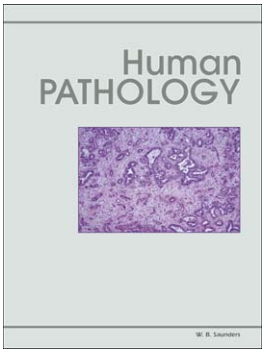

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Nonischemic Left Ventricular Scar and Cardiac Sudden Death in the Young

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Abstract

Nonischemic Left Ventricular Scar (NLVS) is a pattern of myocardial injury characterized by midventricular and/or subepicardial gadolinium hyper enhancement at cardiac magnetic resonance, in absence of significant coronary artery disease. We aimed to evaluate the prevalence of NLVS in juvenile sudden cardiac death and to ascertain its aetiology at autopsy.

We examined 281 consecutive cases of sudden death of subjects aged 1 to 35 years of age. NLVS was defined as a thin, grey rim of subepicardial and/or midmyocardial scar in the left ventricular free wall and/or the septum, in absence of significant stenosis of coronary arteries. NLVS was the most frequent finding (25%) in sudden deaths occurring during sports. Myocardial scar was localized most frequently within the left ventricular posterior wall, and affected the subepicardial myocardium, often extending to the midventricular layer. On histology it consisted of fibrous or fibro-adipose tissue. Right ventricular involvement was always present. Patchy lymphocytic infiltrates were frequent. Genetic and molecular analyses clarified the aetiology of NLVS in a subset of cases. ECG recordings were available in over half of subjects. The most frequent abnormality was the presence of low QRS voltages (< 0,5 mV) in limb leads. In serial ECG tracings, the decrease in QRS voltages appeared, in some way progressive.

NLVS is the most frequent morphologic substrate of juvenile cardiac sudden death in sports. It can be suspected based on ECG findings. Autopsy study and clinical screening of family members are required to differentiate between Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia and chronic acquired myocarditis.

Keywords

Cardiac sudden death; nonischemic left ventricular scar; ARVC/D; LDAC; myocarditis

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Introduction

Nonischemic Left Ventricular Scar is a well-recognised pattern of myocardial injury with features of midventricular and/or subepicardial gadolinium hyper enhancement on cardiac nuclear magnetic resonance (CMR), in patients without significant coronary artery disease (CAD) [1]. The anatomical substrate of gadolinium hyper enhancement is represented by expansion of extracellular tissue [2], which corresponds to fibrous or fibro-adipose myocardial replacement [3]. In the last few years, the finding of NLVS on CMR has been described in subjects with mutations in desmosomal genes, and interpreted accordingly as one end of the phenotypic spectrum of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D) [4-7] i.e. the left dominant form (LDAC), which mirrors the "classic" form of ARVC/D by showing the LV consistently more severely affected than the RV [8]. However, morphological features of NLVS can be found also in chronic or healed acquired myocarditis in patients that are not mutation carriers [5,8]. To achieve a differential diagnosis between these two conditions at autopsy, clinical-cardiologic and CMR screening of the family members, as well as molecular investigations on the probands are required.

Interestingly, NLVS has been recently reported at autopsy as the anatomical substrate of sudden cardiac death in two young competitive athletes [3, 9]. In both cases, NLVS was the main cardiac finding, although small foci of right ventricular fibro adipose replacement were observed at histology, suggesting a diagnosis of LDAC. These preliminary reports prompted us to systematically evaluate the prevalence of NLVS in a consecutive autopsy series of young individuals who died suddenly, either at rest or during sports activity, and to establish its aetiology.

Materials and Methods

Autopsy series

From January 2001 to July 2013, 281 cases of sudden death of subjects from 1 to 35 years of age were consecutively referred to our Department (which is the Lazio Region Referral Centre for sudden deaths in this age range) after a complete autopsy, either from Medical Examiners or from Hospitals autopsy services. All the pathologic studies were performed on routine autopsy examinations required for diagnostic purposes with the approval of the local ethical committee. The need for consent from relatives was waived. Sudden death was defined as an event occurring for natural causes, within 6 hours from the onset of symptoms in a healthy subject or in a subject in stable medical conditions [10]. If death was un-witnessed, subjects were not included in the study, unless they had been observed in healthy conditions at least 24 hours before death. Extra cardiac causes of death were ruled out by a complete autopsy in 76/281 cases (27%) Thus, whole hearts of 205 cases were received for gross and histologic examination. The results of toxicological screening were available in 83/205 cases.

Pathologic evaluation

All hearts were weighted, photographed and examined according to a standardized protocol [11]. After external inspection, the origin and course of epicardial coronary arteries were recorded. They were subsequently cut into 3- to 4-mm thick cross sections and processed for microscopic analysis. Hearts were sectioned according to the short-axis echocardiographic view. The ventricular chamber size, the thickness of the left and right ventricular free walls and the septum were measured at the mid-ventricular level, half way between the atrio-ventricular valves and the apex. Heart valves and myocardium were carefully inspected, and multiple samples were routinely taken from both ventricles and the ventricular septum for histology. The finding of gross lesions led to additional sampling of the myocardium. The conduction tissue was dissected only in cases with clinical history and/or previous electrocardiogram (ECG) records (either referred or available for review) of

conduction defects. Twenty to thirty-five blocks were examined for each case, including the coronary arteries and heart valves. A minimum of two histological sections for each block was stained with Hematoxylin-Eosin and Azan-Mallory trichrome stain. When acute lymphocytic myocarditis was suspected based on the findings of inflammatory infiltrates and myocyte necrosis on Hematoxylin and Eosin–stained slides, immunohistochemistry was performed to characterize the inflammatory infiltrates.

Nonischemic Left Ventricular Scar was defined as the gross finding of a thin, grey rim of subepicardial and/or midmyocardial linear discoloration in the LV free wall and/or the septum, in absence of significant coronary artery disease. The macroscopic features of NLVS were confirmed by histologic findings of fibrous or fibro-adipose myocardial replacement. When fresh or frozen myocardial tissue was available (n=4 cases), both molecular analyses for viral genomes detection and mutation screening for the genes most commonly involved in ARVC/D were performed according to standardized protocols [12-14] to establish the etiology of NLVS.

Toxicology screening

A complete toxicological screening had been performed on blood (after deproteinizing treatment) and urinary samples, including ethanol search was performed by head-space gas chromatography; and screening test based on immunoassay (TRIAGE, ASCEND Multimmunoassay, Merck, Darmstadt, Germany) to detect amphetamines, opiates, barbiturates, tetra- hydrocannabinol, and tricyclic antidepressants, according to the manufacturer's instruction. This had been followed by gas chromatography/mass spectrophotometry analyses on all samples found positive in the screening analysis.

Genetic analysis

Mutation screening of the five desmosomal genes most frequently involved in ARVC/D was performed by direct sequencing. Briefly, genomic DNA was extracted from frozen myocardial

tissue, when available (n=4), with Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Intronic Plakophilin 2 and Plakoglobin primers flanking each exon were designed by Beacon Designer according to genomic sequences obtained from the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) (Supplemental Table 1). Desmoglein *2*, Desmocollin 2 and Desmoplakin primers were as previously described [13, 14]. Amplifications were performed following a standard PCR protocol, and PCR products were analysed by denaturing gradient gel electrophoresis.

After amplification, PCR products were purified and labelled using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Life Technologies, Grand Island, NY, USA) and sequenced on ABI Prism 3130 Genetic Analysis System (Applied Biosystems, Life Technologies, Grand Island, NY, USA).

Detection of viral genomes by PCR analysis

Total DNA was extracted from frozen LV myocardial tissue by Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Total RNA was isolated using the SV total RNA isolation kit (Promega, Madison, WI, USA). RNA amount was measured with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, DE USA) and total RNA was reverse-transcribed to cDNA using System Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Life technologies Italia, MB, Italy) according to manufactures guidelines. The genomes of the following viruses were screened by PCR: adenovirus, enterovirus, human cytomegalovirus, herpes simplex virus, Epstein-Barr virus, parvovirus B19, influenza A and B virus. Primers and PCR conditions were as previously published [15]. Nucleic acids isolated from infected cultures or tissues were used as positive controls.

In case in which viral genomes could be amplified, the specificity of the amplicon was verified by direct sequencing analysis (ABI Prism 3130 Genetic Analysis System, Applied Biosystems).

Review of electrocardiographic recordings

When available (n=7 cases), electrocardiographic recordings from subjects with findings of NLVS were reviewed together with clinical and familial history by a dedicated cardiologist (PZ).

Results

Our study group consisted of 205 cases of juvenile sudden cardiac deaths (SCD). There were 44 females and 161 males. The mean age at death was 26 years. In 161 cases (78%) death occurred at rest or during normal daily activities. In the remaining 44 cases (22%) death occurred during or immediately after sports activity, either competitive or recreational. Results of toxicological analysis, available in 83/205 cases, were positive in seven (2 for opiates, 2 with alcohol levels higher than 1.0 mg/dl, two for cannabinoids and one for amphetamine, all at non toxic levels).

Morphologic analysis of nonischemic left ventricular scar

Nonischemic left ventricular scar was the morphologic substrate in 6.3% of sudden cardiac deaths (13/205). The affected subjects were all males, with a mean age at death of 24.6 years. Gross and histologic features of NLVS are detailed in Fig. 1.

Gross features: hearts had a normal weight or were mildly hypertrophic (up to 420 g). In all but one case the transverse diameter of ventricular cavities was within normal limits. There was no evidence of aneurysmal dilation of the right ventricle. Myocardial scar was localized most frequently within the LV free wall (12/13 cases, 92%) especially in the posterior wall (12/13 cases, 92%), and was associated with septum involvement in 4/13 hearts (31%). Exclusive involvement of the septum was observed only in one case (Fig. 1). Myocardial scar more frequently affected the subepicardial myocardium, often extending to the mid-ventricular layer. It consisted either of a thin linear rim of fibrous tissue or of small, discrete and contiguous foci of fibrosis, admixed with variable amounts of adipose tissue (Fig. 2A, 1D, 1F). The depth of LV myocardial replacement from the subepicardium towards the endocardium ranged from a minimum of 2 mm to a maximum

of 4 mm. Right ventricular involvement was always present, affecting mainly the infundibulum (9/13 cases, 69%) and the lateral wall (8/13 cases, 62%). It represented the prevalent feature in 3/13 hearts (23%) while in 3/13 (23%) right and left ventricles were equally involved. In these cases, a putative diagnosis of biventricular ARVC/D was made. Interestingly, in 7/13 cases (53,8%) right involvement consisted of a small focus of fibro-adipose myocardial replacement, which could be detected only at histology in all but two cases (see Fig. 1).

Microscopic features: left ventricular myocardial replacement was mostly fibrous, with a slight amount of mature adipose tissue (up to 30%), while in the RV adipose tissue was more represented (up to 65%) (Fig.2B-C, 2E, 2G, 2J). Residual cardiac myocytes invariably showed cardiomyopathic changes (i.e. hypertrophy, vacuolization and myofibril loss). Patchy lymphocytic infiltrates within the areas of myocardial scarring were a frequent finding (9/13 cases, 69%) (Fig.3A-C). Interestingly, in 5/9 subjects showing lymphocytic infiltrates within the scars, additional foci of CD3+ lymphocytic infiltrates, occasionally surrounding necrotic myocytes and consistent with active myocarditis were observed also apart from the subepicardial areas of scarring, towards the endocardium (Fig. 3B, 3D-F) in the right and left ventricular free walls but not in the ventricular septum and in the infundibulum. We never observed granulomatous lesions, suggestive of sarcoidosis.

Looking for the etiology of NLVS: molecular and familial screening.

In our series, frozen myocardial tissue was available only in 4/13 cases (SD5, 8,10, and 12 in Fig.1). Screening for viral genomes was positive for adenovirus in subject SD5 (Supplemental Fig. 1) while genetic analysis and family history were negative, consistent with the diagnosis of chronic acquired myocarditis. Subject SD12, previously reported in a collaborative study by our group [3] harboured a heterozygous nonsense mutation of desmoplakin (DSP) at position c.448C>T in exon 4, resulting in a premature stop codon and truncation (Arg150X) at the N-terminal domain of the protein. Clinical and molecular screening of the family revealed affected relatives. Thus, in this

subject the diagnosis of genetic ARVC/D was feasible due to the positive results of both molecular analysis and family screening. In the remaining two cases in which frozen tissue was available (SD8 and SD10), both viral genome screening and mutation analysis were negative, however in subject SD8 family history was positive for sudden cardiac death at young age, suggesting a genetic aetiology.

Of the remaining nine cases in which frozen tissue was not available, family history and clinical screening of the siblings were negative in six, and not available in three. Thus, in this series the aetiology could be clearly defined only in a subset of cases, in which both molecular and family screening were feasible.

Nonischemic left ventricular scar is the most frequent morphologic substrate of sudden cardiac death during sport.

Remarkably, 11/13 subjects with NLVS died during or immediately after sports activity. Four of these (SD1, SD4, SD7, SD10) were competitive athletes.

This finding prompted us to evaluate the relative frequency of NLVS as a pathologic substrate of sudden deaths during sports, as compared with events occurring at rest. To this purpose we restricted our analysis to subjects whose age at death ranged from 6 to 35 years, in the assumption that in the pre-scholar age sports activities are not regularly performed, at least in our Country. Accordingly, we analysed a total of 200 subjects, 44 dying during sports and 156 at rest or during normal daily activities. As shown in Table 1, NLVS represents the most frequent finding in sudden deaths occurring during sports activity (11/44 cases, 25%, vs. 2/156, 1.3% of deaths at rest), followed by HCM (7/44 cases, 16%, vs. 6/156, 3.8%). Thus, according to our results, physical effort is a specific trigger for sudden arrhythmic death in these two conditions, in line with previous observations [16-20]. Conversely, structurally normal heart was the most frequent finding in SCD at rest (58/156, 37%, as compared to 16% of events during sports) followed by coronary atherosclerosis (28/156, 18%, vs. 4.5%).

Low QRS voltages on peripheral leads are the most common finding on ECG.

Both clinical history and ECG recordings were available for seven of the 13 subjects diagnosed with NLVS at autopsy (5/5 competitive athletes and 2/8 recreational athletes). Data are reported in Table 2. Four subjects were asymptomatic, while 3 had symptoms: one referred sporadic palpitations, one "epileptic attacks" and one had a diagnosis of probable dilated cardiomyopathy at magnetic resonance, for which he was disqualified from competitive soccer.

ECG at rest was normal in 2 cases. Five cases had ECG abnormalities, the most frequent being negative T waves in left precordial leads (in 4 out 5 cases) and ventricular extrasystoles (in 3 out of 5 cases), with prevalent right bundle branch block morphology in two cases. Low QRS voltages in limb leads (< 0,5 mV) were present in all 5 cases (Figs 4-7). In serial ECG tracings, the decrease in QRS voltages appeared in some way progressive (Figs. 4A-4C, 5A-5C, 6A-6B).

Discussion

To our knowledge this study is the first to show the prevalence of NLVS in a large series of consecutive cases of juvenile cardiac sudden deaths, and to provide a detailed description of the topographic distribution and the histologic features of this lesion. According to our results, NLVS is found in a relatively low percentage of juvenile SCDs. However, it represents the most frequent anatomic substrate (25%) of SCD cases occurring during sports in the age range from adolescence to younger adulthood. In the last few years, CMR findings of late gadolinium enhancement affecting the left ventricular wall with a subepicardial and/or mid-myocardial pattern have been described by Sen-Chowdhry et al. in probands and asymptomatic desmosomal mutations carriers, and defined as left dominant ARVC/D (LDAC) [8]. Findings of left ventricular myocardial replacement consistent with NLVS, and often associated with RV involvement are also increasingly reported at autopsy. In this setting, this morphologic pattern is usually defined as ARVC/D, although genetic confirmation of the disease is not provided in the majority of cases [21, 22, 18, 9].

In this study we provided a detailed description of gross and histologic features of NLVS. On gross examination the hallmark of NLVS was the presence of a thin, linear subepicardial and/or mid-mural discoloration, almost invariably located in the posterior-lateral wall and eventually affecting also the anterior wall and the posterior aspect of the septum, in agreement with the CMR findings described by Sen-Chowdhry et al. [8]. Similar findings have also been reported in a small number of cases of SCD and labelled as Idiopathic Myocardial Fibrosis by John et al [23]; however, it is reasonable that this lesion is part of the same disease spectrum of NLVS. In our series of NLVS the fibro-adipose scar also affected the RV, either as microscopic foci detected only by histology or as more extensive lesions, already evident on gross examination. A prevalent right ventricular involvement was observed in 23% of cases. At histology, myocardial replacement was always associated with cardiomyopathic changes, consisting in myocyte hypertrophy/atrophy and vacuolization. Focal inflammatory infiltrates were observed in over 2/3 of cases, both in the areas of scar and within the right and left ventricular myocardium, pointing to inflammation as an important contributor to the pathophysiology of myocardial damage and disease progression. Indeed, left ventricular nonischemic scar can also be the expression of chronic/healed myocarditis, and a differential diagnosis with a genetic cardiomyopathy is required [3,5,9]. To this purpose, fresh myocardial tissue should be collected at autopsy for both mutation analysis of the genes most frequently involved in ARVC/D and molecular screening for viral genomes. Moreover, clinical and molecular screening of the families, when they are accessible, should be carried out according to published protocols on post-mortem investigation of sudden cardiac death [11]. In our series, frozen myocardial tissue was available only in 4/13 of referred cases (30%). Mutation analysis allowed a diagnosis of ARVC/D in one subject, which was further confirmed by genetic and clinical screening of the family previously reported by Pilichou et al [3]. Viral genomes were found in one case with negative family history, consistent with the diagnosis of chronic myocarditis. Both viral genome screening and mutation analysis were negative in the remaining two subjects, one of which showed a family history positive for juvenile SCD. Interestingly, inflammatory infiltrates were a prominent

finding in all these cases, including the one with genetically proven ARVC/D. The latter observation is not surprising, in light of previous reports both in transgenic mouse models and in patients with genetically proven ARVC/D [24, 25, 8]. A possible explanation for this finding is that myocarditis is part of the natural history of ARVC/D. In fact, the genetic defect could be responsible both for the developmental abnormalities and for the inflammatory reaction to the genetically determined myocardial damage [6,24]. Alternatively the genetic mutation may increase susceptibility to cardiotropic viruses.

All this considered histologic features of inflammatory infiltrates do not help to discriminate between acquired myocarditis and cardiac inflammation reflecting the active phase of genetically determined ARVC/D. In absence of the results of molecular and familial screening, caution should be taken at autopsy in assigning a specific etiology to the finding of LV myocardial fibro-adipose replacement, with chronic inflammatory infiltrates, in absence of coronary artery disease.

Apart from the discussion on the etiology and physiopathology, the relatively high prevalence of NLVS in young subjects dying suddenly during sport in our series confirms that this morphological substrate predisposes to life-threatening ventricular arrhythmias during physical exercise [20]. Moreover, in our series five out of 13 subjects with NLVS were competitive athletes. This is not surprising, since NLVS minimally affects left ventricular contractility, not impairing the capability of subjects to perform strenuous physical activity. Importantly, preparticipation screening failed to recognize the disease in four out of five subjects. Two of them (SD10 and SD12 in Table 3) had a normal ECG and were asymptomatic, while two (SD1 and SD7) had low QRS voltages in limb leads. Thus, the presence of low QRS voltages in limb (and precordial) leads should be considered with attention in young athletes, in whom high QRS voltages is a very common finding [26, 27] due to the physiological increase in cardiac mass (especially in the left ventricle). This pattern assumes greater significance if a progressive reduction of QRS voltages on serial ECG tracings in the time is observed (Figure 3). The presence of fibrous or fibro-adipose myocardial replacement can explain the progressive loss of electrical forces on resting ECG. However, early and predominant left

ventricular involvement still remains under diagnosed, and only become evident after second level diagnostic tools, like magnetic resonance. Finally, two cases had ventricular extrasystoles with a prevalent right bundle branch morphology, i.e. originating from the left ventricle. As very recently suggested by Zorzi et al [28], this may be an additional important finding for the clinical cardiologist in suspecting left ventricular involvement in this particular setting.

Of note, most of the subjects with LVNS who died on effort were practicing recreational sports, opening the discussion about the opportunity of a routine cardiologic screening with resting ECG in recreational athletes, or at least in those with symptoms and/or a familial history of juvenile sudden death or unexplained ventricular arrhythmias.

Limitations of the Study

Unfortunately, in a number of cases that were referred to our Institution for a detailed pathological analysis, frozen myocardium was not available, thus we could perform genetic and molecular analyses only in a subset of cases.

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References

1. Mahrohldt H, Wagner A, Judd MR, Sechtem U, Kim RJ. Delayed enhancement cardiovascular magnetic resonance assessment of non-ischaemic cardiomyopathies. Eur Heart J. 2005; 15: 1461-1474.
2. Marcu CB, Nijveldt R, Beek AM, Van Rossum AC. Delayed contrast enhancement magnetic resonance imaging for the assessment of cardiac disease. Heart Lung Circ. 2007; 16: 70-8.
3. Pilichou K, Mancini M, Rigato I, et al. Nonischemic left ventricular scar: sporadic or familial? Screen the genes, scan the mutation carriers. Circulation. 2014; 130: e180-182.
4. Maron BJ, Towbin JA, Thiene G, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006; 113: 1807-1816.
5. Fontaine G, Chen HS. Arrhythmogenic right ventricular dysplasia back in force. Am J Cardiol. 2014; 113(10): 1735-1739.
6. Basso C, Thiene G, Corrado D, Angelini A, Nava A, Valente M. Arrhythmogenic right ventricular cardiomyopathy. Dysplasia, dystrophy, or myocarditis? Circulation. 2006; 94:983-991.
7. Vite A, Gandjbakhch E, Prost C, et al. Desmosomal cadherins are decreased in explanted arrhythmogenic right ventricular dysplasia/cardiomyopathy patient hearts. PLoS One. 2013; 8: e75082.

8. Sen-Chowdhry S, Syrris P, Prasad SK, et al. Left-dominant arrhythmogenic cardiomyopathy: an under-recognized clinical entity. J Am Coll Cardiol. 2008; 52: 2175-2187.
9. d'Amati G, De Caterina R, Basso C. Sudden cardiac death in an Italian competitive athlete: Pre-participation screening and cardiovascular emergency care are both essential. Int J Cardiol. 2016 Mar 1;206:84-6.
10. Virmani R, Burke AP, Farb A. Sudden cardiac death. Cardiovasc Pathol. 2001; 10: 275-282.
11. Basso C, Burke M, Fornes P, et al. Guidelines for autopsy investigation of sudden cardiac death. Virchows Arch. 2008; 452: 11-18.
12. Leone O, Veinot JP, Angelini A, et al. 2011 consensus statement on endomyocardial biopsy from the Association for European Cardiovascular Pathology and the Society for Cardiovascular Pathology. Cardiovasc Pathol. 2012; 21: 245-274.
13. Pilichou K, Nava A, Basso C, et al. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. Circulation. 2006; 113: 1171-1179.
14. Rampazzo A, Nava A, Malacrida S, et al. Mutation in human desmoplakin domain binding to plakoglobin causes a dominant form of arrhythmogenic right ventricular cardiomyopathy. Am J Hum Genet. 2002; 71: 1200-1206.
15. Leone O, Rapezzi C, Sinagra G, et al. Consensus document on endomyocardial biopsy of the Associazione per la Patologia Cardiovascolare Italiana. G Ital Cardiol. 2009; 10(9 Suppl 1): 3S-50S.
16. Basso C, Corrado D, Thiene G. Cardiovascular causes of sudden death in young individuals including athletes. Cardiol Rev. 1999; 7: 127-135.
17. Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO. Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980-2006. Circulation. 2009; 119: 1085-1092.

18. Sheppard MN. Aetiology of sudden cardiac death in sport: a histopathologist's perspective. Br J Sports Med. 2012; 46: i15-21.

19. Corrado D, Thiene G, Nava A, Rossi L, Pennelli N. Sudden death in young competitive athletes: clinicopathologic correlations in 22 cases. Am J Med. 1990; 89: 588-596.

20. Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G. Does sports activity enhance the risk of sudden death in adolescents and young adults? J Am Coll Cardiol. 2003; 42: 1959-1963.

21. Tavora F, Zhang M, Franco M, et al. Distribution of biventricular disease in arrhythmogenic cardiomyopathy: an autopsy study. Hum Pathol. 2012; 43: 592-596.

22. Kodikara S. Left-sided ventricular cardiomyopathy with minimal right ventricular involvement. Med Sci Law. 2012; 52(2): 116-118.

23. John BT, Tamarappoo BK, Titus JL, Edwards WD, Shen WK, Chugh SS. Global remodeling of the ventricular interstitium in idiopathic myocardial fibrosis and sudden cardiac death. Heart Rhythm. 2004; 1: 141-149.

24. Pilichou K, Remme C A, Basso C, et al. Myocyte necrosis underlies progressive myocardial dystrophy in mouse dsg2-related arrhythmogenic right ventricular cardiomyopathy. J Exp Med. 2009; 206(8): 1787-1802.

25. Campian ME, Verberne HJ, Hardziyenka M, de Groot EA, van Moerkerken AF, van Eck-Smit BL and Tan HL. Assessment of inflammation in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. Eur J Nucl Med Mol Imaging. 2010; 37: 2079-2085.

26. Madias JE. Low QRS voltage and its causes. J Electrocardiol. 2008; 41: 498-500.

27. Corrado D, Pelliccia A, Heidbuchel H, et al. Recommendations for interpretation of 12-lead electrocardiogram in the athlete. Eur Heart J. 2010; 31: 243-259.

28. Zorzi A, Perazzolo Marra M, et al, Nonischemic Left Ventricular Scar as a Substrate of Life-Threatening Ventricular Arrhythmias and Sudden Cardiac Death in Competitive Athletes. Circ Arrhythm Electrophysiol. 2016;9 (7): e004229.

Fig. 1: Topographic distribution of NLVS and extent of RV involvement.

Grey squares indicate the presence of NLVS, * indicate myocardial lesions detected only at histology, § indicate the presence of lymphocytic infiltrate within replacement fibrosis

Fig. 2: Short-axis sections of representative cases (SD 9, SD12, SD11) of NLVS.

SD 9: myocardium of both the posterior (asterisk) and anterior (arrows) left ventricular wall is replaced by a subtle linear scar, mostly located in the subepicardium. The right ventricle lacks any macroscopic sign of involvement (1A). Histology reveals focal involvement of the RV lateral wall (1B) and confirms the fibro-adipose left ventricular replacement. Asterisk indicates the epicardial side (1C) (Azan Mallory stain, original magnification 5x). SD 12: the subepicardial scar involves both the LV and the septum (1D) (asterisk indicates the insert shown in 1E). Again, RV involvement is focal, and detectable only by histology (1E). The RV involvement, in the anterior wall, is highlighted in the inser. (Azan Mallory stain, original magnification 1.4x). SD12: asterisk indicates the subtle myocardial scar involving the LV lateral and posterior wall (1F). The RV lateral wall shows gross features of fibro-adipose myocardial replacement (arrow, 1F). Findings in the RV and LV are confirmed by histology. Asterisks indicate the epicardial side (1G and 1J) (Azan Mallory stain, original magnification 5x).

Fig. 3: Representative pictures showing histologic findings either of chronic or active myocarditis associated with NLVS.

SD 12: Presence of chronic inflammatory infiltrates within a subepicardial area of scarring, with groups of residual cardiomyocytes (2A) (HE staining, original magnification 10x). (2B, CD3 immunostain, original magnification 20x). SD 8: Fibro-adipose myocardial replacement with scanty inflammatory infiltrates (2C) (HE staining, original magnification 10x). The inflammatory infiltrate is made up mostly of T lymphocytes (2D) (CD3 immunostain, original magnification 20x). SD 11: Focus of active lymphocytic myocarditis in the left ventricle, remote from the area of scar (2E) (HE

stain, original magnification 20x). Scattered T lymphocytes are present within the infiltrate (2F) (CD3 immunostain, original magnification 20x).

Fig. 4: ECG findings from subject SD 1.

Resting ECG at the age of 15 shows low QRS voltages in limb leads, flat/negative T waves in inferior and left precordial leads (V5-V6), and a single premature ventricular beat (PVB) (3A). Exercise ECG showing frequent PVBs with different morphologies and in couplets (3B). Resting ECG at the age of 17, two months before death while playing recreational soccer against medical advice. Low QRS voltages in limb leads and clearly negative T waves in inferior and left precordial lead (V4 to V6) are observed (3C).

Fig. 5: ECG findings from subject SD 4.

Resting ECG at the age of 18 shows right axis deviation, deep-narrow septal Q waves, right ventricular conduction delay and negative T waves in inferior and left precordial leads (4A). Six years later, resting ECG showed the same anomalies, together with premature ventricular beats with a right bundle branch block + right axis deviation QRS morphology (4B). A slight decrease in QRS voltages, both in limb and in lateral leads, is appreciable, but this aspect was even more evident in the ECG tracing (4C) recorded at the age of 25, one year before death during sport (in spite of previous disqualification).

Fig. 6: ECG findings from subject SD 6.

Resting ECG at the age of 29 shows low QRS voltages in limb leads (except for D1) and flat T waves in limb and lateral leads (5A). Four years later, at the age of 34, a slight right axis deviation and a decrease in QRS voltages in limb leads could be observed (in particular in D1, D3 and aVF) (5B).

Fig. 7: ECG findings from subject SD 7.

Resting ECG at the age of 16 shows negative T waves from V1 to V3; QRS voltages in precordial leads were normal, but diffusely low in limb leads (5A). Holter monitoring showing frequent isolated, and coupled premature ventricular beats (polymorphic) (5B).

Table 1: Anatomical substrates of juvenile sudden cardiac death during sport and at rest

Morphologic findings	SCD during sports	SCD at rest
NLVS (a differential diagnosis between genetic ARVC/D and chronic acquired myocarditis is required)	25.0% (11/44)	1.3%(2/156)
HCM	16.0%(7/44)	3,8%(6/156)
Structurally normal Heart	16.0%(7/44)	37.0%(58/156)
CHD	11.4%(5/44)	3.2% (5/156)
CCAA	6.8%(3/44)	1.3%(1/156)
Lymphocytic Myocarditis	6.8%(3/44)	9.0%(14/156)
ATH CAD	4.5%(2/44)	18.0% (28/156)
Non-specific LVH	2.3%(1/44)	16.0%(25/156)
Others (MVP, CSD, CV, DCM etc.)	11.4%(5/44)*	11%(17/156)

Abbreviations: NLVS Nonischemic left ventricular scar SCD, Sudden Cardiac Death; HCM, Hypertrophic Cardiomyopathy; CHD, Congenital Heart Disease; CCAA, Congenital Coronary Arteries Anomalies; ATH CAD, Atherosclerotic Coronary Artery Disease; LVH, Left Ventricle Hypertrophy; CSD, Conduction System Disease; MVP, Mitral Valve Prolapse; CSD, Conduction System Disease; CV: coronary vasculitis; DCM, Dilated Cardiomyopathy

Table 2. Clinical features and ECG findings in 7 subjects with NLVS

N.	Sex	Age at death	Sport (competitive or recreational) and death circumstances	Clinical history	ECG findings
SD1	M	17	Competitive soccer † during a soccer game	Sporadic palpitations	ECG at the age of 15: low QRS voltages in limb leads; flat/negative T waves in inferior and left precordial leads (from V5 to V6); monomorphic PVBs. Exercise ECG at the age of 15: frequent polymorphic PVBs, isolated and in couplets. ECG at the age of 17: further decrease in QRSvoltages in limb leads, negative T waves in inferior and left precordial leads (from V4 to V6)
SD4	M	26	Competitive soccer (referee) † during a soccer game	DCM suspect at MR scan	ECG at the age of 18: right axis deviation; deep septal Q waves; right ventricular conduction delay; negative T waves in inferior and left precordial leads ECG at the age of 24: single PVB with right bundle branch morphology and superior-right axis deviation ECG at the age of 25: similar to the first ECG. In addition, reduced QRS voltages.
SD6	M	34	Recreational jogging, †during a jogging session	Asymptomatic	ECG at the age of 29: low QRS in limb leads and flat T waves in inferior and left precordial leads (from V4 to V6). ECG at the age of 34: similar to the fist ECG. Further reduction of QRS voltages in limb leads
SD7	M	16	Competitive soccer † during a soccer game	Asymptomatic	PVBs (after exercise ECG) with left bundle branch block morphology; low QRS voltages in limb leads and negative T waves from V1 to V3
SD9	M	33	Recreational jogging †during a jogging session	"Epileptic attacks"	Diffuse low QRS voltages; negative T waves in I-aVL and V5-V6

	M	25	Competitive soccer	Asymptomatic	Normal ECG
SD10			† during a soccer game		
	M	20	Competitive soccer	Asymptomatic	Normal ECG
SD12			† watching television		

	LEFT VENTRICLE			SEPTUM	RIGHT VENTRICLE			
	ANT	LAT	POST		ANT	LAT	POST	INFUND
SD 1								*
SD 2								
SD 3								
SD 4								
SD 5		§	§			*		*
SD 6			§				§	
SD 7		§	§			§	§	
SD 8			§			§		
SD 9			§			*		
SD 10		§	§			*		
SD 11	§	§	§			§		
SD 12		§	§		*		*	
SD 13						§		

Figure 1

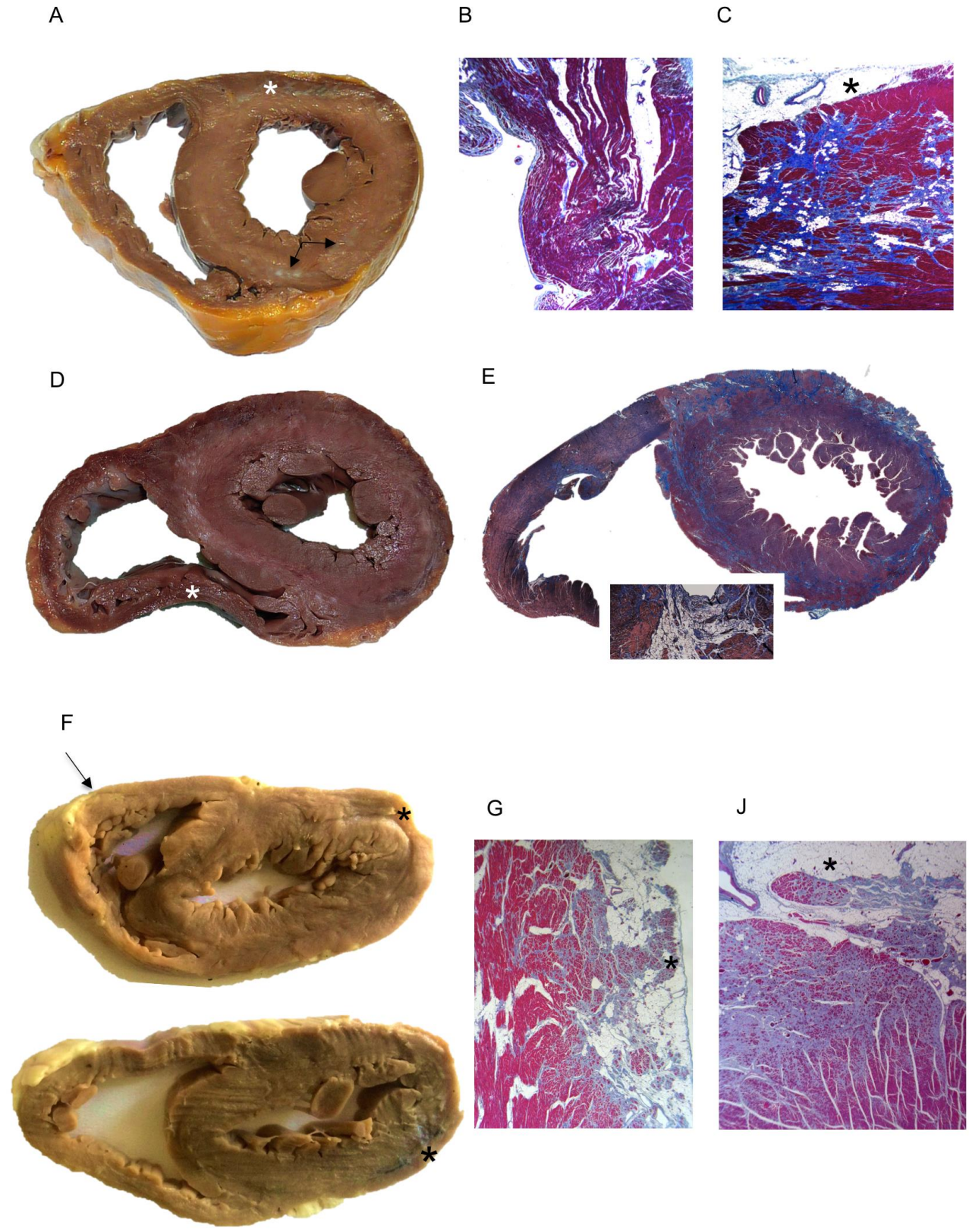


Figure 2

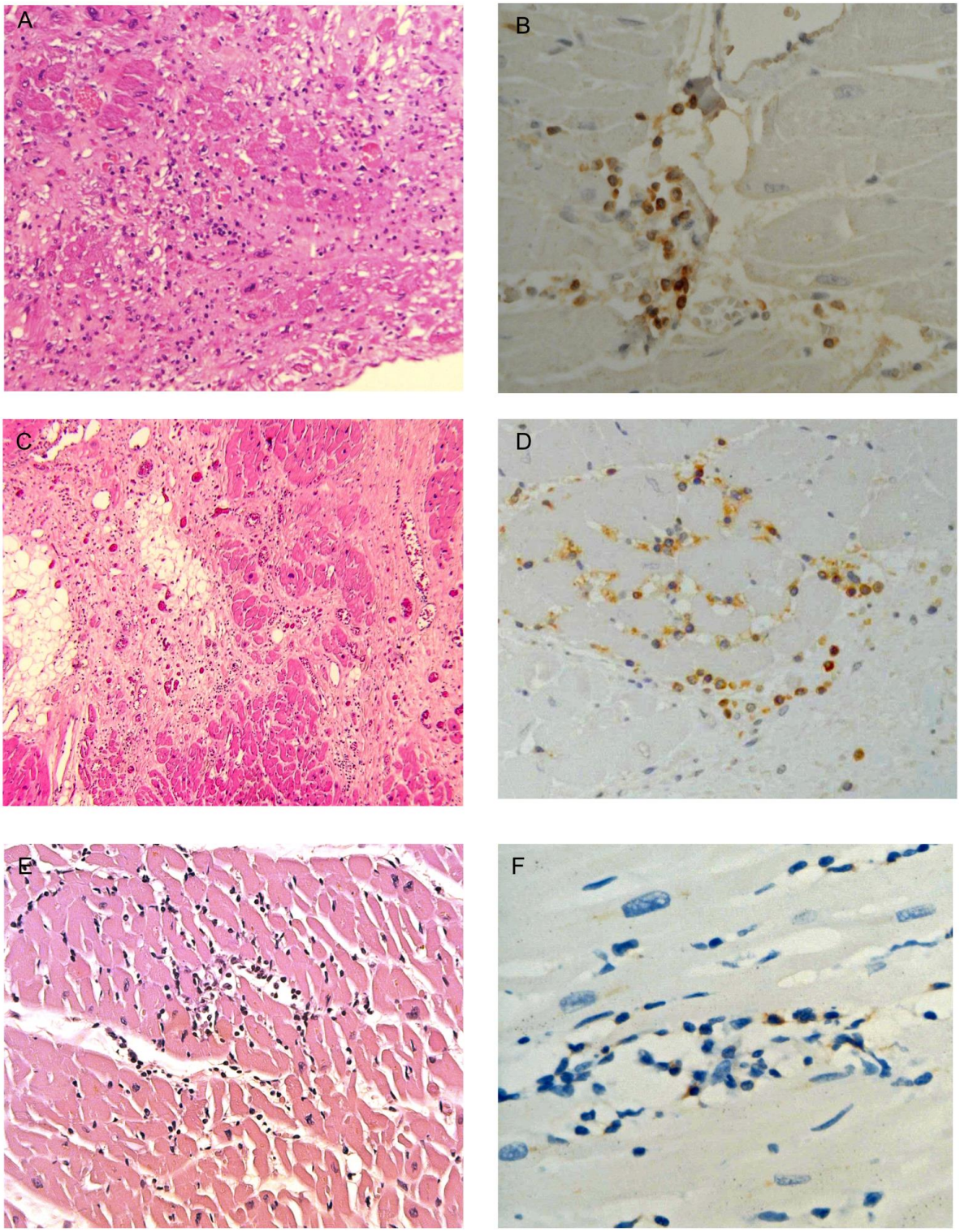


Figure 3

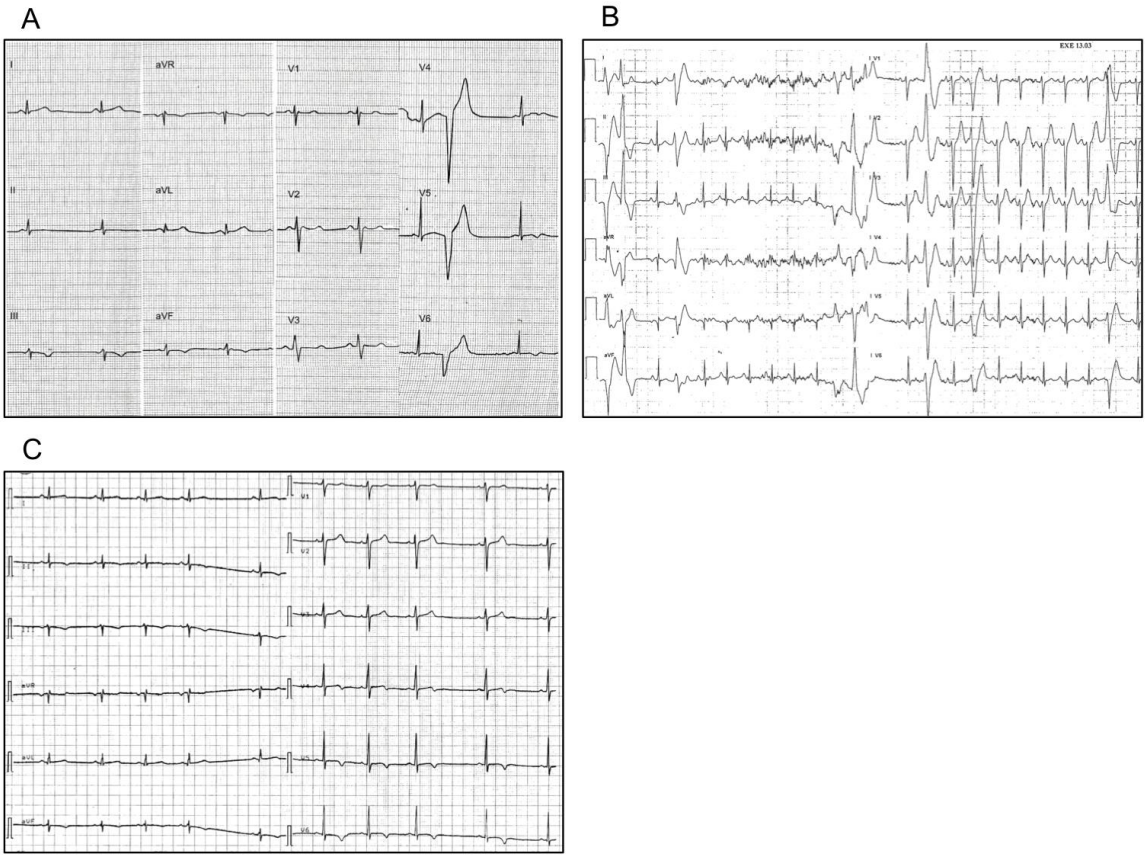


Figure 4

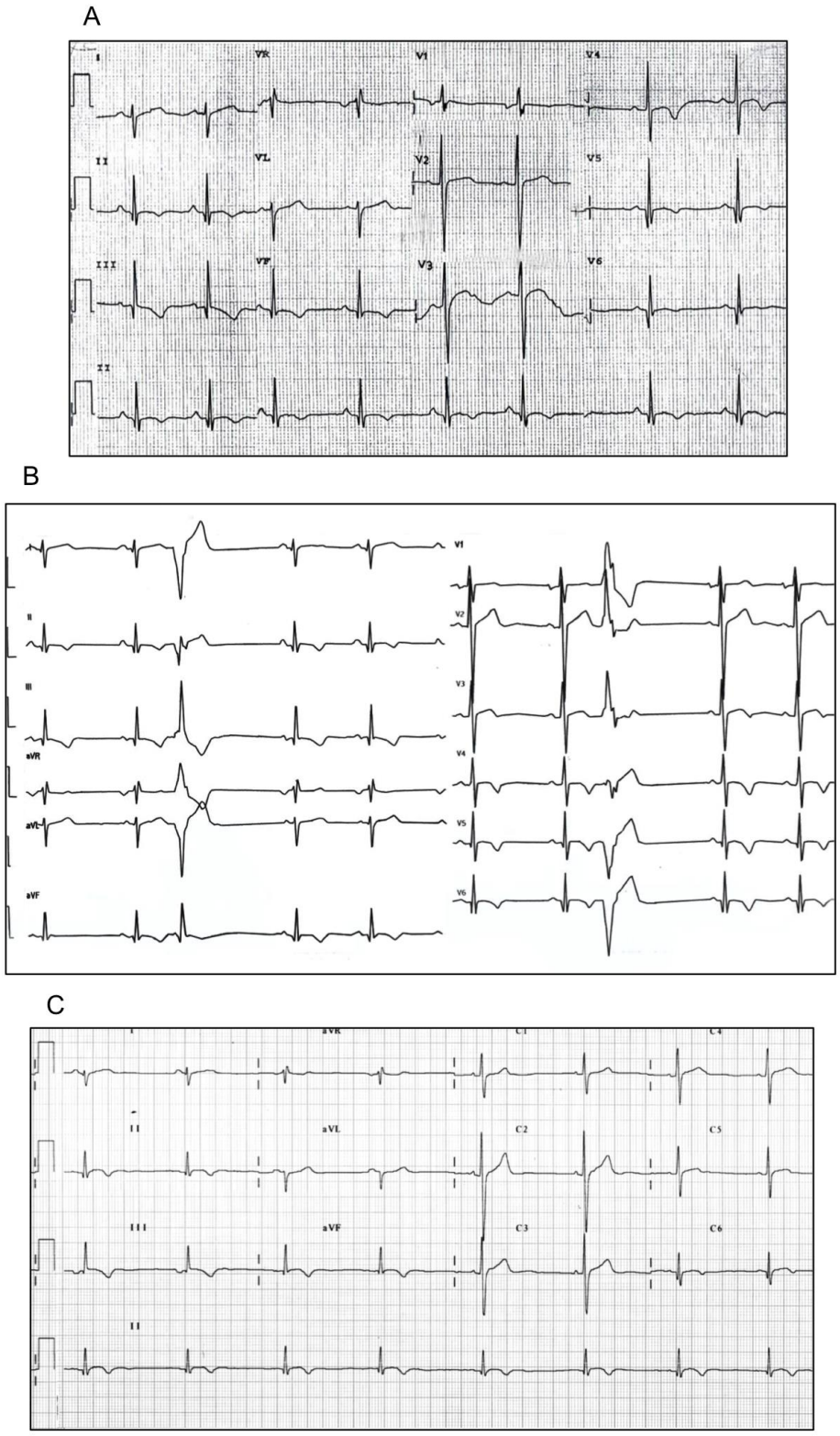


Figure 5

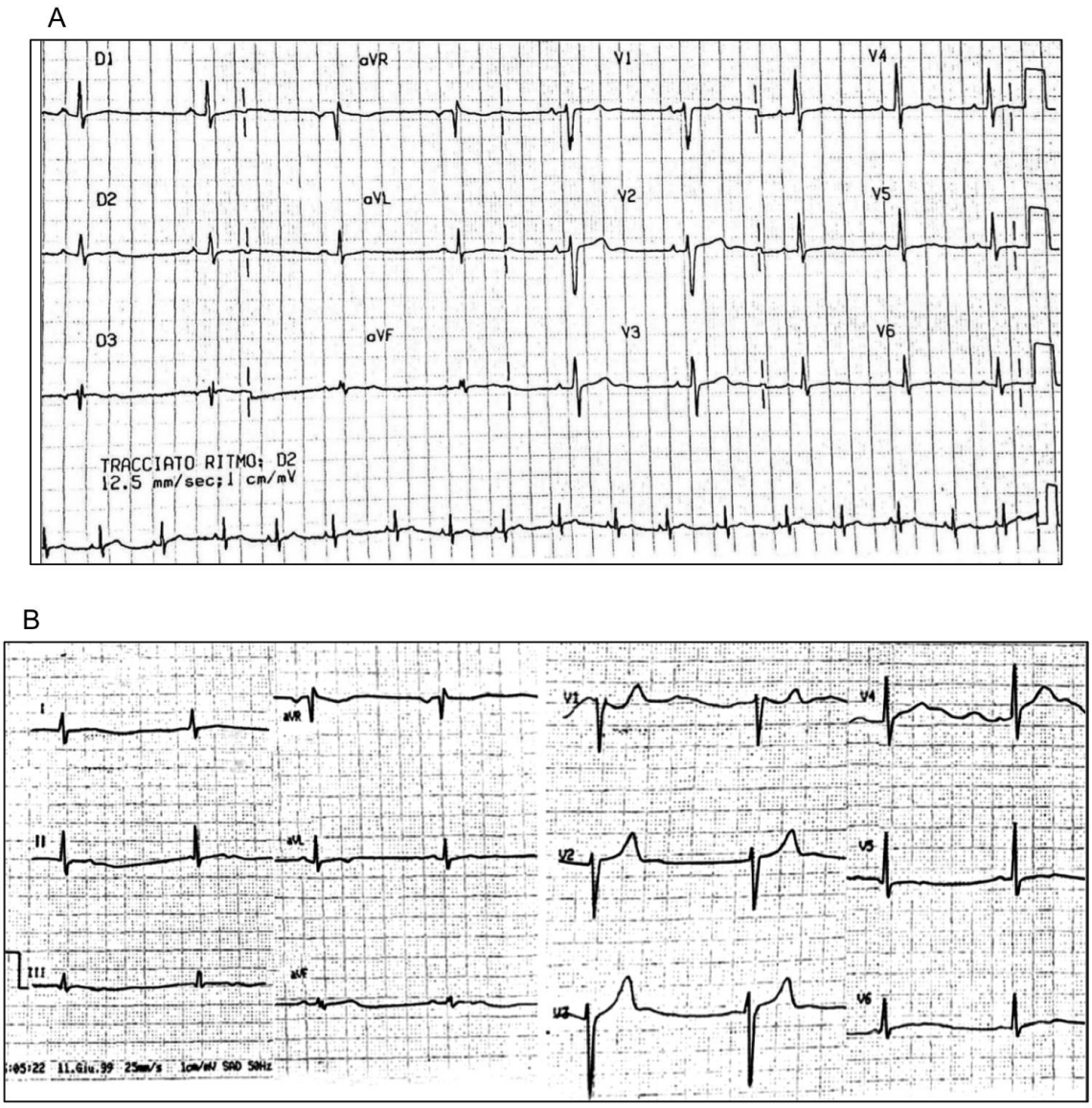
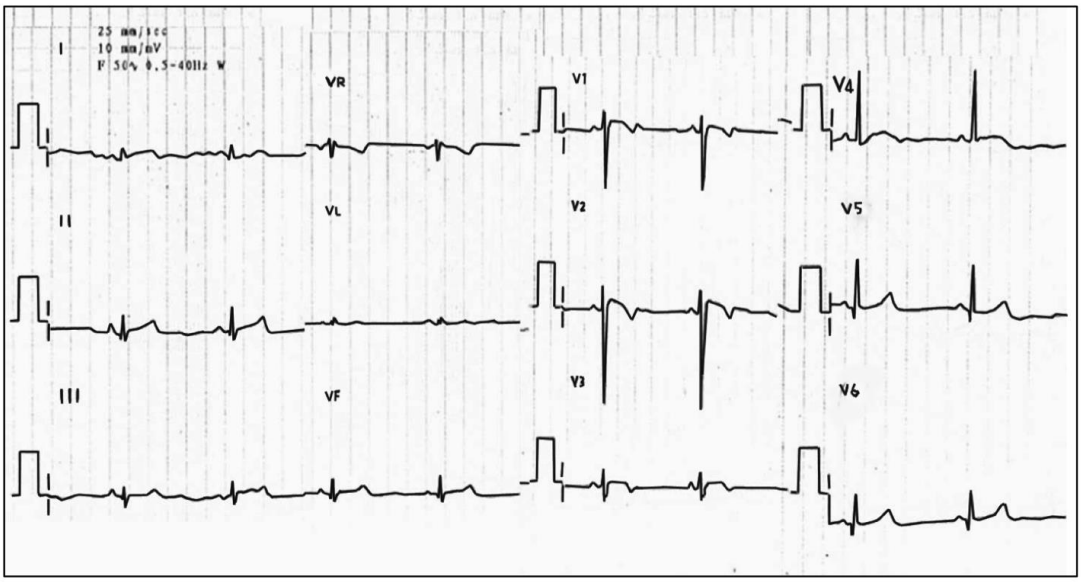


Figure 6

A



B

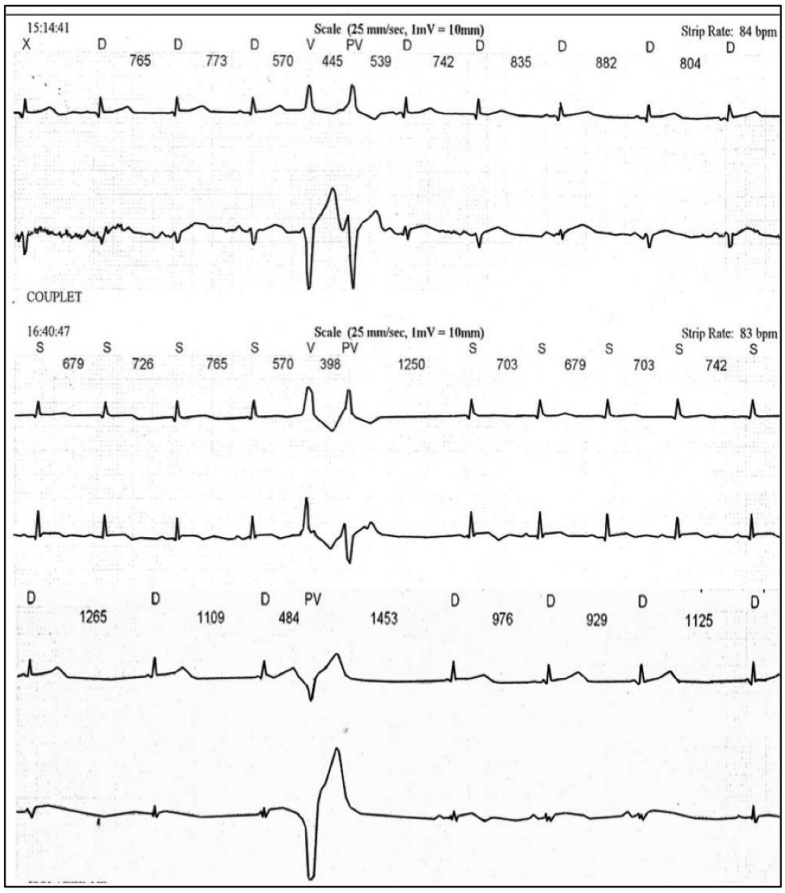


Figure 7