmia, histopathological findings, as well as structural and functional parameters of the RV were included. The TF diagnostic criteria were grouped into 6 different categories encompassing the spectrum of clinical manifestations of ARVC. The criteria were classified into "major" and "minor" according to their specificity for ARVC and the diagnosis fulfilled in the presence of 2 major criteria or 1 major plus 2 minor or 4 minor criteria from different categories. However, the main limitations of the 1994 TF diagnostic criteria relate to the qualitative and subjective assessment of the clinical features of the disease. In details, the TF criteria lack quantitative values to assess RV dilatation/dysfunction regarding the morphofunctional abnormalities. Furthermore, to exclude potential overlap with DCM forms, patients should not show signs of left-sided involvement. From the arrhythmic point of view, a high arrhythmic burden was defined as the presence of >1000 PVCs in 24h, or the presence of a sVT with LBBB morphology. Both criteria were designated as minor, as they were difficult to distinguish from idiopathic LV arrhythmias, which are characterized by a benign prognosis. Moreover, the lack of a precise definition of fibro-adipose infiltration at biopsy (major criterion) and of the epsilon wave at ECG, left much to the judgement of the individual clinician, with the consequent risk of over-diagnosis. In summary, the 1994 criteria were a first attempt to bring order

to a complex clinical context and were characterized by a good specificity for ACM, nonetheless they lack sensitivity for certain phenotypes and early forms of the disease.

For these reasons, a revised version of the previous criteria was published in 2010. The aim was to increase sensitivity while keeping diagnostic specificity unchanged. The multi-parametric scoring system, based on the presence of major and minor criteria, was retained, although revised. In detail:

- 2010 revised TF criteria provided quantitative imaging (echocardiography, ventricular angiography, and CMR) reference values based on sex-specific volumetric measurements indexed to body surface area (BSA) to define normal RV and to categorize the various degrees of structural and functional RV abnormalities. To optimize the diagnostic specificity of morpho-functional criteria, the 2010 TF criteria required the association of global RV dilatation or RV systolic dysfunction with regional wall motion abnormalities. These criteria were classified as “major”, or “minor” based on the severity of RV dilatation and/or systolic impairment.
- They provided a definition and quantitative histomorphometry for proper grading of fibrofatty replacement of the myocardium on EMB.
- ECG and arrhythmic features: in the 2010 TF criteria, T-wave inversion in V1–V3 as well as sVT with a LBBB morphology with superior/indeterminate QRS axis, either sustained or non-sustained, became major criteria. Other findings were included among the minor criteria: T-wave inversion in V1 and V2 in the absence of right bundle branch block (RBBB) and from V1 to V4 in the presence of complete RBBB; prolongation of right precordial QRS duration with delayed S-wave upstroke (terminal activation delay >55 ms); positivity of any 1 of the 3 signal-averaged ECG parameters for late potentials; and PVCs >500 per 24 hours on Holter monitoring.
- 2010 TF diagnostic criteria were modified to include genetic information in the category of “family history” criteria as a major criterion for diagnosis of ARVC.

Although at the present time 2010 TFC represent the latest universally recognized diagnostic criteria, the advancement of imaging techniques, and in particular of tissue characterization at CMR highlighted the presence of several limitations, especially for left-dominant forms. In this regard, an International Expert report in 2019 emphasized some criticisms of the 2010 criteria considering new achievement on the wide phenotypic spectrum of the disease. Among these authors underlined the of tissue characterization, which has become the gold standard for assessing LV involvement. In 2020, a new consensus document published the so called 'the Padua criteria', with the aim to overcome limitations of previous diagnostic criteria, with particular regard to diagnosis of left dominant forms of the disease. Beside revising certain parameters included in the diagnostic categories, the Padua criteria provided a phenotypic classification of the disease (Figure 2):

- the “dominant-right” variant (i.e., the classic ARVC phenotype characterized by the predominant RV involvement, with no LV abnormalities).
- the “biventricular disease” variant, characterized by the involvement of both RV and LV.
- the “dominant-left” variant (also referred to as ALVC) characterized by LV involvement, with no RV abnormalities.

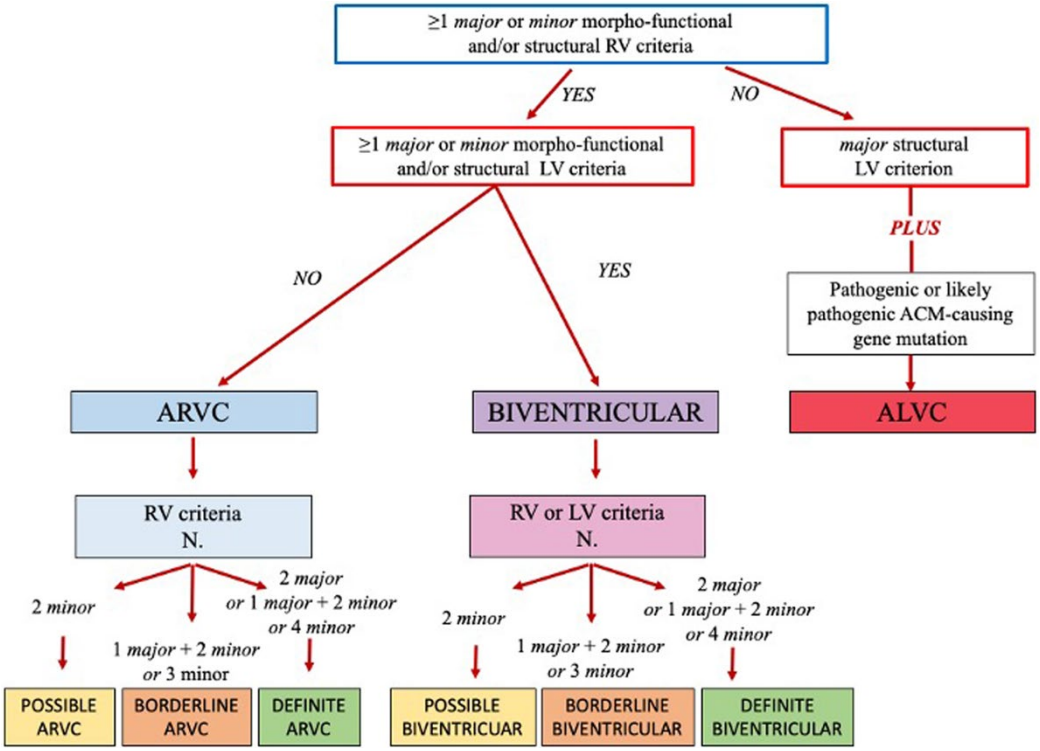

Figure 2. **Diagnostic flow-chart for ACM phenotypic variants.** ACM indicates arrhythmogenic left ventricular cardiomyopathy; ALVC, arrhythmogenic left ventricular cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; LV, left ventricle; and RV, right ventricle. From Corrado et al (1).

The multiparametric diagnostic approach was maintained with criteria grouped in 6 categories encompassing functional and structural ventricular abnormalities, tissue characterization findings, depolarization and repolarization electrocardiographic alterations, ventricular arrhythmias, and familial/genetic factors (Table. 1).

Comparison of 2010 TF Criteria and 2020 International Criteria for Diagnosis of ARVC		
Category	**2010 TF criteria**	**2020 International criteria**
I. Global or regional dysfunction and structural alteration	*Major* *By 2D echocardiogram:* • Regional RV akinesia, dyskinesia, or aneurysm *and* one of the following (end diastole)	*Major* *By 2D echocardiogram, CMR, or angiography:* • Regional RV akinesia, dyskinesia, or bulging

Comparison of 2010 TF Criteria and 2020 International Criteria for Diagnosis of ARVC		
Category	**2010 TF criteria**	**2020 International criteria**
	- PLAX RVOT ≥32 mm (corrected for body size [PLAX/BSA] ≥19 mm/m^2) - PSAX RVOT ≥36 mm (corrected for body size [PSAX/BSA] ≥21 mm/m^2) - Fractional area change ≤33% *By MRI:* • Regional RV akinesia or dyskinesia or dyssynchronous RV contraction *and* one of the following: • - Ratio of RV end-diastolic volume to BSA: ≥110 ml/m^2 (male) or ≥100 ml/m^2 (female) - *or* RV ejection fraction ≤40% *By RV angiography:* • Regional RV akinesia, dyskinesia, or aneurysm *Minor* *By 2D echocardiogram:* • Regional RV akinesia or dyskinesia *and* one of the following (end diastole): - PLAX RVOT ≥29–<32 mm; (corrected for body size [PLAX/BSA] ≥16–<19 mm/m^2) - PSAX RVOT ≥32–<36 mm; (corrected for body size [PSAX/BSA] ≥18–<21 mm/m^2) - or fractional area change >33%–≤40% *By MRI:* • Regional RV akinesia or dyskinesia or dyssynchronous RV contraction *and* one of the following: - Ratio of RV end-diastolic volume to BSA ≥ to <110 ml/m^2 (male) or ≥90 to <100 ml/m^2 (female) • - *or* RV ejection fraction >40% to ≤45%	*plus* 1 of the following: • Global RV dilatation (increase of RV EDV according to the imaging test specific nomograms for age, sex, and BSA) *or* • Global RV systolic dysfunction (reduction of RV EF according to the imaging test specific nomograms for age and sex) *Minor* *By 2D echocardiogram, CMR, or angiography:* • Regional RV akinesia, dyskinesia or aneurysm of RV free wall
II. Tissue characterization	*Major* *By EMB* • Residual myocytes <60% by morphometric analysis (or 50% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without	*Major* *By CE-CMR:* • Transmural LGE (stria pattern) of ≥1 RV region(s) (inlet, outlet, and apex in 2 orthogonal views)

Comparison of 2010 TF Criteria and 2020 International Criteria for Diagnosis of ARVC		
Category	**2010 TF criteria**	**2020 International criteria**
	fatty replacement of tissue on endomyocardial biopsy *Minor* *By EMB* • Residual myocytes 60% to 75% by morphometric analysis (or 50–65% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy	*Major* *By EMB (limited indications):* • Fibrous replacement of the myocardium in ≥1 sample, with or without fatty tissue
III. Repolarization abnormalities	*Major* • Inverted T waves in right precordial leads (V1, V_2, and V_3) or beyond in individuals >14 y of age (in the absence of complete RBBB QRS ≥120 ms) *Minor* • Inverted T waves in leads V_1 and V_2 in individuals >14 y of age (in the absence of complete RBBB) or in V4, V5, or V6 • Inverted T waves in V_1, V_2, V_3, and V_4 in individuals >14 y of age in the presence of complete RBBB	*Major* • Inverted T waves in right precordial leads (V_1, V_2, and V_3) or beyond in individuals with complete pubertal development (in the absence of complete RBBB) *Minor* • Inverted T waves in leads V1 and V2 in individuals with completed pubertal development (in the absence of complete RBBB) • Inverted T waves in V1, V2, V3 and V4 in individuals with completed pubertal development in the presence of complete RBBB
IV. Depolarization and conduction abnormalities	*Major* • Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V1 to V3) *Minor* • Late potentials by SAECG in ≥1 of 3 parameters in the absence of a QRS duration of ≥110 ms on the standard ECG - Filtered QRS duration (fQRS) ≥114 ms - Duration of terminal QRS <40 μV (low-amplitude signal duration) ≥38 ms - Root-mean-square voltage of terminal 40 m ≤20 μV	*Minor* • Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V1 to V3) • Terminal activation duration of QRS ≥55 ms measured from the nadir of the S wave to the end of the QRS, including R', in V1, V2, or V3 (in the absence of complete RBBB)

Comparison of 2010 TF Criteria and 2020 International Criteria for Diagnosis of ARVC		
Category	**2010 TF criteria**	**2020 International criteria**
	• Terminal activation duration of QRS ≥55 ms measured from the nadir of the S wave to the end of the QRS, including R', in V1, V2, or V3, in the absence of complete right bundle-branch block	
V. Arrhythmias	*Major* • Non-sustained or sustained ventricular tachycardia of left bundle-branch block morphology with superior axis (negative or indeterminate QRS in leads II, III, and aVF and positive in lead aVL) *Minor* • Nonsustained or sustained ventricular tachycardia of RV outflow configuration, left bundle-branch block morphology with inferior axis (positive QRS in leads II, III, and aVF and negative in lead aVL) or of unknown axis • >500 ventricular extrasystoles per 24 h (Holter)	*Major* • Frequent ventricular extrasystoles (>500 per 24 h), non-sustained or sustained ventricular tachycardia of LBBB morphology* *Minor* • Frequent ventricular extrasystoles (>500 per 24 h), non-sustained or sustained ventricular tachycardia of LBBB morphology with inferior axis ("RVOT pattern")
VI. Family history/genetics	*Major* • ACM confirmed in a first-degree relative who meets diagnostic criteria • ACM confirmed pathologically at autopsy or surgery in a first-degree relative • Identification of a pathogenic or likely pathogenetic ACM mutation in the patient under evaluation *Minor* • History of ACM in a first-degree relative in whom it is not possible or practical to determine whether the family member meets diagnostic criteria • Premature sudden death (<35 y of age) due to suspected ACM in a first-degree relative • ACM confirmed pathologically or by diagnostic criteria in second-degree relative	

Table 1. ACM indicates arrhythmogenic cardiomyopathy; BSA, body surface area; CMR, cardiac magnetic resonance; EDV, end diastolic volume; EF, ejection fraction; EMB, endomyocardial biopsy; ITF, International Task Force; LBBB, left bundle-branch block; LGE, late gadolinium enhancement; LV, left ventricle; MRI, magnetic resonance imaging; PLAX, parasternal long axis; PSAX, parasternal short axis; RBBB, right bundle-branch block; SAECG, signal-averaged ECG; RV, right ventricle; and RVOT, right ventricular outflow tract. Adapted from Corrado et al(19).

The main innovation of the 2020 International criteria was the introduction of tissue characterization findings by LGE for detection of fibro (-fatty) myocardial replacement of both ventricles. Other

revisions were made to the morpho-functional parameters and to the cut-offs that were updated to international guidelines, the presence of epsilon waves being downgraded to a minor criterion (due to poor sensitivity and specificity). Other revisions were made to the morpho-functional parameters and to the cut-offs that are updated to international guidelines, the presence of epsilon waves being downgraded to a minor criterion (due to poor sensitivity and specificity). In addition to the latter, several criteria were added to increase the diagnostic sensitivity for ALVC (Table. 2).

The 2020 International Criteria for Diagnosis of ALVC	
Category	**Diagnostic criteria**
I. Morpho-functional ventricular abnormalities	*Minor* • Global LV systolic dysfunction (depression of LV EF or reduction of echocardiographic global longitudinal strain), with or without LV dilatation (increase of LV EDV according to the imaging test specific nomograms for age, sex, and BSA) *Minor* • Regional LV hypokinesia or akinesia of LV free wall, septum, or both
II. Structural myocardial abnormalities	*Major* • LV LGE (stria pattern) of ≥1 Bull's Eye segment(s) (in 2 orthogonal views) of the free wall (subepicardial or midmyocardial), septum, or both (excluding septal junctional LGE)
III. Repolarization abnormalities	*Minor* • Inverted T waves in left precordial leads (V_4–V_6) (in the absence of complete LBBB)
IV. Depolarization abnormalities	*Minor* • Low QRS voltages (<0.5 mV peak to peak) in limb leads (in the absence of obesity, emphysema, or pericardial effusion)
V. Ventricular arrhythmias	*Minor* • Frequent ventricular extrasystoles (>500 per 24 h), non-sustained or sustained ventricular tachycardia with a RBBB morphology (excluding the "fascicular pattern")

VI. Family history/genetics	*Major* • ACM confirmed in a first-degree relative who meets diagnostic criteria • ACM confirmed pathologically at autopsy or surgery in a first-degree relative • Identification of a pathogenic or likely pathogenetic ACM mutation in the patient under evaluation *Minor* • History of ACM in a first-degree relative in whom it is not possible or practical to determine whether the family member meets diagnostic criteria • Premature sudden death (<35 y of age) because of suspected ACM in a first-degree relative • ACM confirmed pathologically or by diagnostic criteria in second-degree relative

Table 2 ACM indicates arrhythmogenic cardiomyopathy; ALVC, arrhythmogenic left ventricular cardiomyopathy; BSA, body surface area; CMR, cardiac magnetic resonance; EDV, end diastolic volume; EF, ejection fraction; LBBB, left bundle-branch block; LGE, late gadolinium enhancement; LV, left ventricle; and RBBB, right bundle-branch block. Adapted from Corrado et al *(19)*.

Arrhythmogenic left ventricular cardiomyopathy

Arrhythmogenic left ventricular cardiomyopathy (ALVC) is defined as an ACM form characterized by an early and predominant involvement of the LV (18). In contrast to the biventricular variant, where degree of ventricular dysfunction is similar in the two ventricles, in this case RV involvement, if present, has a minor significance. Soon after the clinical characterization of the disease, several autopsies and histological studies found a LV involvement in the majority of ACM patients (76%) (17). In recent autopsy studies, 87% of patients who died suddenly due to ACM had LV lesions (98). The LV involvement is often subepicardial, less frequently intramyocardial, with a preferential topographic distribution of the inferior and lateral wall, although there may be septal involvement(47,99). The big advance in the recognition and definition of this disease phenotype came with the introduction of contrast enhanced CMR, allowing in vivo tissue characterization.

Early clinical evidence of left ventricular involvement was reported by Bauce et al.in 2005. In particular, the authors analyzed the clinical-instrumental, anatomopathological, and outcome findings of 38 patients carrying mutations in the *DSP* gene. The study demonstrated that familial ACM due to *DSP* mutations is characterized by a high occurrence of SCD and that LV involvement is not a rare feature. In addition, myocardial damage, characterized by ST-segment elevation on ECG, chest pain, and increased myocardial enzymes, was observed in two cases (47). The first systematic clinical description of ALVC was made in 2008 by Sen Chowdhry et al (18). The study described the clinical and instrumental characteristics of 48 patients, diagnosed on the basis of the presence of arrhythmias from the LV, LGE at CMR and the exclusion of other pathologies that could explain their origin. The study provides an accurate description of the phenotype (18):

- Twelve-lead ECG: unexplained T-wave inversion in V5, V6 ± V4, I, and aVL
- Ventricular arrhythmia: presence of sustained or non-sustained ventricular tachycardia of RBBB configuration documented on ECG or Holter monitoring or during exercise

testing. Frequent ventricular extrasystoles (RBBB morphology) exceeding the degree of systolic dysfunction.

- Imaging: presence of LV aneurysms, mild LV dilation and/or systolic impairment (with arrhythmic presentation).
- Endomyocardial biopsy: presence of myocytes loss with fibrofatty replacement on histology
- CMR: extensive LGE of LV myocardium (with subepicardial/midmyocardial distribution).
- Difficulties in arrhythmic risk stratification: unlike DCM, major arrhythmic events were present in patients with mildly reduced or preserved LV function

Another commonly described feature is the presence of low QRS voltages in peripheral leads (22). It has been speculated that this may be secondary to fibro-fatty replacement of the left ventricle, but conclusive studies are not yet available.

The 2010 TFC increased the diagnostic specificity for the classic forms of the disease, nonetheless they were found to be insensitive in ALVC diagnosis (50). However, in 2020, an international consensus document, named 'the Padua criteria', was published with the aim to include the clinical, instrumental (with regards to tissue characterization at CMR) and genetic features of left-dominant forms in the diagnostic criteria (20).

Indeed, CMR plays a pivotal role in diagnosis mostly thorough the possibility to detect the presence of fibrous tissue by means of LGE detection. The peculiar pattern of LGE distribution is represented by LV subepicardial stria, most frequently located on infero-lateral basal segments with variable extension and in some cases leading to a circumferential involvement of the entire ventricle (Figure 3). Unfortunately, this pattern is not exclusive of ACM and enters into differential diagnosis with other diseases, mainly myocarditis. Recent studies demonstrated that in patients with acute myocarditis LGE can be found in 41% of cases on subepicardial layers of LV inferior and lateral

walls (100) with not significant changes only in 30% of patients at six months' follow-up (101). Conversely, in ALVC, because of its progressive pathogenesis, LGE is unchanged or increased in almost all patients. For these reasons, the diagnosis cannot be based solely on instrumental examinations but must take account of family history, genetic findings, and, in sporadic cases, endomyocardial biopsy.

Similarly, DCM forms showing clinical and instrumental features similar to those of ALVC have been described (so-called “dilated cardiomyopathies with an arrhythmogenic phenotype”) (102). However, studies comparing clinical and CMR characteristics of a group of DCM and ACM patients demonstrated that the amount of LGE and its distribution are significantly different between the two groups. As described above, ACM LV-LGE showed a peculiar pattern, while in DCM, an intramural stria at the septal level constituted a common finding. In addition, the amount of LGE was significantly higher in ACM group (103). The explanation for these findings can be obtained by analyzing the different pathophysiology of the two cardiomyopathies. In DCM, fibrosis is a secondary phenomenon due to ventricular enlargement, while in ACM, it is a primary phenomenon resulting from the death of cardiomyocytes through necrosis and apoptosis(103).

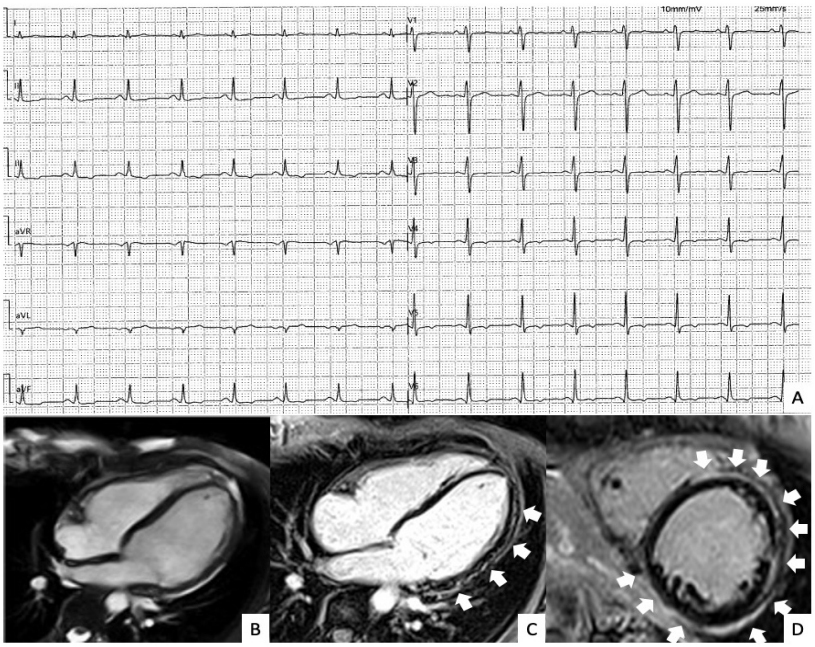

Figure 3. Electrocardiographic and CMR features of an ALVC patient. ECG shows TWI V4-V6 and inferior leads with the presence of low QRS voltages in limb leads (A), while CMR shows a mild dilatation of left ventricle with moderate reduction of EF (B). After gadolinium administration circumferential LGE is evident (C, D).

The hot-phase episodes in ACM patients

Patients with ALVC may experience 'hot phases', characterized by chest pain, enzymatic release and frequent ECG modifications. Unfortunately, differential diagnosis with acute myocarditis can be quite difficult. It is estimated that "hot phase" phenomenon has an incidence that ranges from 5% to 25% of patients in different series (50,104). In a recent paper Bariani et al. analyzed clinical and instrumental findings of a series of 23 patients affected by ACM, manly with a ALVC or BIV phenotype, who experienced one or more episodes of myocardial injury (104). The study demonstrated that myocarditis like picture seems to be a rather uncommon clinical presentation of ACM, often occurring in the pediatric age, and CMR is the first-choice examination for the differential diagnosis between ACM and acute myocarditis. Moreover, in patients with this clinical presentation EMB can have a pivotal role in differential diagnosis as well as family screening and genetic test (104). The gene most frequently involved ids DSP (47). Recently, several publications described the clinical characteristics and the outcome pathogenic *DSP* mutation carriers. Smith et al. analyzed a cohort of 107 patients carrying a truncating mutation in *DSP* gene, compared to 81 patients

with pathogenic *PKP2* mutations. A LV involvement was observed only in patients with *DSP*, with LGE reported in 40% of patients in the *DSP* cohort. Episodes of myocardial damage were observed in 15% of patients. Furthermore, major arrhythmic events were strongly associated with reduced LV function (< 55%), in contrast to the *PKP2* group where RV function was a predictor of arrhythmic events (50). However, to date, it is unclear why some ACM patients develop episodes of myocardial injury, and it has been speculated that this phenomenon could be in relation with the wall thickness extent, considering that these episodes are more intense and symptomatic when involving the LV as in ALVC forms (50). Moreover, their role in disease progression and arrhythmic risk remains to be elucidated. Wang et al. described the follow-up of 91 patients with pathogenic variants in the *DSP* gene over a median follow-up of 4.5 years. The authors concluded that *DSP* cardiomyopathy affects both ventricles and carries high risk for ventricular arrhythmia and heart failure and that myocardial injury is associated with worse disease outcomes (51). Finally, Bariani et al recently described a cohort of 73 ACM patients carrying pathogenic/likely pathogenic mutations. Authors found that the phenotype changed during follow-up (mean 11 years; range 1-39 years) in 25 patients (35%), with ALVC becoming the most frequent form (36%), followed by BIV (27%) and ARVC (22%) forms. Major ventricular arrhythmias were detected in 21 patients (29%) and were more common in the ARVC (n = 6, 56%) and BIV (n = 8, 40%) forms than in the ALVC forms (n = 4, 15%). Interestingly, in patients with ALVC, major ventricular arrhythmias occurred in the presence of normal or mildly reduced systolic function. Heart failure occurred in 6 patients (8%), none with ALVC. Females more commonly showed LV involvement, whereas ARVC was found more frequently in males, who showed a higher incidence of major ventricular arrhythmias, heart failure and cardiac death.

Beside *DSP*, FLNC gene has been found to be significantly related to ALVC. Several studies demonstrated an association between mutations of this gene and a peculiar LV phenotype, characterized by extensive non-ischemic LV fibrosis, life-threatening VAs, and SCD (72,105,106). A description of the main studies can be found in the section on genetics. However, a recent study by Celeghin et al found that FLNC-associated cardiomyopathy is characterized by a late onset and a

predominantly left-dominant phenotype. Typical ECG abnormalities consist of low QRS voltages and inferolateral/lateral TWI, and frequent complex VA. Non-ischemic LV scarring is detectable by CMR or postmortem analysis. The presence of low QRS voltages in limb leads, inferolateral/lateral TWI and LV LGE/fibrosis, but not LV dilatation or severe systolic dysfunction, was found to be associated with SCD (75).

Phospholamban gene has also been associated with ALVC (22). It represents a rare causative gene of ACM (see dedicated section in the genetics section). The p.Arg14del variant has been extensively studied and appears to be associated with the left forms of the disease, characterized by low QRS voltages at ECG, presence of LGE at CMR and risk of sudden death (79).

However, in contrast to classical variants, no conclusive data exist for ALVC as far as prognosis and arrhythmic risk stratification. While parameters such as degree of RV dilatation and dysfunction, extension of T wave inversion, degree of electrical instability exist for right and biventricular variants arrhythmic risk stratification (29), no validated predictors are present for ALVC forms to date. In 2020, a risk score has been developed to help the clinician in the decision to implant an ICD in primary prevention (107). Several studies sought to evaluate the arrhythmic risk in ALVC. Casella et al. investigated the follow-up of a cohort of ACM patients with ALVC a BIV phenotype. Over a median time of 5.4 years, they observed that ALVC and BIV ACM forms appear more prone to VAs than classical forms. The novel prediction model effectively predicted arrhythmic risk in the classical ARVC cohort but seemed to underestimate it in non-classical forms(108). Similarly, Aquaro et al. evaluated the role of CMR in the arrhythmic risk stratification of a cohort of 140 ACM patients, comparing it with the 5-year risk score. The authors reported that in a median follow-up of 5 years LV involvement was associated with a worse prognosis than lone-RV presentation, and together with the LV-dominant pattern and the 5-year ARVC risk score, it was an independent predictor of the major cardiac endpoint. The 5-year ARVC score was found to be valid to predict risk in patients with lone-RV presentation but underestimates the risk in case of LV involvement(101). Recently, a

specific risk score for PLN p.Arg14del variant carriers that considers the number of PVCs, the extent of T-waves inversion and the presence of low QRS complex voltages has been proposed (80).

Therapeutic strategies in ACM

Physical restriction: sports activity has been proved to enhance ACM progression and to worsen the disease arrhythmic substrate(85,87,89,90,109). Conversely, the risk of ventricular arrhythmias (VAs) and mortality can be lowered by reducing exercise (8,89,90,110).

Different categories of ACM patients show a dose-dependent association between exercise exposure and disease penetrance. Genotype-positive relatives undergoing competitive sports and high-intensity physical exercise are affected by an increased risk of VAs and heart failure as documented by clinical studies (86,111). With this regard, pre-symptomatic genetic testing has a role because it can detect those individuals in whom a lifestyle change can reduce the risk of developing. Likewise, in patients with an overt phenotype, preparticipation screening and disqualification may prevent SCD (94).

Accordingly, both European and American guidelines recommend restriction from competitive sports activity of ACM patients and at-risk relatives as measure aimed to reduce the risk of SCD(95,96).

Drug therapy

Beta-blockers: ventricular arrhythmias and cardiac arrest in ACM are usually promoted by adrenergic stimulation and occur typically during or early after a physical effort. Thus, beta-blockers are recommended in ACM patients symptomatic for frequent premature ventricular complex (PVCs) and non-sustained ventricular tachycardias (NSVT), patients with recurrent sVT, appropriate ICD therapies, or inappropriate ICD interventions resulting from sinus tachycardia, supraventricular tachycardia, or atrial fibrillation/flutter with high-ventricular rate. In addition, since they reduce the ventricular wall stress they are considered as first-line medications in the management of heart failure, they should be offered in all patients with a definite diagnosis of ACM, irrespective of arrhythmias. So far, in phenotype-negative gene-carriers prophylactic use of these drugs is not justified (112,113).

Antiarrhythmic drugs: when beta-blockers alone are not sufficient to control the arrhythmic burden, anti-arrhythmic drug therapy is indicated for symptomatic patients with frequent PVCs and/or NSVT.

In particular, sotalol and amiodarone (alone or associated with beta-blockers) are the most effective drugs with a relatively low proarrhythmic risk(112,113).

Heart failure drugs: the standard pharmacological treatment for heart failure (angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, betablockers, and diuretics) is recommended in patients who develop right, left, or biventricular heart failure(113).

New drugs: therapeutic strategies targeting the Wtn/β and NFκB pathways appear to lower the disease in animal models and thus may be promising options in the future(114).

Catheter Ablation: catheter ablation should be considered as therapeutic option for patients symptomatic for PVCs or VT or frequent appropriate ICD interventions on sVT despite optimal medical therapy, in order to improve symptoms and prevent ICD shocks, respectively (112). The initial experience with this technique reported high acute success rates followed by high rates of recurrences due to the progressive nature of the disease leading to the development of multiple arrhythmogenic foci over time(115–117). Moreover, regions of fibrofatty replacement - that are regarded as the arrhythmogenic substrate for VT - are mostly located in the subepicardial RV layers thus partially explaining the failure of the traditional endocardial approach. Epicardial catheter ablation appears to be a feasible and more effective approach for patients in whom one or more endocardial procedures have been unsuccessful (113,118). Importantly, neither antiarrhythmic drugs nor catheter ablation proved to reduce the risk of SCD. Thus, they should be considered as measures to reduce arrhythmic episodes' frequency rather than improve prognosis. The only effective therapy for the prevention of SCD in such patients is ICD implantation(113).

ICD implantation: regarding recommendation of ICD implantation in ACM patients, three risk categories (“high,” “moderate,” and “low”) have been defined. Those with a history of cardiac arrest or hemodynamically unstable sVT or severe ventricular dysfunction (either right, left, or of both ventricles) are considered “high risk” subjects and receive a Class I recommendation for ICD implantation.

Patients with significant risk factors, such as syncope, non-sustained VT, or moderate dysfunction of the RV, LV, or both ventricles, are classified as “intermediate risk” subjects and receive a class IIa recommendation for ICD implantation. Recently, a scoring system including ECG, CMR, and degree of electrical instability has been proposed (107). Since the presence of scars in ACM may not affect the LV performance but can still trigger adverse arrhythmic events, an ICD implant for primary prevention should be considered in the presence of extensive LGE/fibrosis even if the LV systolic function is not severely depressed(113).

Heart transplantation: heart transplantation represents the final therapeutic option for ACM patients with advanced stages of the disease who suffer refractory congestive heart failure and/or uncontrollable arrhythmic storms, despite previous attempts with catheter ablation and ICD therapy (113).

AIM OF THE STUDY

To characterize the genetic background, clinical features, and outcome of the left dominant Arrhythmogenic Cardiomyopathy (ALVC) and to make a comparison with the right dominant (ARVC) and biventricular (BIV) ACM phenotype. Moreover, we sought to investigate possible prognostic predictive factors for life-threatening ventricular arrhythmias.

MATERIALS AND METHODS

From the entire cohort of 544 patients (probands and family members) followed at the Inherited Arrhythmogenic Cardiomyopathy Unit of the University of Padua, we selected 436 patients (80%) fulfilling ACM diagnosis (20,119). Subsequently, based on phenotypic expression, patients were divided into three subgroups (**Figure 4**):

- ALVC forms were defined in presence of epicardial LV late gadolinium enhancement (LGE) and a spared or mildly involved RV. In addition, to increase the diagnostic specificity, at least one of the following parameters had to be present:
    - Presence of pathogenic/likely pathogenic genetic variant associated with ACM.
    - Presence of family history of ACM or DCM.
    - Presence of family history of SCD with autopsy finding of ACM.
    - Patients with clinical features in keeping with ALVC, in the absence of anamnestic, clinical, and instrumental features suggesting other diagnosis, such as myocarditis or sarcoidosis. Patients of this group entered the group of "Idiopathic ALVC".
    - ARVC forms were defined as patients with ACM forms with prevalent RV involvement and preserved LV systolic function.
- Biventricular (BIV) forms defined as parallel involvement of the two ventricles.

The reference values used to define the degree of ventricular dilatation and dysfunction are those of the international guidelines(120,121).

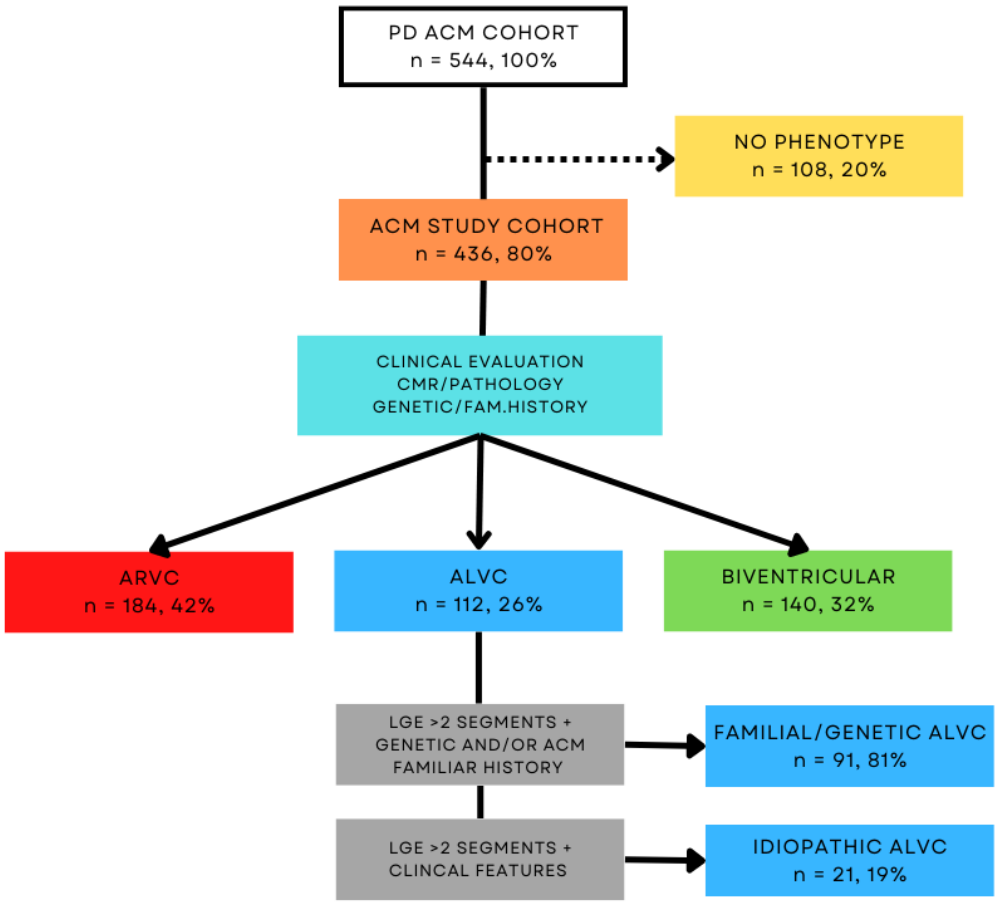

Figure 4. Patient selection process from the ACM general population.

The study protocol included a detailed familial and personal anamnesis, 12-lead ECG, 24-Hour Holter ECG, two-D echocardiogram with Doppler analysis, and CMR.

Anamnestic data: patient ID, gender, date of birth; date of diagnosis and calculated age at diagnosis; previous diagnosis of myocarditis or dilated cardiomyopathy; family history of SCD, DCM; episodes of chest pain (if available, troponin levels), syncope, atrial fibrillation. 2010-ITF criteria: number of major and minor criteria, 2010 ACM diagnosis (definite, borderline, possible). 2020 Padua criteria (number of major and minor criteria), type of diagnosis of ALVC (definite, borderline, possible).

ECG analysis: twelve-lead ECGs were performed for all patients on a standard speed paper (25 mm/sec, 10 mm/mV, 0.05-150 Hz) at each evaluation. The following parameters were considered: type of rhythm (sinus rhythm, sinus arrhythmia, atrial fibrillation); the presence of atrioventricular

block; fragmentation of QRS; complete right bundle branch block (RBBB): QRS duration > 120ms, rSR' in V1-V2 and deep and broadened S wave in V5-V6; complete left bundle branch block: QRS duration > 120ms, deep S-wave in V1- V2 with small or absent initial r-wave, and widened R-wave with a notch in V5-V6, often also in DI and aVL, with absent q-wave; low voltages: defined as QRS of amplitude <5mm in peripheral leads or <10mm in precordial leads: repolarizations abnormalities (T wave inversion divided according to the location in anterior V1-V3, lateral V4-V6, and inferior DII, DIII, and aVF).

24-hour ECG Holter monitoring: each patient performed at least one 24-Holter ECG evaluation. The data collected were analyzed using dedicated software to identify atrial fibrillation, supraventricular ectopic beats, and the presence of ventricular arrhythmias. The latter are classified as follows: single premature ventricular complexes (PVCs), couplets, run of non-sustained ventricular tachycardia (NSVT), or sustained ventricular tachycardia (sVT) according to the duration (under or over 30 seconds respectively). When available, the ventricular arrhythmias' morphology (RBBB or LBBB) was noted.

Echocardiography: echocardiographic examination was performed using a standardized protocol, which included both traditional (2D echocardiography). All examinations were performed with a commercially available GE S6 ultrasound machine (GE Vingmed Ultrasound AS, Horten, Norway). Echocardiographic protocols and measurements were performed according to international recommendations (21–23).

Cardiac magnetic resonance: biventricular morpho-functional assessment was performed using Steady State Free Precession (SSFP) sequences on kinetic images. Calculation of volume, mass, and function was performed in post-processing using specific software (Circle Cardiovascular Imaging, CVI42, Circle International; Calgary, Alberta, Canada). For segmental kinetics of the two ventricles, normal or pathological aspects were defined based on the presence or absence of akinesia and/or dyskinesia, according to TFC-2010 criteria and/or Padua criteria for ALVC forms. Tissue characterization was independently assessed by two experienced examiners; the presence of adipose

infiltration was evaluated using black blood double-inversion recovery T1-weighted Turbo Spin-Echo (TSE) sequences. Triple Inversion Recovery Magnitude (TIRM) sequences were used to identify myocardial edema. Assessment of fibrosis was performed with segmented Inversion Recovery (IR) or GradientEcho (GRE) sequences, obtained at least 10-15 minutes after intravenous injection of 0.2 mmol/Kg gadolinium-based contrast medium (Gadobenate Dimeglumine, Multihance; Bracco), in the same views acquired for kinetic imaging. Inversion times were manually adjusted to null the normal myocardium. The presence/absence of signs of late biventricular gadolinium impregnation and the type of pattern (ischemic, non-ischemic, or junctional) were evaluated. Late gadolinium enhancement (LGE) was defined as positive if visible at the same site in two orthogonal projections. Moreover, to graphically represent LGE's distribution, LV was segmented using the standard 16-segment AHA model. The bullseye was then reconstructed by Matlab (MATLAB 2022, version R2022a, Natick, Massachusetts: The MathWorks Inc).

Genetic analysis: Each patient underwent genetic testing using venous blood samples at the University Hospital of Padua in accordance with the guidelines and directives of the local ethics committee. Coding exons and intronic boundaries of 174 genes related to inherited cardiovascular diseases and sudden death were captured for each proband by using the Trusight Cardio kit (Illumina, San Diego, California). Sequencing was performed using the Miseq platform (Illumina, San Diego, California) with 2 X 150 base read length following Illumina protocols. Bioinformatics analysis was performed by means of a custom pipeline including software for variant calling, genotyping, and annotation. Mean coverage for all the evaluated genes ranged between 250 and 400. All synonymous and intronic (other than canonical splice sites) variants were excluded. Genetic variants were also interrogated in the 1000 Genomes project (www.1000genomes.org), the Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org), and gnomAD databases (http://gnomad.broadinstitute.org/). Predicted functional effect of a coding variant was surveyed using Polyphen-2 (http://genetics.bwh.harvard.edu/pph/), SIFT (http://sift.jcvi.org/), MutationTaster and Combined Annotation Dependent Depletion (CADD) (http://cadd.gs.washington.edu/). The

allele frequency threshold to consider a variant clinically relevant was ≤0.02%. Pathogenicity of variants was classified according to current guidelines [5]. Those variants considered clinically relevant were validated and first-degree relatives were evaluated by direct sequencing (ABI3500Dx, Life Technologies).

Outcome analysis: patients' follow-up was analyzed in order to assess the presence of a primary outcome. Primary outcome was defined as: presence of life-threatening arrhythmias (LTA), heart failure (HF), cardiac transplantation, death from cardiac causes (including SCD) and "hot phase" episodes. In detail:

- LTA: presence of one or more episode of ventricular fibrillation (VF), defined as irregular or regular tachycardia with regard to polarity, amplitude, morphology, with a mean cycle length of ≤240 milliseconds; sVT defined as regular tachycardia with a mean cycle length >240 milliseconds that lasted >30 s. In those patients with an ICD, we considered an appropriate intervention when the HR of ventricular arrhythmia was > 200 bpm (cycle length < 300 ms).
- HF: defined as the presence of signs and symptoms of HF which required hospitalization.
- Death from cardiac causes: death for HF or SCD.
- Hot phase: defined as the presence of myocarditis like episodes, characterized by chest pain, troponin elevation and ECG abnormalities in the setting of normal coronary arteries.

Statistical analysis: continuous quantitative variables were reported as mean, minimum value - maximum value, while the categorical variables as absolute number and relative frequency (%). The normality of the quantitative variables was evaluated through the Shapiro-Wilkins test, while their comparison was performed through T-test or Mann-Whitney test when appropriate. Categorical variables were compared by Chi Square test and Fisher's exact test when appropriate. Event free survival of the different phenotypic groups were analyzed with Kaplan-Meier's curves and compared with log-rank test. Statistical significance for all tests was set for probability values $p<0.05$. Univariate Cox regression analysis was used to evaluate the impact of each significant variable in analysis to predict the occurrence of outcome. The predictive model was built using Cox regression

with adaptive elastic-net penalty to account for the high number of candidate predictors in front of the low number of LTA events. Model validation was performed considering 2000 bootstrap iterations. Model performance was evaluated by calculating the area under the receiving operating curve (AUC) and the time-dependent plot of the model performance was presented. Finally, a nomogram based on the model was developed to predict 1-year event-free probability.

Analyses were performed using the SPSS version 27 software for MAC (SPSS, Inc., Chicago, Illinois), R software version 4.1.3 (122) within the “hdnom” package (123).

RESULTS

General population

The study population was composed of 436 ACM patients, of whom 273 (63%) were male and 284 (65%) were probands. The first clinical sign leading to the diagnosis was in 175 (40%) patients' family history of ACM, in 42 (10%) LTA, in 87 (20%) ECG abnormalities, in 67 (15%) presence of PVCs at Holter ECG or stress test, in 31 (7%) presence of abnormalities at imaging exams, in 25 (6%) chest pain episodes and 9 (2%) SCD. Thirty-seven patients (9%) had received a previous diagnosis of myocarditis and 28 (6%) of DCM. The median age at diagnosis was 35 years (IQR 22-49).

Positive family history of SCD was present in 134 (31%) patients, while 27 (6%) had a family history of DCM and 183 (42%) of ACM. At the first evaluation, 82 individuals (19%) reported a previous syncopal event, 63 (14%) chest pain, and 21 (5%) a history of atrial fibrillation. A total of 202 (46%) patients underwent ICD implantation, of whom 103 (24%) were in primary prevention, and 99 (22%) were in secondary prevention.

Regarding the diagnosis of ACM, according to 2010 Task Force criteria, 323 patients (74%) had a definite, 53 (12%) a borderline, and 58 (13%) a possible diagnosis, while one (1%) with an ALVC phenotype no criterion was met. Patients with an ALVC phenotype were also evaluated through the 2020 Padua criteria. This resulted in a significant increase in defined diagnoses (54, 48%).

As described above, according to the phenotype, the general population was divided into three phenotypic groups:

- 184 patients (42%) with a right-dominant phenotype (ARVC).
- 112 (26%) with left-dominant phenotype (ALVC).
- 140 (32%) with biventricular phenotype (BIV).

The genetic test result was available in 400 patients (92%). In 219 (55%), a pathogenic/likely pathogenic variant (P/LP) was found. The most commonly genes involved were *DSP* (82, 19%), *PKP2* (78, 18%), *DSG2* (24, 6%), *DSC2* (3, 1%), *DES* (4, 1%), *FLNC* (16, 4%).

Electrocardiographic features

Alterations on 12-lead ECG were found in 351 patients (81%). In detail, 199 (46%) showed TWI in V1-V3, 99 (23%) in V4-V6 leads, and 62 (14%) in inferior leads. The ε wave was detected in 6 subjects (1%), and fragmentation of the QRS complex in 34 (8%). In addition, three subjects (1%) had a left bundle-branch block (LBBB) while a right bundle-branch block (RBBB) was present in 30 (7%). Low QRS complex voltages were found in limb leads in 183 patients (42%) and the precordial leads in 52 (12%). At the Holter ECG performed at the first evaluation, a median value of 702 PVCs (IQR 541-1044) was recorded, and at the last assessment a median value of 659 PVCs (IQR 474-1000, p=0.193).

Cardiac magnetic resonance imaging features

The examination was performed on 337 patients (77%). Analysis of the RV revealed a median EDV of 102 ml/m^2 (IQR 82-118) and a median EF of 50% (IQR 41-58). RV wall motion abnormalities were found in 223 patients (66%). RV-LGE was detected in 148 subjects (44%). Regarding the LV, a median EDV of 87 ml/ m^2 (IQR 776-102) and a median EF of 56% (IQR 49-61) were found. Wall motion alterations (WMA) were found in 134 patients (40%) and LGE in 247 subjects (73%) with an extent of at least two segments in 205 (61%) or with an extent exceeding three segments in 124 (37%). Fatty infiltration was observed in 148 (44%) in RV, while in LV, it was observed in 141 (43%) subjects.

Follow-up

The follow-up had a median duration of 6 years (IQR 5-7, range 1-46). During this period, 125 (29%) LTAs were observed. In detail, 88 (74%) sVT, 19 (16%) VF and 12 (10%) appropriate ICD shocks in subjects with ICDs implanted in primary prevention were recorded. In 57 cases (13%), there was at least one arrhythmic recurrence, which was treated through transcatheter ventricular ablation in 36 patients (8%). Regarding anti-arrhythmic therapy, at the first evaluation, 194 patients (44%) were

taking beta-blocker therapy (the most frequently used drug was bisoprolol, followed by metoprolol), 69 (16%) sotalol, 13 (3%) amiodarone and 4 (1%) flecainide. Furthermore, 156 (36%) were not taking any therapy. At the last follow-up 233 patients (53%) were taking beta-blocker therapy (mostly metoprolol), 145 (33%) sotalol, 23 (5%) amiodarone and 5 (1%) flecainide; 30 patients (7%) did not take any therapy. In addition, in 72 patients (17%), ACE inhibitor was prescribed for HF prevention. Thirty-four patients (8%, 71% males) showed at least one episode of HF (mean age at first episode 37.7± 18.8 years). The mean time interval between ACM diagnosis and HF development was 11 years (min 1, max 20). Among the 34 patients with HF episodes, 8 (24%) were younger than 18 years. In 14 (41%), HF was the initial symptom that led to the diagnosis. Overall, 16 patients (47% of HF group, the median age at diagnosis of ACM was 27 (IQR 17-39)) underwent cardiac transplantation (median age 44 years [IQR 41-56 years]). In this subgroup of patients, the median age at first HF symptoms was 33 years (IQR 16-52); on average, they underwent transplantation 4 years (±3.5) after the onset of symptoms. Among the 8 patients who developed HF before the age of 18 years, 6 (75%) underwent cardiac transplantation. Finally, 11 patients (32%) died due to refractory HF. Overall, 11 patients (3%) had SCD, and in 9 cases (81%), this was the first clinical manifestation of the disease. Finally, 32 patients (7%) presented at least one hot phase episode.

Left dominant arrhythmogenic cardiomyopathy cohort

Based on the described inclusion criteria, 112 patients (26%) with ALVC phenotype were selected. In detail, 106 (95%) subjects had LGE at CMR with an extent of at least two segments, plus:

- 61 patients (54%) resulted in carrying a P/LP variant on a causative gene
- 18 patients (17%) had a family history of ACM in first-degree relatives
- 4 patients (3%) had a family history of DCM in first-degree relatives
- 2 patients (2%) had a family history of SCD with evidence of ACM at post-mortem examination
- 21 patients (18%) showed only clinical and instrumental features in keeping with ALVC, in the absence of anamnestic, clinical, and instrumental features suggesting other diagnosis, such as myocarditis or sarcoidosis. Patients of this group entered the group of "Idiopathic ALVC."

In 8 (7%) subjects, CMR was not available. The diagnosis was reached by means of autopsy findings after SCD (4 patients, 4%), heart specimen examination after cardiac transplantation (2 patients, 1%), and because of pathological analysis of the heart following the patient death due to refractory heart failure (2 patients, 1%.)

In ALVC groups, 70 patients (63%) were males, and 66 (59%) were probands. The median age at diagnosis was 38 years (IQR 26-49). Eighteen patients (16%) had a previous diagnosis of myocarditis and 14 (13%) of DCM. At the clinical evaluation, syncope and chest pain episodes were reported in 18 (16%) and 22 (20%) subjects, respectively, while atrial fibrillation was documented in 6 (5%).

The first clinical signs/symptoms leading to diagnosis in the ALVC population were in 52 (47%) patients family history of ACM, in 15 (13%) LTA, in 11 (10%) ECG abnormalities, in 15 (13%) the presence of PVCs at Holter ECG or stress test, in 6 (5%) the presence of abnormalities at imaging evaluation (2D-echo/CMR), in 11 (10%) chest pain episodes and in 2 (2%) SCD.

A total of 37 patients (33%) were ICD carriers, of whom 18 (16%) as primary prevention and 19 (17%) as secondary prevention.

According to the 2010 Task Force criteria, a definite diagnosis of ACM was obtained in 33 subjects (30%), a borderline diagnosis in 34 (30%), and a possible diagnosis in 44 (39%). Moreover, one patient did not fulfill the ITF criteria.

Genetic analysis identified the presence of a P/LP genetic variant in a causative gene in 61 patients (54%). In detail, the disease-gene was *DSP* in 39 patients (63%), *PKP2* in 6 (10%), *DSG2* in 2 (33%), *DSC2* in 1 (2%), *DES* in 3 (5%) and *FLNC* in 10 (16%), **Figure 5**.

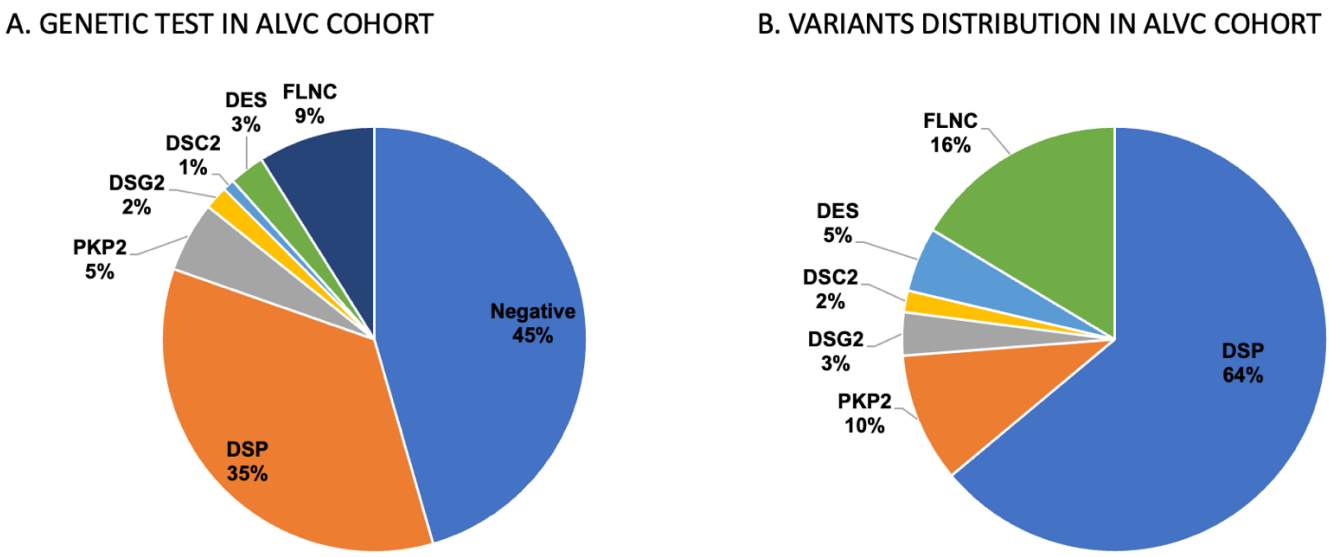

Figure 5. Genetic test result (Panel A) and variants distribution (panel b) inside the ALVC population. "DSP" Desmoplakin, "PKP2" Plakophilin-2, "DSG2" Desmoglein-2, "DSC2" Desmocollin-2, "DES" Desmin, "FLNC" Filamin-C.

Electrocardiographic features

The ECG was abnormal in 74 (66%) patients. In detail, 13 (12%) showed a T-wave inversion in V1-V3, 22 (20%) in V4-V6 leads and 13 (12%) in inferior leads. No patient showed an ε-wave, while fragmentation of the QRS complex was found in 4 (4%). One subject (1%) showed a LBBB and 3 (3%) a RBBB. Low QRS complex voltages in the precordial leads were observed in 14 patients (13%) and in the peripheral leads in 52 (46%).

CMR features

A total of 106 patients (95%) underwent CMR. At RV evaluation the median EDV was 82 ml/m^2 (IQR 72-93) and the median EF was 59% (IQR 55-63). RV-WMA were detectable in 22 patients (21%) and RV-LGE in 9 (8%). Regarding the LV, the median LV EDV was 88 ml/m^2 (IQR 78-106) and the median was EF 53% (IQR 47-60). LV-WMA were observed in 50 subjects (47%), while all patients had LGE of variable extent. In detail, the ALVC patient population had a median number of affected segments of 6 (IQR 4-8, range 2-10), with a higher involvement of the infero-lateral segments of the basal sections, **Figure 6**. Regarding LGE distribution, it was mostly subepicardial in 88 (90%) subjects, sometimes with midmural (n=26, 27%) and transmural (n=10, 10%) involvement. Fatty infiltration of the LV was observed in 41 patients (39%).

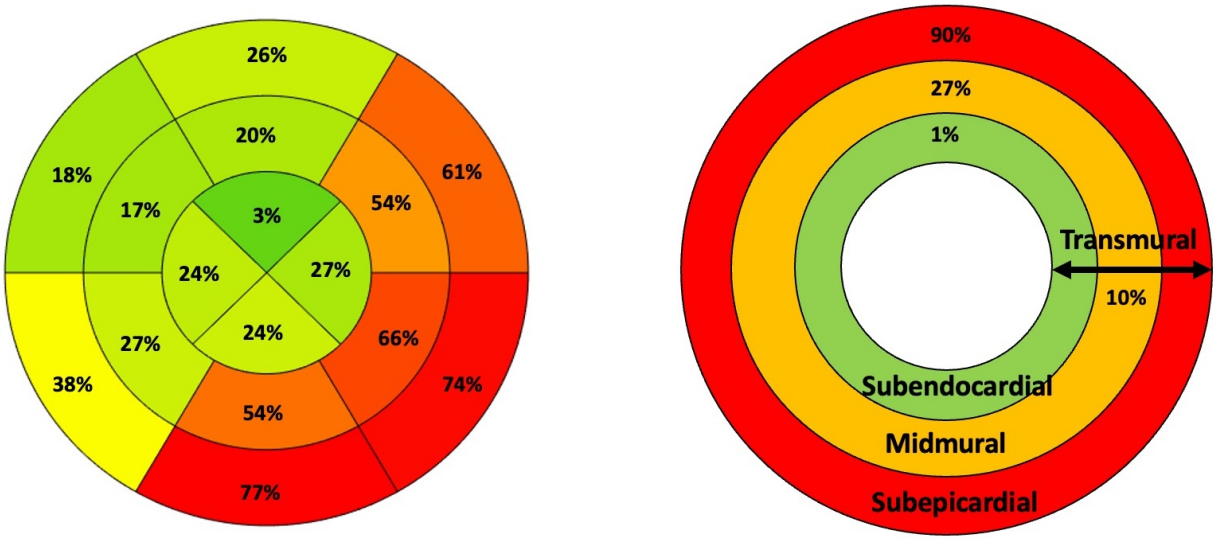

Figure 6. AHA bullseye for LGE distribution inside the ALVC cohort.

A significant negative association was observed between EF-LV and the extent of LV-LGE in terms of the number of segments involved (β= -0.853, 95%CI -1.47, -0.24, p=0.007), **Figure 7**. However, extension of LGE is not associated with significant increased risk of LTA (HR 1.12, 95%CI 0.97-1.30, p=0.120).

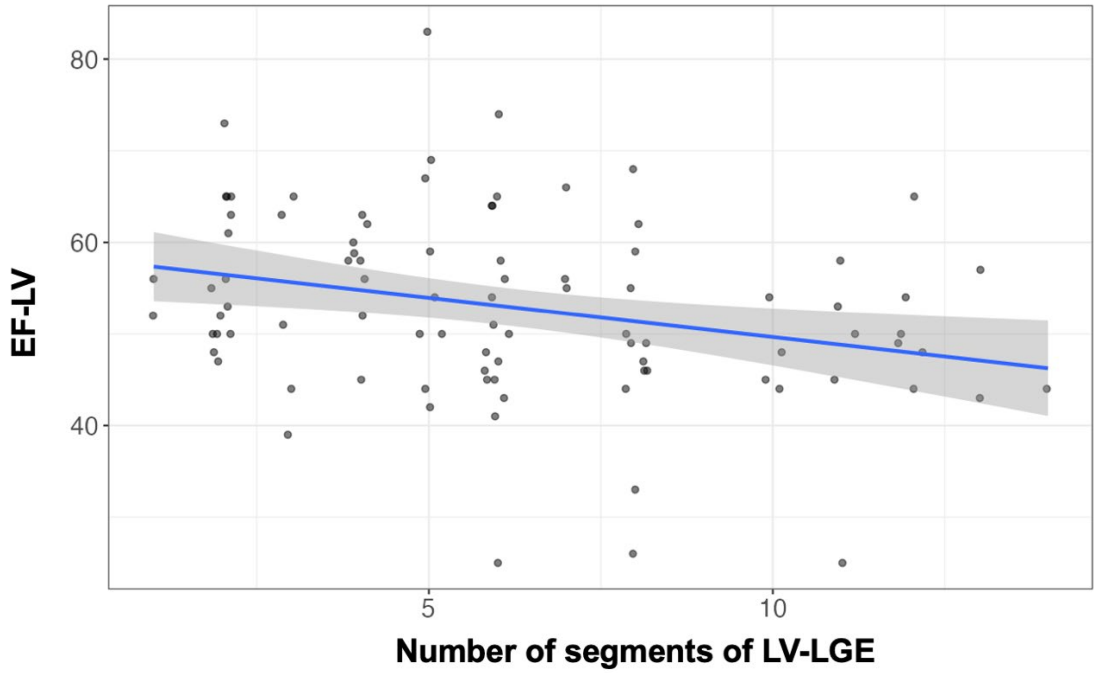

Figure 7. Association between left ventricle ejection fraction and extent of late gadolinium enhancement.

Follow-up

The median duration of follow-up was 3 years (IQR 1-6, range 1-37). At the first evaluation, 43 patients (38%) were not taking any therapy, 54 (48%) were taking beta-blockers (mainly bisoprolol), 12 (11%) sotalol, 2 (2%) flecainide and 1 (1%) amiodarone. A median number of PVCs of 643 (IQR 35-2197, range 0-25933) was observed at 24-hour Holter ECG. In 13 patients (12%), at least one episode of NSVT was recorded. The predominant morphology of ventricular arrhythmias was RBBB with superior axis deviation. At last follow-up, 12 patients (11%) were not taking therapy, 83 (74%) were taking beta-blockers (metoprolol most frequently), 12 (11%) sotalol, 2 (2%) flecainide and 3 (3%) amiodarone. At the 24-hour Holter ECG, a median number of PVCs of 807 (IQR 68-2372, range 0-9636, p vs first follow-up = 0.368) was observed. A reduction in arrhythmia complexity was observed, with 7 (6%) patients having at least one episode of NSVT.

Overall, 18 patients (16%) had LTA episodes and in 9 (8%) this was the first clinical manifestation of the disease leading to ACM diagnosis. In detail, 10 (55%) showed sVT, 6 (33%) VF and 2 (11%) appropriate ICD shock. Notably, all LTA occurred in the presence of normal or mildly reduced LV EF values. Moreover, among the 18 patients who underwent ICD implantation, 3 (17%) experienced an appropriate device intervention (median time to event: 4 years, IQR 2-9).

Five patients (5%) experienced HF (median age 56 years [IQR], range 34-74). The median time interval between ACM diagnosis and HF was 2 years (IQR 1-6). Overall, two patients (2%) underwent cardiac transplantation (median age 49 years, range 34-63), while 2 (2%) died due to refractory HF (median age 77 years, range 72-82). Four patients (4%) suffered SCD (median age 35 years, range 28-56), in all cases as first manifestation of the disease. Finally, 11 patients (10%) presented at least one hot phase episode (median age 35 years, range 28-56).

Idiopathic vs familiar ALVC

In 21 patients (18%), the diagnosis was made due to the presence of LGE exceeding two segments and of clinical features suggestive of ALVC, but in absence a family history of cardiomyopathy and/or of a causative genetic variant. Therefore, we investigated the clinical instrumental and outcome differences of the two populations, **Table 3**.

Patients with idiopathic ALVC presented a higher age at diagnosis (median 41, IQR 35-55), compared to those with ALVC (median 36, IQR 25-49), but without reaching statistical significance. A higher incidence of syncopal events was observed in the idiopathic ALVC cohort (33%) compared with the familial/genetic ALVC group (30%, p=0.017). Moreover, idiopathic subgroup presented a higher number of ICD implantations in secondary prevention following LTA episodes (38% vs 12%, p=0.016) and of atrial fibrillation (n=4, 19%), compared with familial/genetic ALVC patients (p= 0.002). No significant differences in ECG features were observed. In contrast, at CMR, idiopathic ALVC patients had a significantly higher EDV RV (93ml/m^2, IQR 81-99) and a significantly lower EF RV (56%, IQR 50-60) when compared to the ALVC cohort (p=0.005 and p=0.007, respectively), **Table. 3**. Similarly, LV analysis found a significantly higher EDV in idiopathic ALVC (108 ml/m^2, IQR 94-118), compared to the familial/genetic patients (88 ml/m^2, IQR 76-102, p=0.001), in association with a higher LV WMA (idiopathic ALVC 75% vs familial/genetic ALVC 43%, p=0.010). No significant differences were observed regarding the extent of LGE and its distribution.

Patients with idiopathic ALVC presented a median follow-up of 2 years (range 1-5) and showed a higher incidence of LTA (n=7, 33%), compared to the other ALVC patients (n=11, 12%), p=0.017. It should be noted that in the former group of patients, LTA was the first clinical event leading to diagnosis. Moreover, all LTA episodes occurred in presence of normal or mildly reduced LV systolic function. In contrast, no differences were observed regarding incidence of HF, overall death (also SCD), heart transplantation and hot phase episodes.

Table 3. Idiopathic vs familial/genetic ALVC patients.

	Total (N=112, 100%)	Idiopathic ALVC (N=21, 19%)	Familial/genetic ALVC (N=91, 81%)	P
Age of diagnosis	38 (26 - 49)	41(35-55)	36 (25-49)	0.114
Sex (male)	70 (63%)	16 (76%)	54 (59%)	0.151
Previous myocarditis diagnosis	18 (16%)	5 (24%)	13 (14%)	0.284
Previous DCM diagnosis	14 (13%)	3 (14%)	11 (12%)	0.784
Chest pain	22 (20%)	4 (19%)	18 (20%)	0.939
History of syncope	18 (16%)	7 (33%)	11 (12%)	0.017
Atrial fibrillation	6 (5%)	4 (19%)	2 (2%)	0.002
ICD	37 (33%)	10 (48%)	27 (30%)	0.115
Prevention				0.016
I	18 (16%)	2 (10%)	16 (18%)	
II	19 (17%)	8 (38%)	11 (12%)	
Diagnosis 2010 TFC				0.483
No diagnosis	1 (1%)	0 (0%)	1 (1%)	
Definite	33 (30%)	6 (29%)	27 (30%)	
Borderline	34 (30%)	4 (19%)	30 (33%)	
Possible	44 (39%)	11 (52%)	33 (36%)	
Proband status	66 (59%)	21 (100%)	45 (50%)	< 0.001
Abnormal ECG	74 (66%)	14 (67%)	60 (66%)	0.949
TWI V1-V3	13 (12%)	3 (14%)	10 (11%)	0.671
TWI V4-V6	22 (20%)	6 (29%)	16 (18%)	0.253
TWI INF	13 (12%)	3 (14%)	10 (11%)	0.671
Fragmented QRS	4 (4%)	1 (5%)	3 (3%)	0.744
LBBB	1 (1%)	0 (0%)	1 (1%)	0.629
RBBB	3 (3%)	1 (5%)	2 (2%)	0.512
LQRSv precordial leads	14 (13%)	1 (5%)	13 (14%)	0.234
LQRSv limb leads	52 (46%)	8 (38%)	44 (48%)	0.396
EDV RV	82 (72-93)	93 (81-99)	80 (70-91.0)	0.005
EF RV	59 (55-63)	56 (50-60)	60 (56-64)	0.007
WMA RV	22 (23%)	4 (20%)	18 (23%)	0.748
LGE RV	9 (9%)	1 (5%)	8 (10%)	0.459
FAT RV	10 (10%)	3 (15%)	7 (9%)	0.439
EDV LV	89 (78-106)	108 (94-118)	88 (76-102)	0.001
EF LV	53 (47-60)	49 (46-55)	54 (49-61)	0.058
WMA LV	48 (50%)	15 (75%)	33 (43%)	0.010
LV LGE n segments	6 (4-8)	7 (6-8)	6 (3-8)	0.286
PVCs Holter I follow-up	643 (44-2153)	756 (520-1747)	620 (23-2274)	0.416
PVCs Holter II follow-up	807 (92-2299)	242 (40-300)	1251 (130-2551)	0.126

	Total (N=112, 100%)	Idiopathic ALVC (N=21, 19%)	Familial/genetic ALVC (N=91, 81%)	P
LTA	18(16%)	7 (33%)	11 (12%)	0.017
TYPE				0.169
VT	10 (56%)	3(43%)	7(64%)	
VF	6 (33%)	4 (57%)	2 (18%)	
ICD shock	2 (11%)	0 (0%)	2 (18%)	
Recurrency	5 (5%)	1 (5%)	4 (4%)	0.942
HF	5 (5%)	1 (5%)	4 (4%)	0.942
DEATH	2 (2%)	0 (0%)	2 (2%)	0.493
Heart transplantation	2 (2%)	0 (0%)	2 (2%)	0.493
SCD	4 (4%)	0 (0%)	4 (4%)	0.744
Hot phase	11 (10%)	3 (14%)	8 (10%)	0.446

"SCD" sudden cardiac death, "DCM" Dilated Cardiomyopathy, "ACM" Arrhythmogenic Cardiomyopathy, "TFC" task force criteria, "TWI" T wave inversion; "LBBB" left bundle branch block; "RBBB" right bundle branch block; "LQRSV" low QRS voltages; "CMR" cardiac magnetic resonance; "EDV" end-diastolic volume; "EF" ejection fraction; "RV" right ventricle; "LV" left ventricle; "LGE" late gadolinium enhancement; "WMA" wall motion alterations "PVCs" premature ventricular complexes; "LTA" life threatening arrhythmias; "VT" ventricular tachycardia; "VF" ventricular fibrillation; "HF" heart failure.

Kaplan Meier analyses show lower LTA-free survival (p<0.001), HF(p<0.001), hot phases (p=0.047) in idiopathic ALVC forms compared with familial/genetic forms, while no differences were observed concerning free survival from death for cardiac causes (**Figure 8 and 9**).

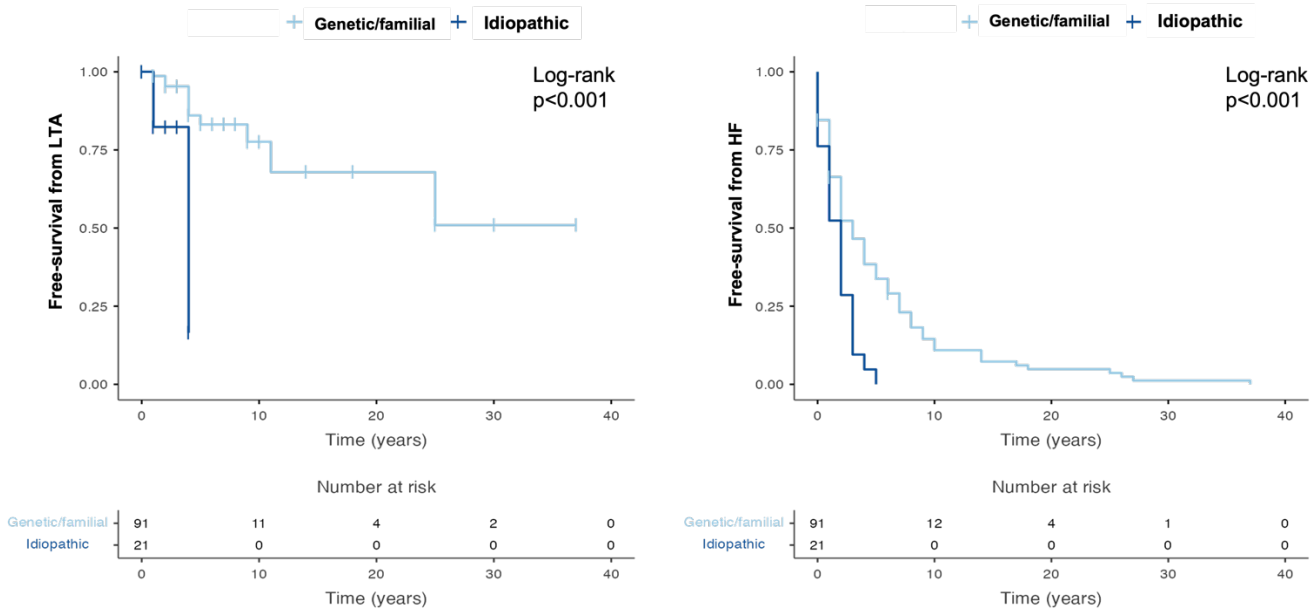

Figure 8. Kaplan Meier analysis from life threatening arrhythmias (LTA) and heart failure (HF).

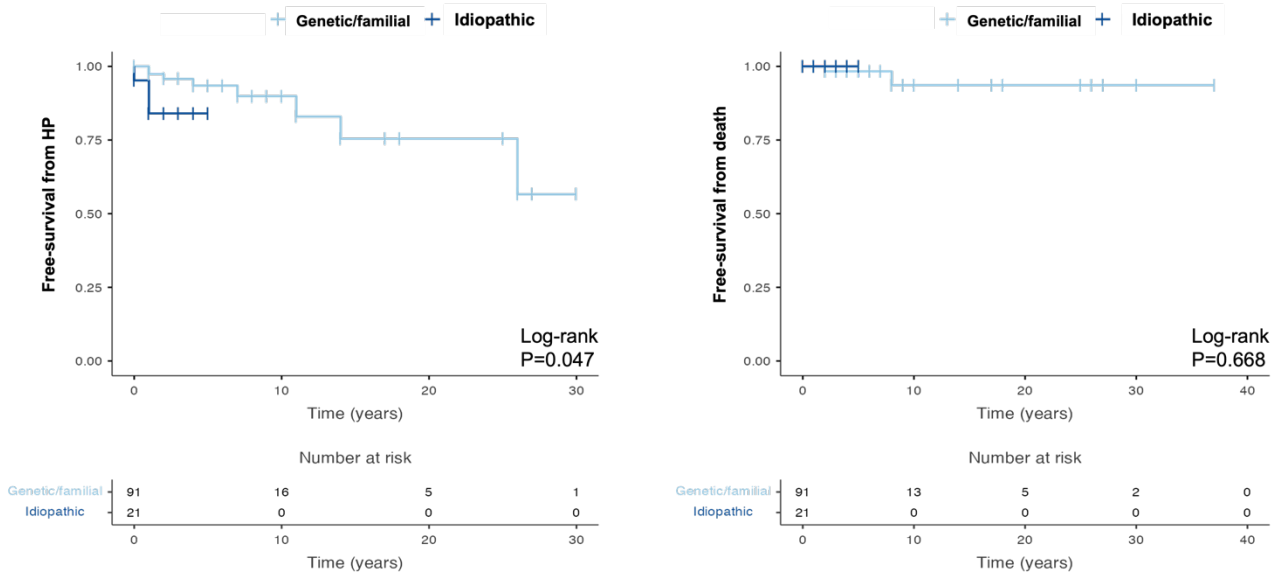

Figure 9. Kaplan Meier analysis from hot phases (HP) and death for cardiac causes.

Furthermore, considering that the cohort of idiopathic ALVC was composed only by probands, we performed a survival analysis which considered only probands of both cohorts. In details, probands with an idiopathic ALVC forms had worst LTA-free survival (Log-rank p=0.047), but no differences were found regarding HF (Log-rank p=0.998), hot phases (Log-rank p=0.608) and death for cardiac causes (Log-rank p=0.462) free survival.

Risk stratification

According to results of the Cox regression with adaptive elastic-net penalty, proband status (HR 1.21), history of syncope (HR 2.53), T wave inversion in V4-V6 (HR 1.26), were associated with an increased likelihood of LTA (**Table 4**). On the other hand, family history of SCD (HR 0.63) and family history of ACM (0.86) were associated with a reduced probability of LTA (**Table 4**).

Table 4. Regularized Cox HR models results

VARIABLES		HR
Sex	Male vs female	1.00
Proband status	Yes vs no	1.21
History of syncope	Yes vs no	2.53
Family history of SCD	Yes vs no	0.63
Family history of ACM	Yes vs no	0.86
Family history of DCM	Yes vs no	1.00
Low QRS voltages limb leads	Yes vs no	1.00
TWI V1-V3	Yes vs no	1.00
TWI V4-V6	Yes vs no	1.26

"SCD" sudden cardiac death; "DCM" Dilated Cardiomyopathy; "ACM" Arrhythmogenic Cardiomyopathy; "TWI" T wave inversion.

Model performance was very good at predicting LTA-free survival at 6 months (AUC of 0.71), 1 year (AUC 0.77), and 3 years (AUC 0.75), **Figure 10**. A nomogram to predict for 1 year LTA-free probability was built based on the predictive model (**Figure 11**).

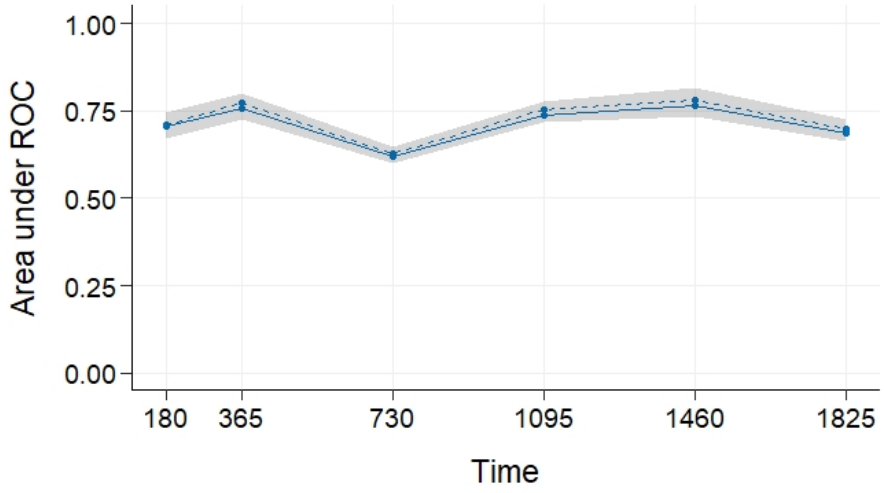

Figure 10. ROC values of the regularized elastic net Cox regression. Time is expressed in days from first evaluation.

Each factor in the nomogram was assigned a weighted number of points, and the sum of points for each patient was associated with the 1-year LTA-free probability. Using the nomogram, a higher score was associated with worse prognosis. For example, a proband patient, who presents history of syncope and at ECG presence of T wave inversion from V4-V6, has a total score of 152 corresponding to a 1-year LTA free probability of approximately 85%.

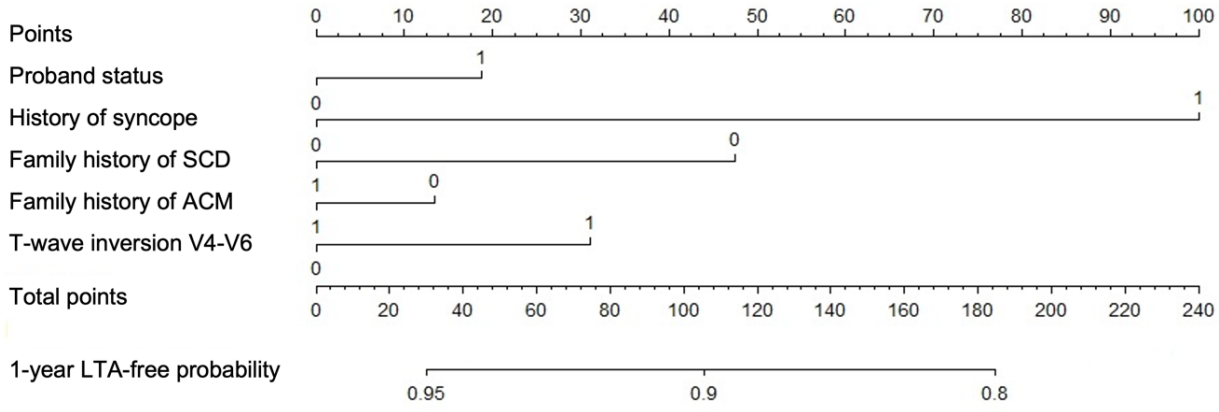

Figure 11. Prognostic nomogram for one year LTA-free probability.

Comparison of the left-dominant phenotype with the right-dominant and biventricular phenotype

The anamnestic, clinical, genetic, instrumental and follow-up data presented above were then compared between the different disease phenotypes.

The median age of diagnosis was slightly higher in ALVC group without reaching a statistical significance. Regarding the distribution of the three phenotypes between the two sexes, no differences were observed. Similarly, the number of probands was similar in all three groups, **Table. 5**.

ALVC group showed more frequently a previous diagnosis of myocarditis (16%) compared to ARVC (3%) and BIV (10%) group, $p<0.001$. Similarly, a previous diagnosis of DCM was more common in ALVC (13%) than in ARVC (1%) and BIV forms (9%), $p<0.001$. Furthermore, ALVC patients had a significantly higher prevalence of family history of SCD (38% vs 33% and 22%, $p=0.021$) and DCM (15% vs 3% and 4%, $p<0.001$) compared to patients with ARVC and BIV phenotypes. Furthermore, no differences were observed among the three groups regarding family history of ACM ($p=0.454$). Chest pain episodes were more common in patients with ALVC and BIV forms (**Table 5**) Syncopal episodes occurred more frequently in ARVC group, without reaching a statistical significance (Table 5). No difference od atrial fibrillation incidence among the three groups was detected (ALVC=5%, BIV= 6%, ARVC=3%, $p=0.399$)

The prevalence of subjects who received an ICD was higher in the cohort of BIV forms (74%) than in ALVC (33%) and ARVC (33%), $p<0.001$.

During the follow-up period, considering the 103 patients (24%) who received an ICD implantation as primary prevention, 24 (23%) presented an appropriate device intervention. The majority of ICD shocks involved patients with ARVC (10, 40%), while they were similar in ALVC (3, 17%) and BIV (11, 18%) patients.

Regarding diagnostic capability, the 2010 TFCs resulted to have significantly higher definite diagnosis rate in ARVC (84%) and BIV (96%) forms, while in ALVC forms this rate resulted to be

significantly lower (30%), p<0.001. On the other hand, when assessed through the 2020 PD criteria, patients with ALVC received a definite diagnosis in 54% of cases.

Table 5. Clinical features of ACM cohort.

	OVERALL (N=436, 100%)	**ARVC (N=184, 42%)**	**ALVC (N=112, 26%)**	**BIV (N=140, 32%)**	**p**
Age of diagnosis	35 (22-49)	34 (21-46)	38 (26-49)	38 (20-51)	0.074
Range	3.0 - 74.0	10.0 - 73.0	10.0 - 70.0	3.0 - 74.0	
Sex					0.099
Male	273 (63%)	106 (58%)	70 (63%)	97 (69%)	
Female	163 (37%)	78 (42%)	42 (38%)	43 (31%)	
Previous myocarditis diagnosis	37 (9%)	5 (3%)	18 (16%)	14 (10%)	< 0.001
Previous DCM diagnosis	28 (6%)	1 (1%)	14 (13%)	13 (9%)	< 0.001
Family history of SCD	134 (31%)	61 (33%)	42 (38%)	31 (22%)	0.021
Family history of DCM	27 (6%)	5 (3%)	17 (15%)	5 (4%)	< 0.001
Family history of ACM	183 (42%)	77 (42%)	52 (46%)	54 (39%)	0.454
Chest pain	63 (14%)	19 (10%)	22 (20%)	22 (16%)	0.076
History of syncope	82 (19%)	44 (24%)	18 (16%)	20 (14%)	0.062
Atrial fibrillation	21 (5%)	6 (3%)	6 (5%)	9 (6%)	0.399
ICD	202 (46%)	62 (34%)	37 (33%)	103 (74%)	< 0.001
Prevention					< 0.001
I	103 (24%)	25 (14%)	18 (16%)	60 (43%)	
II	99 (23%)	38 (21%)	19 (17%)	42 (30%)	
Diagnosis 2010 TFC					< 0.001
No diagnosis	1 (1%)	0 (0%)	1 (1%)	0 (0%)	
Definite	323 (74%)	155 (84%)	33 (30%)	135 (96%)	
Borderline	53 (12%)	18 (10%)	34 (30%)	2 (1%)	
Possible	58 (13%)	11 (6%)	44 (39%)	3 (2%)	
Proband status	284 (65%)	117 (64%)	66 (59%)	101 (72%)	0.077
Positive genetic test	219 (54%)	94 (56%)	61 (54%)	64 (50%)	0.480
Gene variant					< 0.001
DSP	82 (19%)	16 (9%)	39 (35%)	27 (20%)	
PKP2	78 (18%)	56 (30%)	6 (5%)	16 (12%)	
DSG2	24 (6%)	12 (7%)	2 (2%)	10 (7%)	
DSC2	3 (1%)	1 (1%)	1 (1%)	1 (1%)	
DES	4 (1%)	0 (0%)	3 (3%)	1 (1%)	
FLNC	16 (4%)	2 (1%)	10 (9%)	4 (3%)	
JUP	1 (0.2%)	1 (1%)	0 (0.0%)	0 (0%)	
LMNA	1 (0.2%)	0 (0%)	0 (0.0%)	1 (1%)	

	OVERALL (N=436, 100%)	ARVC (N=184, 42%)	ALVC (N=112, 26%)	BIV (N=140, 32%)	p
SCN5A	1 (0.2%)	1 (1%)	0 (0.0%)	0 (0%)	
TMEM43	2 (1%)	0 (0%)	0 (0.0%)	2 (1%)	
delDSG2+DSC2	1 (0.2%)	0 (0%)	0 (0.0%)	1 (1%)	

"SCD" sudden cardiac death, "DCM" Dilated Cardiomyopathy, "ACM" Arrhythmogenic Cardiomyopathy, "TFC" task force criteria, *"DSP*" Desmoplakin, "*PKP2*" Plakophilin-2, "*DSG2*" Desmoglein-2, "*DSC2*" Desmocollin-2, "*DES*" Desmin, "*FLNC*" Filamin-C, "*JUP*" Plakoglobin, "*LMNA*" Lamin-A, "*SCN5A*" sodium voltage-gated channel alpha subunit 5, "*TMEM43*" transmembrane protein 43.

The prevalence of a positive genetic test was similar in the three phenotypic groups (p=0.480), with different prevalence of genetic variants in a specific disease-gene among the three groups, p<0.001. In detail, the most involved gene was *PKP2* (30%) in ARVC forms and *DSP* (35%) in ALVC forms,

Figure 12.

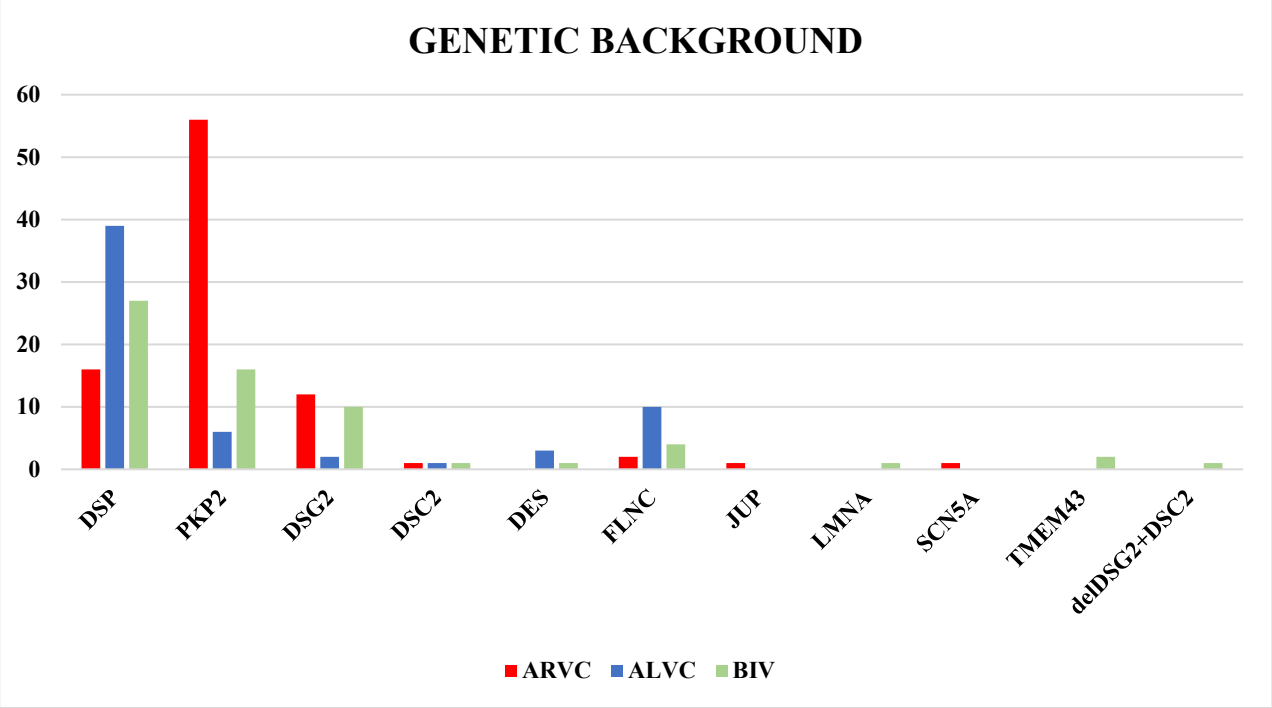

Figure 12. Genetic background divided according to phenotype. *"DSP*" Desmoplakin, "*PKP2*" Plakophilin-2, "*DSG2*" Desmoglein-2, "*DSC2*" Desmocollin-2, "*DES*" Desmin, "*FLNC*" Filamin-C, "*JUP*" Plakoglobin, "LMNA" Lamin-A, "*SCN5A*" sodium voltage-gated channel alpha subunit 5, "*TMEM43*" transmembrane protein 43.

Instrumental findings

Analysis of the ECGs demonstrated that ALVC forms show more frequently a normal ECG, compared to the other cohorts, p<0.001, **Table 6**.

In detail, inverted T waves in V1-V3 were more common in ARVC (58%) and BIV (57%) phenotypes compared to ALVC cohort (12%) p<0.001. In contrast, inverted T-waves in V4-V6 leads were more frequent in the BIV (34%) than in the ALVC (20%) and ARVC (16%) cohorts, p<0.001. Similarly, T waves inversion in inferior leads were more commonly found in BIV (21%) compared to ARVC (11%) and ALVC (12%) cohorts, p=0.028. The ε-wave was not detected in any patients of the ALVC cohort, in 3 (2%) of the ARVC cohort and in 3 (2%) of the BIV cohort, p=0.324. Fragmentation of the QRS complex was more commonly observed in ARVC (7%) and BIV (12%) patients than in the ALVC cohort (4%), p=0.037. Finally, low QRS voltages in precordial leads showed no significant differences within the three groups, whereas low QRS voltages in limb leads were more frequent in ALVC (46%) and BIV (53%) compared to ARVC (31%) forms, p<0.001.

Table 6. Electrocardiographic features

	Overall (N=436, 100%)	ARVC (N=184, 42%)	ALVC (N=112, 26%)	BIV (N=140, 32%)	p
Abnormal ECG	351 (81%)	149 (81%)	74 (66%)	128 (91%)	< 0.001
TWI V1-V3	199 (46%)	106 (58%)	13 (12%)	80 (57%)	< 0.001
TWI V4-V6	99 (23%)	30 (16%)	22 (20%)	47 (34%)	< 0.001
TWI INF	62 (14%)	20 (11%)	13 (12%)	29 (21%)	0.028
Epsilon wave	6 (1%)	3 (2%)	0 (0%)	3 (2%)	0.324
Fragmented QRS	34 (8%)	13 (7%)	4 (4%)	17 (12%)	0.037
LBBB	3 (1%)	0 (0%)	1 (1%)	2 (1%)	0.291
RBBB	30 (7%)	13 (7%)	3 (3%)	14 (10%)	0.073
LQRSv precordial leads	52 (12%)	16 (9%)	14 (13%)	22 (16%)	0.151
LQRSv limb leads	183 (42%)	57 (31%)	52 (46%)	74 (53%)	< 0.001
PVCs Holter I follow-up	702 (541-1044)	500 (300-899)	643 (490-1181)	1775 (918-2588)	< 0.001
PVCs Holter II follow-up	659 (474-1000)	466 (117-720)	807 (175-2158)	1000 (712-1506)	0.080

"TWI" T wave inversion; "LBBB" left bundle branch block; "RBBB" right bundle branch block; "LQRSv" low QRS voltages; "PVCs" premature ventricular complexes.

CMR was available in a total of 337 patients (77%): 137 (75%) with ARVC, 106 (95%) with ALVC and 94 (66%) with BIV forms, p=0.005.

Analysis of the RV revealed that the median RV-EDV in ALVC subjects (median=82mL/m^2, IQR 72-93) was significantly lower than in ARVC (median=109 ml/m^2, IQR 91-121, p=0.015) and BIV forms (median=115 ml/m^2, IQR 99-135), p<0.001. Similarly, RV-EF was within limits in all patients belonging to ALVC cohort (median=59%, IQR 55-63), whereas it was reduced in both ARVC (median=48%, IQR 41-54) and BIV cohorts (median=42%, IQR 35-49), p<0.001. RV-WMAs were more commonly observed in ARVC (84%) and BIV (87%), compared to the ALVC cohort (21%). Similarly, RV-LGE was detected in 8% of ALVC, in 53% of ARVC and 67% of BIV patients (p<0.001). Similar results were found regarding RV fatty infiltration **Table. 7**.

Analysis of the LV demonstrated a significantly higher median LV-EDV values in ALVC (median=88 ml/m^2, IQR 78-106) and BIV (median=100 ml/m^2, IQR 85-115) compared ARVC cohorts (median=81 ml/m^2, IQR 74-88) cohort, p<0.001. The median LV-EF in ALVC (median=53%, IQR 47-60) and BIV groups (median=48%, IQR 44-55) resulted to be lower than in ARVC patients (median=60%, IQR 57-65), p<0.001. LV-WMA were less commonly found in ARVC (16%) than in ALVC (47%) or BIV (63%) phenotypes, p<0.001. The presence of LV LGE was observed in 38% of ARVC, in 95% of BIV and in 100% ALVC forms, p<0.001. LV fatty infiltration was less common in ARVC (27%) compared to ALVC (39%) and BIV (68%) forms, p<0.001.

Table 7.CMR feature divided according to phenotype.

	Overall (N=436, 100%)	**ARVC (N=184, 42%)**	**ALVC (N=112, 26%)**	**BIV (N=140, 32%)**	**p**
CMR performed	337 (77%)	137 (75%)	106 (95%)	94 (68%)	0.005
EDV RV	102 (82-118)	109 (91-121)	82 (72-93)	115 (99-135)	< 0.001
EF RV	50 (41-58)	48 (41-54)	59 (55-63)	42 (35-49)	< 0.001
WMA RV	223 (66%)	115 (84%)	22 (21%)	86 (87%)	< 0.001
LGE RV	148 (44%)	73 (53%)	9 (8%)	66 (67%)	< 0.001
FAT RV	130 (39%)	62 (45%)	10 (9%)	58 (59%)	< 0.001
EDV LV	87 (76-102)	81 (74-88)	88 (78-106)	100 (85-115)	< 0.001
EF LV	56 (49-61)	60 (57-65)	53 (47-60)	48 (44-55)	< 0.001
WMA LV	134 (40%)	22 (16%)	50 (47%)	62 (63%)	< 0.001
LGE LV	247 (73%)	52 (38%)	100 (100%)	95 (95%)	< 0.001
LGE LV > 2 segments	205 (61%)	31 (23%)	100 (100%)	74 (75%)	< 0.001
LGE LV > 3 segments	124 (37%)	0 (0%)	86 (89%)	38 (27%)	< 0.001
FAT LV	141 (43%)	37 (27%)	41 (39%)	66 (67%)	< 0.001

“CMR” cardiac magnetic resonance; “EDV” end-diastolic volume; “EF” ejection fraction; “RV” right ventricle; “LV” left ventricle; “LGE” late gadolinium enhancement; “WMA” wall motion alterations.

Outcome analysis

Table 8. Outcome analysis divided according to phenotype.

	Overall (N=436, 100%)	ARVC (N=184, 42%)	ALVC (N=112, 26%)	BIV (N=140, 32%)	p
LTA	125 (29%)	55 (30%)	18 (16%)	52 (37%)	0.001
Type					0.248
VT	88 (74%)	40 (77%)	10 (56%)	38 (78%)	
VF	19 (16%)	6 (12%)	6 (33%)	7 (14%)	
ICD shocks	12 (10%)	6 (12%)	2 (11%)	4 (8%)	
LTA recurrency	57 (13%)	33 (18%)	5 (5%)	19 (14%)	0.004
Heart failure	34 (8%)	1.0 (1%)	5 (5%)	28 (20%)	< 0.001
Death	19 (4%)	2 (1%)	2 (2%)	15 (11%)	< 0.001
Cause of death					0.097
Cardiac	12 (63%)	0 (0%)	2 (100%)	10 (67%)	
Non cardiac	7 (37%)	2 (100%)	0 (0%)	5 (33%)	
Heart transplantation	16 (4%)	0 (0%)	2 (2%)	14 (10%)	< 0.001
SCD	11 (3%)	3 (2%)	4 (4%)	4 (3%)	0.560
SCD as first manifestation	9 (2%)	2 (1%)	4 (4%)	3 (2%)	0.344
Hot phase	32 (7%)	8 (4%)	11 (9%)	13 (9%)	0.121

"LTA" life threatening arrhythmias; "VT" ventricular tachycardia; "VF" ventricular fibrillation; "SCD" sudden cardiac death.

Patients belonging to ALVC group showed a significantly lower incidence of LTA (16%), compared to ARVC (30%) and BIV (37%), p=0.001. Furthermore, a higher frequency of LTA recurrence was observed in subjects with right-dominant involvement, ARVC (18%) and BIV (14%), compared to ALVC (5%), p=0.004. From 24-Holter ECG evaluation, we recorded a higher prevalence of PVCs in biventricular disease (ARVC vs BIV p<0.001 and ALVC vs BIV p=0.003) than in ARVC and ALVC, which showed similar values (p=0.625).

HF was more frequent in BIV forms (20%), compared to ALVC (5%) and ARVC (1%), p<0.001. Similarly, mortality rate was observed to be significantly higher in BIV patients (11%), compared to ALVC (2%) and ARVC (1%), as well as heart transplantation (0% in ARVC, 2% in ALVC and 10% BIV, p<0.001. Similarly, those with a left ventricular involvement had a higher incidence of death, compared to ARVC (p<0.001). However, no differences among the three-group regarding SCD were

observed **Table. 8**. Even if hot phases episodes were more common in patients with LV involvement (ALVC: 10%, BIV: 9%), compared to patients with only RV involvement (4%), no statistical differences between the three phenotypes were observed, p=0.121.

Kaplan Meier analysis showed a similar LTA-free survival in the three groups, p=0.44, but they demonstrated a significantly lower free survival from HF, hot phases, and death in those with a LV involvement (**Figure 13**).

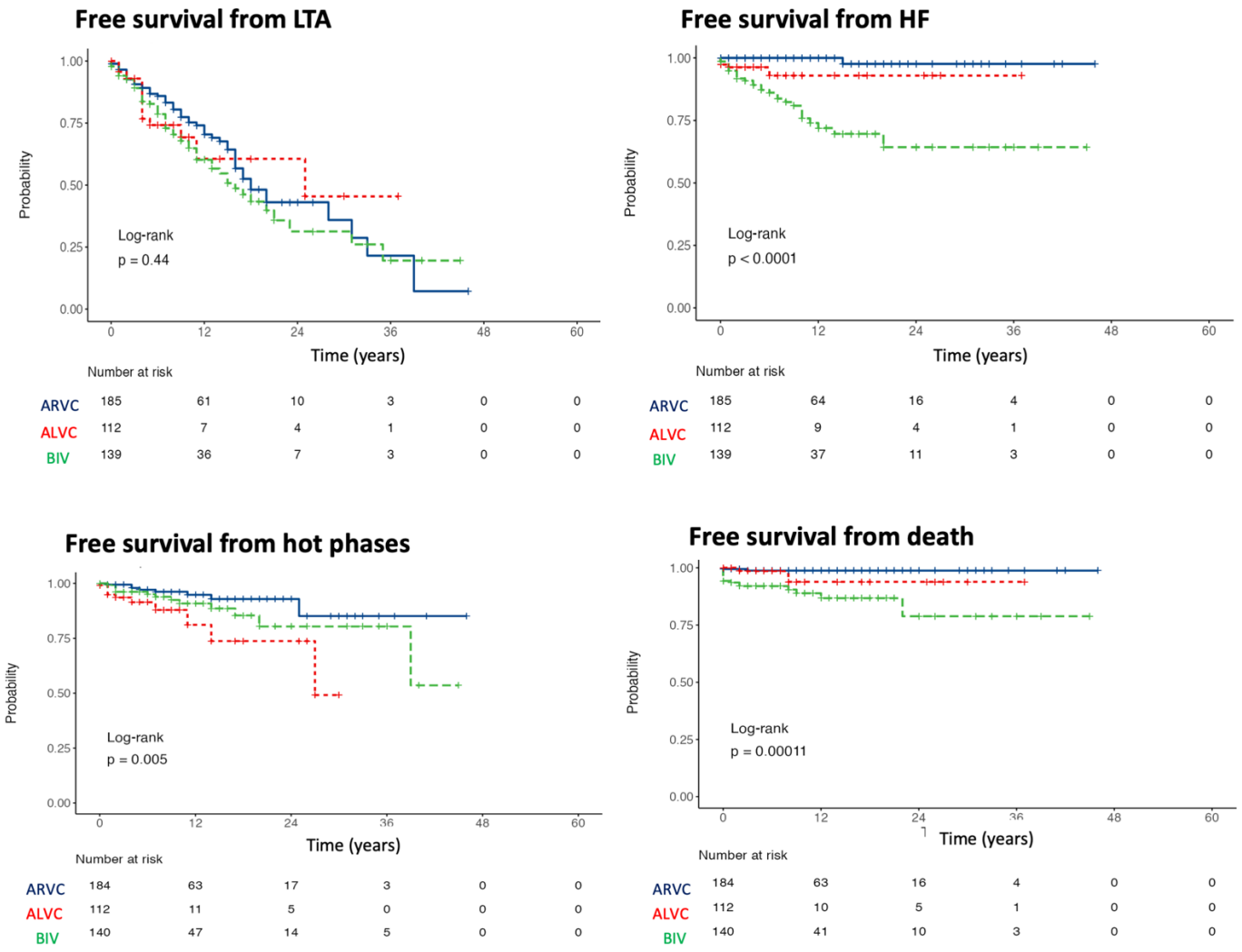

Figure 13. Kaplan Meier analysis according to phenotype for life threatening arrhythmias (LTA), heart failure (HF), hot phases and death for cardiac cause

DISCUSSION

Arrhythmogenic Cardiomyopathy (ACM) is a genetically determined myocardial disease characterized by myocytes necrosis and fibrofatty replacement. The main clinical aspects are ventricular morphological alterations and presence of ventricular arrhythmias, which can even lead to SCD (1,2). After the first descriptions that considered the disease to be confined to the RV, in the last years CMR studies demonstrated that the LV is frequently involved (1, 2). Furthermore, the existence of a phenotype that involves exclusively LV (ALVC) has become evident (22). To date, studies on large cohorts of ALVC patients are lacking. Indeed, diagnosis in ALVC patients can be frequently challenging, due to the clinical overlap with several cardiac diseases, with particular regard to myocarditis and DCM. Furthermore, no reliable predictors of arrhythmic risk in ALVC patients have been identified so far.

The aim of our study was to characterize a cohort of patients with ALVC and to improve arrhythmic risk stratification through follow-up analysis.

Clinical features of analyzed patients

The study population consisted of 436 patients, divided according to phenotype in three subgroups: ALVC form (26%), ARVC form (42%) and BIV form (32%).

The median age at diagnosis of ALVC patients was similar to that of BIV, patients but higher compared to ARVC patients (p=0.074). This differs from what was observed by Sen Chowdhry et al., in which ALVC patients were significantly older compared to the other forms (18). This could be explained by the important increase of CMR exams number in the most recent years, due to its important diagnostic capability. Indeed, our data on ALVC age at diagnosis are similar to those reported in recent publications (50,103,124).

In our cohort of ALVC patients we found a higher prevalence of males (70, 63%, M: F=1.4:1). This differs from what observed by Sen-Chowdhry et al. who did not find a significant difference in gender

distribution (M:F=1.2:1) (18). Notably, Smith et al. observed a female predominance (61%) in a large cohort of ALVC linked to truncated mutations on *DSP* gene (50).

In ALVC forms, the most frequent reason leading to diagnosis was the presence of a family history of ACM, followed by presence of LTA or PVCs at instrumental examinations, ECG abnormalities, imaging alterations, chest pain and, rarely, SCD. In a recent paper, Graziosi et al. analyzed the spectrum of clinical presentations of a ALVC population and, similarly our data, found that five main clinical contexts could be identified: ventricular arrhythmias, chest pain, heart failure, familial screening and SCD as presenting event (125).

The clinical overlap between ALVC and DCM form has been already emphasized (126). Cipriani et al. tried to differential these two clinical entities on the basis of morpho-structural detected through CMR: in ACM form with LV involvement the fibro-fatty substitution constitutes the primum movens of the pathologic process, with typical localization in the infero-lateral segments. This pattern of distribution was also observed in our ALVC cohort. Differently, in DCM patients the fibrous tissue is more nuanced and often located in the intramural portions of the septum since it probably develops because of wall stress due to dilatation (103). A proper diagnosis has important prognostic and therapeutic consequences, as an increasing number of studies indicate that LV systolic function, that in DCM patients constitutes a very important parameter in arrhythmic risk stratification, is unreliable in the stratification of ALVC forms (50–52).These data have been confirmed by recent studies and are consistent with our results (50–52).

A previous diagnosis of myocarditis was observed more frequently in patients with LV involvement (ALVC=16% and BIV=10% vs ARVC=2% $p<0.001$). A recent study of Smith et al. compared patients carrying a pathogenic mutation in the *PKP2* gene with those carrying a *DSP* truncating mutation and reported in the latter group a 25% of episodes of myocardial damage, clinically characterized by chest pain with release of myocardiocytolysis enzymes release.

The authors speculated that, given the greater thickness of LV, the inflammatory process could be more symptomatic in ALVC forms (50). Similarly, Bariani et al observed that hot phase episodes

occur more often in pediatric patients who carry *DSP* gene mutations and showed LV epicardial scar. Thus, tissue characterization, family history and genetic testing are confirmed as effective diagnostic tools for ALVC diagnosis (104).

Diagnostic criteria in ACM. In our study only 30% of patients with ALVC reached a definite diagnosis according to 2010 Task Force criteria, whereas a higher diagnostic sensitivity was demonstrated for the classic disease phenotypes (ARVC=84% and BIV=96%). With the aim to improve diagnostic accuracy in ALVC forms, in 2020 an international consensus document, named 'the Padua criteria', was published. These criteria considered the clinical, instrumental (with regards to tissue characterization at CMR) and genetic features of ALVC forms (19,20). Notably, by means of 2020 Padua criteria, in our cohort a definite diagnosis was reached in 55% of the ALVC forms.

Family history represents one of the more important factors that can lead to ACM diagnosis. In our cohort, a higher prevalence of family history of SCD (ALVC=38%, ARVC=33%, BIV=22%, p=0.021) and DCM (ALVC=15%, ARVC=7%, BIV=4%, p<0.001) was observed in the ALVC cohort, whereas a similar incidence of ACM family history was reported (ALVC=46%, ARVC=42%, BIV=38%, p=0.454).

It is noteworthy that in ALVC patients with negative genetic investigation, family history of SCD and/or cardiomyopathy can have an important role in differential diagnosis with a previous myocarditis. Moreover, analysis of family members can reveal the presence of other ACM phenotypes, as recently highlighted by Bariani et al in a study investigating the phenotypic expression of patients carrying *DSP* P/LP mutations (52).

Idiopathic ALVC. Currently, cardiomyopathy characterized by a non-ischemic LV scar, absence of a causative genetic variant and absence of family history of ACM is defined as an idiopathic ALVC (19). Therefore, we compared the idiopathic ALVC forms with genetic/familial ALVC group. Notably, idiopathic ALVC showed an increased prevalence of LTA (33%), which also represent the first clinical event leading to the diagnosis in this subset of patients. Interestingly, they also reported a higher incidence of syncopal events, compared to the remaining ALVC cohort (33% vs 12%,

p=0.017). Furthermore, idiopathic ALVC forms were characterized by a greater RV/LV EDV and a lower RV/LV EF values. In all ALVC patients, LTAs occurred in the setting of preserved or mildly reduced LV EF. During follow-up (median duration of 2 years, range 1-5), we observed a lower LTA-free survival (p<0.001), HF (p<0.001), hot phases (p=0.047) in idiopathic ALVC forms compared with familial/genetic forms, while no differences were observed concerning free survival from death for refractory heart failure, **Table 3**. Thus, it seems that idiopathic ALVC forms are characterized by greater disease extent and a higher prevalence of LTA episodes compared to genetic/familial ALVC patients, even if further studied on larger cohorts are needed to confirm these data.

Indication for ICD implantation. In our cohort, the prevalence of ICD implantation in ALVC forms did not different from the one observed in ARVC forms (33% vs 34%), but significantly lower than that the one reported in BIV forms (74%, p<0.001).

It is noteworthy that in the majority of patients affected with ACM forms ICD was implanted in primary prevention (ARVC 13%, ALVC 16%, BIV 44%). However, in ARVC a significant percentage of patients received an ICD in secondary prevention (ARVC 21%, ALVC 17%, BIV 30%). This may be surprising, since in ARVC and BIV forms indication for ICD implantation have been codified in an international consensus document (113), however, we have to consider that some of these patients received the ICD in other centers and/or before the 2015 consensus on treatment (113).

Role of genetic investigation.

Within the ALVC cohort, 54% of patients were found to carry a P/LP variant in an ACM-related gene. These findings were similar to those observed in ARVC phenotype (56%) and slightly higher than in the BIV phenotype (50%), but without statistical significance (p=0.480). On the other hand, in 2020 Padua criteria a positive genetic test constitutes an important diagnostic criterion for ALVC diagnosis.

Genetic analysis revealed a significant prevalence of *DSP* mutations in ALVC group (ALVC=35%, ARVC=9%, BIV=20%, p<0.001), consistent with previous studies (18,47,50–52,127). In details,

Smith et al. demonstrated that in patients carrying a truncated *DSP* mutation, aLV involvement was present in the majority of cases and for this reason they coined the term "*Desmoplakin Cardiomyopathy*" to define a cardiac disease characterized by episodic myocardial inflammation, fibrosis, a propensity to deterioration of LV function and a predisposition to the development of major arrhythmic events (50).

Consistently with previous observations, we found an association between *PKP2* mutations and ARVC phenotypes (128,129).

Among non-desmosomal protein mutations, we found a high prevalence of *FLNC* P/LP variants (9%) within the ALVC cohort. The association between FLNC genetic variants and ACM was initially reported described by Ortiz-Genga et al. (72) and Begay et al. (73) who described an arrhythmogenic DCM phenotype in families carrying truncating FLNC mutations. Augusto et al. (105) and Hall et al. (106) subsequently better characterized the clinical phenotype of FLNC cardiomyopathy, reporting a frequent LV involvement and a high arrhythmic burden. Moreover, they also demonstrated that the presence of LGE with a subepicardial ring pattern at CMR could be the only instrumental sign of disease (in absence of ECG alterations or other morpho-functional abnormalities). Similar results were recently reported by recent studies of Celeghin et al. and Gigli et al., thus confirming that the high propensity for LTA does not correlate with the degree of LV systolic dysfunction (75,76).

Event-free survival data didn't indicate an association between the presence of a causative genetic variant and the presence of LTA (**Figure 9**). The difference observed from the papers described above may be determined by the selection criterion of the population under investigation.

Role of instrumental tools

Electrocardiography. In our cohort of patients showing ALVC phenotype, we found a statistically lower prevalence of inverted T-waves in the V1-V3 leads compared to ARVC and BIV groups. On the other hand, inverted T-waves inversion in V4-V6 and in inferior leads were more common in the cohorts with LV involvement (ALVC and BIV), as already reported by Sen-Chowdhry et al. (18).

Another ECG feature that has been proved to be frequently present in ALVC patients is low QRS voltages in limb leads (22). These data were confirmed by our results, as we found a significant higher prevalence of low QRS voltages in limb leads in patients with LV involvement (ALVC=46%, BIV=52%, ARVC=31%, p<0.001). In contrast, the prevalence of low QRS voltages in precordial leads was similar in the three phenotypes (ALVC=13%, BIV=16%, ARVC=9%, p=0.151). The mechanism underlying the reduction in QRS complex voltages remains debated. It was speculated that the loss of LV myocardial mass secondary to fibro-adipose replacement causes a reduction in surface voltages. From the evaluation of the 24h-Holter ECG at first follow-up, we recorded a higher prevalence of PVCs in BIV forms (ARVC vs BIV p<0.001 and BIV vs ALVC p=0.003) than in ARVC and ALVC forms, which showed similar values (p=0.625). However, at last follow-up there was a trend towards a higher incidence of PVCs in ACM forms with LV involvement (ALVC: 807, IQR 175-2158, BIV:1000 IQR 712-1506), compared to ARVC forms (466, IQR 117-720), p=0.080. Moreover, we have to consider that follow-up period was significantly shorter in ALVC group compared to the other two ACM cohorts and this could at least partially explain the difference in arrhythmic burden during follow-up.

Cardiac magnetic resonance. In ALVC and BIV patients, we observed a significant increase in LV-EDV and a significant reduction in LV-EF. Similarly, in the study by Cipriani et al. (103) LV LGE was reported in 73% of ACM patients, confirming its important involvement in the pathogenetic process of ACM. Specifically, LV-LGE (ALVC=100%, BIV=95%, ARVC=38%, p<0.001) as well as LV-WMA (ALVC=47%, BIV=63%, ARVC=16%, p<0. 001) were more frequent in forms with LV involvement while RV-LGE (ALVC=8%, ARVC=53%, BIV=67%, p<0.001) and RV-WMA (ALVC=21%, ARVC=84%, BIV=87%, p<0.001) was more common in those with RV involvement. As shown in previous studies, the presence of a greater extent of LGE determines is associated with a reduction in EF-LV (103). The high sensitivity of the LGE led to its inclusion in the diagnostic algorithm for ACM, although it exhibits a low specificity (19,20). In our ALVC cohort LGE was mostly detected in the subepicardial layer (90%), sometimes with mid-mural involvement (27%) and

less frequently with transmural extension (10%). We did not observe any patients with isolated subendocardial LGE. The fibrotic scar was mostly located in the basal inferior cardiac segments (74%) confirming the topographic features previously described by Sen-Chowdhry et al. (18). Finally, differently from previous descriptions (18), we did not find a frequent septal involvement of fibrosis. Moreover, in ALVC we observed a lower fatty infiltration compared to the other phenotypic forms.

Follow-up analysis and risk stratification

In our cohort, during follow-up a significant lower incidence of LTA was observed in the ALVC group (n=18, 16%), compared to the RV phenotypes: ARVC (n=55, 30%), BIV (n=52, 37%), p=0.001. The follow-up had a median duration of 6 years (IQR 5-7, range 1-46) and it is interesting to note that the free survival is similar in the three phenotypes (**Figure 13**).

Considering that currently ALVC forms lack of universally recognized criteria for arrhythmic risk stratification, we should also consider existing data in arrhythmic risk stratification in DCM. It has already been pointed out that unlike the latter, in ALVC LV systolic function is not associated with LTA occurrence, which may be present even in subjects with preserved or mildly reduced systolic function. On the contrary, interesting insights could come from the role of LGE as an arrhythmic risk factor in DCM. Di Marco et al. published a meta-analysis on a large cohort of DCM patients with the aim to evaluate the association between LGE, LTA and SCD (130). Twenty-nine papers were considered, with a total of 2498 patients and varying degrees of disease extension. The authors concluded that LGE is an independent predictor of ventricular arrhythmias and SCD (HR: 6.7; $p<0.001$) (130). In a second study involving 1020 patients with a nonischemic cardiomyopathy, myocardial fibrosis was found to have a strong and incremental prognostic value for SCD risk stratification (131). Similarly, Aquaro et al. observed a worse prognosis in ACM forms with LV involvement, particularly those with a lone LV involvement and therefore suggest that ICD implantation should be required in these type of patients (132).

In our study some analyzed parameters, including history of syncope, proband status and presence of TWI V4-V6, appear to be associated with an increased risk for LTA in ALVC population. In addition, a scoring system for one-year LTA-free survival estimation was implemented through inclusion of the above cited clinical and instrumental variables. However, it should be considered that the relative small sample size and the low number of LTA observed during the follow-up may have limited the estimation of arrhythmic risk. Unexpectedly, we did not observe an association between the extent of LV-LGE and the onset of LTA. It is possible to postulate that other mechanisms, yet unknown, could have a role in modulating the arrhythmic burden in ALVC patients. On the other hand, no significant difference between the phenotypes were found regarding overall SCD incidence (ARVC 2%, ALVC 4% and BIV 3%, p=0.560). Further studies with a larger cohort of ALVC patients are needed to define possible predictors of arrhythmic risk.

ACM forms with LV-involvement demonstrated an inferior HF-free survivor compared with ARVC, Log-Rank p= 0.001 (**Figure 13**). Similarly, the incidence of heart transplantation was higher in BIV and ALVC forms, p<0.001. Accordingly to previous studies(50,52,104), hot phases appear to be more frequent in ALVC (10%) and BIV (9%) forms compared with ARVC (4%). However, a statistically significant difference was not observed in this cohort.

CONCLUSIONS

Differential diagnosis between ALVC and phenocopies can be challenging due to clinical overlap with ACM phenocopies.

The 2010 ITF diagnostic criteria have proven ineffective in ALVC patients, while the 2020 Padua criteria seem more sensitive in reaching the diagnosis. The genetic test showed the presence of a P/LP genetic variant in 54% of patients, mainly on the *DSP* gene. CMR is confirmed to be the gold standard examination for the morpho-structural definition of patients with ALVC, and the more common LGE pattern is the subepicardial stria at the level of inferolateral, basal segments. Moreover, we found an association between LGE extent and LV-EF reduction, although these parameters were not associated with an increased risk of LTA. Proband status, history of syncope, and presence of TWI from V4-V6 were found to be associated with an increased risk of LTA in the ALVC population.

In the ACM cohort, follow-up analysis showed that free survival from life-threatening arrhythmia did not differ significantly among disease phenotypes. Noteworthy, ACM forms with left ventricular involvement (ALVC and BIV) had a lower free survival from HF, hot phases, and death for cardiac causes.

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