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MORFOLOGICAL ASPECTS OF CARDIAC FIBROSIS IN SUDDEN CARDIAC DEATH

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Abstract

This article presents the findings of a retrospective analysis of medicolegal autopsy reports in cases of sudden cardiac death. This study aimed to identify morphological changes in myocardial connective tissue among individuals aged 18–45 years who experienced sudden cardiac arrest. In instances of sudden unexpected adult death, the arrhythmogenic mechanism of sudden cardiac death tends to be more common than that of mechanical death. Cardiac fibrosis was identified in 78% of the cases, with diffuse interstitial fibrosis (DIF) being the predominant type in 91% of these cases. A significant difference ($p < 0.01$) was found between the area of DIF in the study group and that in the control group. The authors concluded that sudden cardiac death cases, where no clear cause is identified during autopsy, often stem from a functional and/or structural arrhythmogenic substrate induced by cardiac fibrosis (myocardial remodelling).

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1. INTRODUCTION

Sudden unexpected natural death (SUND) is predominantly caused by cardiac pathology [1, 2, 3]. According to World Health Organization guidelines, all cases of sudden death from cardiac causes occurring within one hour of the onset of symptoms from an undiagnosed or previously latent heart disease should be classified as sudden cardiac death (SCD) [2]. In elderly individuals, SCD is mainly due to the complications of coronary atherosclerosis. In younger people, the causes of SCD include genetically determined cardiac arrhythmias (channelopathies and conduction system disorders), cardiomyopathies, inflammatory diseases, and various metabolic disorders of the myocardium. These conditions lead to structural changes in cardiomyocytes and the extracellular matrix (myocardial remodeling or cardiac fibrosis), resulting in the electromechanical dysfunction of the myocardium [1]. Death from cardiovascular disease is the leading cause of non-violent death, occurring suddenly and rapidly in more than 50% of cases after the first appearance of heart disease symptoms [2]. In many cases of sudden death, where no extracardiac cause is found during autopsy, histological and microscopic examination of the heart fails to reveal significant structural pathology of the myocardium or coronary arteries. Accurate interpretation of these "non-critical" myocardial changes and confirmation of a cardiac cause of sudden death in cases with autopsy-negative macroscopic findings, the following are require: (1) detailed knowledge of the histological structure of the myocardium and cardiovascular physiology; (2) understanding of the electrophysiological and biochemical processes in the myocardium under normal conditions and during pathology that affect its contractile function; and (3) a clear understanding of the pathophysiological mechanisms of ion current disorders (electrical imbalance) and myocardial contractility, leading to

electromechanical dysfunction of the myocardium with the development of ventricular tachycardia, transitioning into fibrillation and asystole (the arrhythmogenic mechanism of SCD) in each case of "unexplained" sudden non-violent death.

2. OBJECTIVE

To study morphological changes in myocardial connective tissue in young adults aged 18–45 years who experienced sudden cardiac death (SCD).

3. MATERIALS AND METHODS

A retrospective analysis of forensic autopsy reports from 2018 to 2023 was performed. The study group consisted of 54 forensic autopsies of men aged 18–45 years with autopsy-negative morphological findings of ischemic causes of sudden cardiac death (SCD). The inclusion and exclusion criteria were as follows:

3.1. Inclusion criteria

- Male, aged 18–45 years
- Time of death < 2 days
- ICD-10 code of the primary cause of death – I42.8/I42.9 (cardiomyopathy)
- Presence of forensic histological and chemical examinations
- Blood ethanol concentration at the time of death < 2.5‰
- Negative toxicological results.

3.2. Exclusion criteria

- Time of death 2 days
- Putrefactive transformation
- Coronary atherosclerosis (ischemic heart disease)
- Death in healthcare facilities with a stay > 1 hour
- Severe alcohol and/or drug.
  intoxication at the time of death (blood ethanol concentration 2.5%, presence of $\alpha - PVP$, opiates, opioids (morphine, codeine, promedol, tramadol, fentanyl), methamphetamine, and phenobarbital)
- Presence of morphological markers of chronic alcohol intoxication in the brain and/or liver
- Medical records of endocrine diseases of the pancreas and/or thyroid, chronic alcoholism and/or drug addiction, adrenal tumors, acute/chronic hepatitis, liver/pancreatic cirrhosis, acute/chronic pancreatitis, acute/chronic thyroiditis, malignant neoplasms, HIV infection (AIDS), Down syndrome, or cerebral palsy.

4. PRE-ANALYTICAL AND ANALYTICAL STAGES OF HISTOLOGICAL INVESTIGATION

4.1. Pre-analytical Stage

- Fixation of longitudinal slices of left ventricular myocardium in a neutral 10% formalin.
- Dehydration using increasing concentrations of alcohols.
- Preparation of paraffin blocks.
- Sectioning of the material into 4 μm thick slices.
- Sections were stained with hematoxylin and eosin, MSB (red, orange, blue), and Masson's trichrome.
- Light microscopy using a Leica DM 2500 microscope.

4.2. Analytical Stage

- Microphotography for morphometric analysis was performed in 10 fields of view (40x objective) with a resolution of 1920 × 1080 using a Leica DFC425 microcamera.
- Morphometric analysis using ImagePro Plus v7.0 software.
- Statistical analysis was performed using Microsoft Excel, Statistica 10, and non-parametric statistical tests (Spearman's rank correlation coefficient (rs), Mann-Whitney U-test).

The control group (n=20) included unused explanted hearts from deceased male donors aged 18–45 years with noncardiac causes of death.

5. RESULTS

In the study group, 74% (40 of 54) of the cases of sudden cardiac death (SCD) occurred in men aged > 35 years. The age distribution of the study groups is shown in Figure (1). During the study, it was not possible to thor-

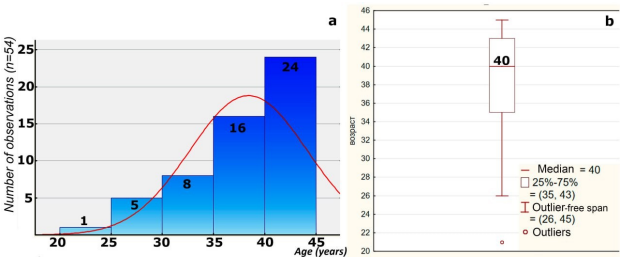

Figure 1. Histogram (a) and Boxplot (b) illustrating the age distribution of SCD cases in the study group.

oughly analyze and systematize data on phenotypic characteristics, minor heart anomalies, extracardiac internal dysmorphisms, heart shape, coronary blood supply type, features of the coronary artery ostia location, coronary arteries and their major branches' origin, course, branching, morphometric parameters of valves, variant anatomy, morphological structure of papillary muscles, and results related to the heart's conduction system and the deceased's previous health status (from medical records).

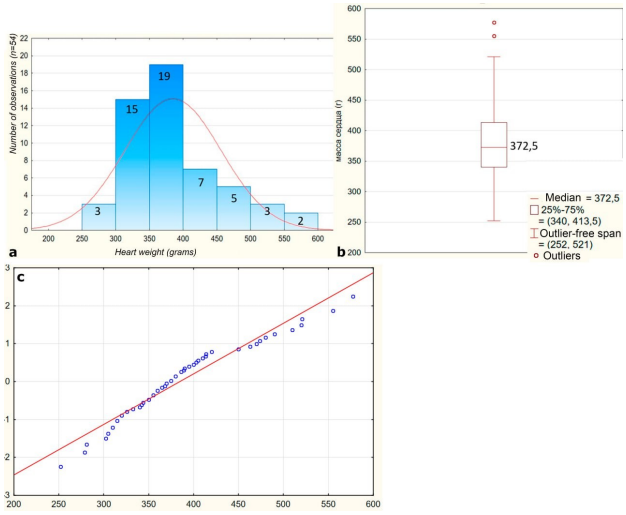

Figure 2. Histogram (a) and boxplot (b) illustrating heart weight in cases of sudden cardiac death (SCD) due to cardiomyopathy in the study group

In the 54 cases of SCD with “cardiomyopathy” autopsy included weighing, measuring linear dimensions, and assessing the thickness of the interventricular septum and ventricular walls (Table 1), without specifying the exact location and level of measurements. Autopsy provided a general descriptive account of the heart’s normal anatomical structure and its major vessels, followed by histological examination of samples (myocardium and coronary artery) without elements of the conduction system and heart valves. In the study group, 63% (34 of 54) of the patients with SCD had a heart weight ranging from 301 to 400 g. The heart weight distribution of the study group is shown in Figure (2).

Table 1. Results of Morphometric Analysis of the Heart in Sudden Cardiac Death (SCD)

Morphometric Parameter	Mean ± SD	Median
Weight	384.87±71.42 g	372.50 g
Length	12.04±1.68 cm	12.0 cm
Width	10.44±1.62 cm	10.45 cm
Thickness	5.25±1.41 cm	5.0 cm
Right Ventricular Myocardial Thickness	0.33±0.12 cm	0.30 cm
Left Ventricular Myocardial Thickness	1.44±0.33 cm	1.45 cm
Interventricular Septal Thickness	1.40±0.34 cm	1.40 cm

No significant correlation was found between the pairwise morphometric parameters of the heart (weight and myocardial thickness of the right ventricle (RV), left ventricle (LV), and interventricular septum (IVS)) and the age of the deceased in the study group. However, statistically significant ($p < 0.05$) moderate positive correlations were established between the pairwise morphometric parameters of the heart (weight, myocardial thickness of the RV, LV, and IVS).

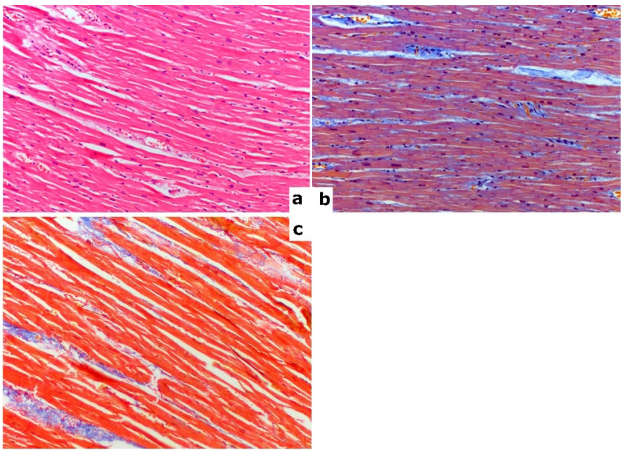

Figure 3. Microphotographs (x200) of the left ventricular myocardium stained with hematoxylin and eosin (a), MSB (b), and Masson’s trichrome (c); collagen fibers are stained blue (b, c).

In the myocardium of the left ventricle in the study group, MSB (Martius-Scarlett-Blue) staining revealed ischemic changes in cardiomyocytes of varying degrees; the cardiomyocytes shifted from their normal "cold” spectrum with a purple-violet hue to a “hot” spectrum with a predominance of orange color. Necrotic areas were not observed (Figure (3 b)). Along with color changes, there were also alterations in the “pattern” of the cardiomyocytes’ cytoplasm and their groups—heterogeneity in the myocardium was observed between intercalated discs in most fields of view of the “hot” areas (Figure (3a, b)). Histological visualization of cardiac fibrosis and morphometric analysis of its extent were performed using Masson’s trichrome staining (Figure (3c)). Cardiac fibrosis was identified in 78% (42 of 54) of the cases, and both focal perivascular and focal interstitial types of cardiac fibrosis were noted. However, diffuse interstitial fibrosis (DIF) was the predominant type in 91% (3842) of the cases. Morphometric Results: area of DIF (median [25%-75%], μm^2) in the study group (n=38): 8315 [8054-8895]; in the control group, 4733 [4312-5046]. A significant difference ($p < 0.01$) was found between the area of DIF in the myocardium of the study group and that in the control group.

6. DISCUSSION

The myocardium is a morphological structure composed of cardiomyocytes (CMCs) surrounded by a dense network of collagen, the main structural protein of the connective tissue. The cardiac interstitium represents a unique and highly adaptable extracellular matrix (ECM) that provides a "comfortable environment" for the interaction and function of various cells, including CMCs, fibrob-

lasts, and endothelial cells. Myocardial remodeling is an adaptive-dysadaptive process involving structural reorganization of the ECM and the muscular-vascular component. This remodeling occurs under functional stress or overload, and is triggered by various factors, often in combination. These factors include ischemia, necrosis, inflammation, disruption of central and/or peripheral neurohumoral regulation, psycho-emotional stress, metabolic damage of various origins, and apoptosis. Chronic myocardial hypoxia, resulting from either organic (hemodynamically significant coronary atherosclerosis) or functional (non-coronary) ischemia, is the predominant primary cause of myocardial remodeling. This remodeling manifests as focal perivascular, interstitial, or diffuse interstitial cardiomyopathy leading to cardiac fibrosis. Sudden Cardiac Death (SCD) is a diagnosis of exclusion and can only be classified as the primary cause of death with ICD-10 code I46.1, after all possible causes of both violent and non-violent death have been ruled out. Despite the numerous methods available to standardize heart autopsies and systematic collection of materials for histological examination, these methods are not always applied in cases of SCD. When no organic structural pathology is found in the myocardium and coronary arteries, the diagnosis of cardiomyopathy (CMP) with ICD-10 codes I42.8 or I42.9 is often determined as the primary cause of death. This can lead to the overdiagnosis of CMP as the primary cause of death, and discussing the justification, objectivity, and scientific validity of such an approach to determining the cause of death is beyond the scope of this study. Diagnosing sudden death requires meticulous, standardized macro- and micromorphological investigations, complemented by toxicological analysis.

- Exclude violent deaths (e.g., poisoning, electrical trauma, suffocation).
- Exclude extracardiac causes of sudden death (e.g., pulmonary embolism and sudden death associated with epilepsy).

Determine the mechanism of SCD (mechanical or arrhythmic) and the nature of cardiac disease (including light and electron microscopy, immunohistochemical, and molecular genetic studies) [4]. The diagnostic criteria for SCD are determined by the immediate mechanisms leading to death, whether mechanical or arrhythmic, which are influenced by the underlying morphological substrate. This includes the presence of congenital or acquired acute or chronic macro- and/or microstructural pathologies of the myocardium and coronary arteries. Such pathology does not exclude triggering factors (e.g., ischemia, physical exertion, psycho-emotional stress, disturbances in neurohumoral regulation of the cardiovascular system, endocrine-metabolic disorders, or toxic agents affecting the electromechanical function of the myocardium through medications) that can activate the immediate mechanisms of SCD. Reliable morphological criteria for the arrhythmic mechanism of SCD in young individuals and in the absence of structural heart pathology and vascular abnormalities are lacking. In these cases, only morphological signs of fibrillation and associated rheological disturbances in local and systemic blood flow can be observed. These findings do not rule out genetic non-coronary cardiopathies as the cause of "unexplained" sudden death. Such conditions include inherited channelopathies (e.g., such as Brugada syndrome (BrS) and long QT syndrome (LQTS)), cardiomyopathies (e.g., hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular dysplasia (ARVD), dilated cardiomyopathy (DCM)), and congenital or acquired abnormalities of the cardiac conduction system [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. Focal and/or diffuse interstitial fibrosis may represent the only initial structural manifestation of compensated (latent) non-coronary cardiopathies, such as HCM, ARVD, DCM, BrS, or LQTS, which are not detectable by macromorphometric heart examination (autopsy-negative macroscopic findings of cardiac and extracardiac causes of sudden non-violent death). Therefore, a standardized diagnostic approach is necessary to determine the cause of sudden death in young individuals. This approach should include thorough autopsy (macromorphometric), toxicological, histological, and molecular genetic investigations in cases with autopsy-negative macroscopic findings for both extracardiac and cardiac causes of sudden death [4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. This study revealed that in the myocardium of the research group, diffuse interstitial fibrosis (DIF) with ischemic changes in cardiomyocytes, but without necrosis, predominated. A statistically significant difference ($p < 0.01$) was found between the area of DIF in the myocardium of the study group and the control group. Excessive connective tissue (collagen) proliferation in the myocardial interstitium leads to relative morphofunctional separation of cardiomyocytes or their groups, resulting in impaired microcirculatory hemodynamics and non-coronary ischemia (hypoxia) of the myocardium [3, 12]. Chronic myocardial hypoxia induces premature necrobiotic changes in the intracellular structures of cardiomyocytes and their apoptosis, followed by replacement of necrotized cardiomyocytes with connective tissue (focal cardiomyopathy) [3, 12]. Thus, in cases of sudden unexpected death (SUND) in young individuals, where cardiac and extracardiac causes are not evident from autopsy findings and with thorough autopsy and histological examinations of the heart (coronary arteries, myocardium, valve apparatus, and cardiac conduction system elements) and negative toxicological results, identified DIF can be considered as an arrhythmogenic structural substrate. This substrate contributes to the electromechanical dysfunction of the myocardium and arrhythmogenic sudden cardiac death (SCD, ICD code I46.1). Accurate determination of the

primary cardiological cause of SCD in such cases is not possible without molecular autopsy, especially when dealing with latent inherited noncoronary cardiopathies. Postmortem molecular genetic testing (molecular autopsy) is a diagnostic method that allows the identification of mutant genes responsible for inherited non-coronary cardiopathies with a high risk of sudden cardiac death (SCD) in young individuals. Determining the hereditary (congenital) arrhythmogenic substrate is crucial not only for the accurate diagnosis of the cause of SCD but also holds significant importance for public health, as it aids in the prevention of "familial" cases of SCD among the deceased's relatives.

7. CONCLUSION

In the study group, various types of cardiac fibrosis were identified in 78% of cases, with diffuse interstitial fibrosis (DIF) found in 70% of cases. A statistically significant difference ($p < 0.01$) was observed between the area of DIF in the myocardium of the study group and the control group. The myocardial remodeling observed in the study group (DIF) could serve as a structural arrhythmogenic substrate, which, in combination with a specific trigger (ischemia), could lead to electromechanical dysfunction of the myocardium, initiating the arrhythmogenic mechanism of sudden cardiac death (SCD). Focal and/or diffuse interstitial fibrosis may be the sole initial structural manifestation of compensated (latent) non-coronary cardiopathy in cases of autopsy-negative macroscopic sudden non-violent death, signaling a potential genetically determined arrhythmogenic substrate that could result in fatal arrhythmia. The results of a molecular autopsy are crucial not only for accurately diagnosing the genetically determined cause of SCD, but also for public health efforts to prevent cases of "familial" SCD among deceased relatives.

REFERENCES

[1] P. Markwerth, T. Bajanowski, I. Tzimas, et al. "Sudden cardiac death-update". In: *Int. J. Leg. Med.* 135.2 (2021), pp. 483–495. DOI: 10.1007/s00414-020-02481-z.

[2] J. Obrova, E. Sovova, E. Kocianova, et al. "Sudden cardiac death - a known unknown?" In: *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc* 166.3 (2022), pp. 258–266. DOI: 10.5507/bp.2021.065.

[3] A. J. Camm et al. *The ESC Textbook of Cardiovascular Medicine*. 3rd. The European Society of Cardiology Series. Oxford University Press, 2018. DOI: 10.1093/med/9780198784906.001.0001.

[4] C. Basso, B. Aguilera, J. Banner, et al. "Guidelines for autopsy investigation of sudden cardiac death: 2017 update from the Association for European Cardiovascular Pathology". In: *Virchows Arch.* 471.6 (2017), pp. 691–705. DOI: 10.1007/s00428-017-2221-0.

[5] B. A. Koplan and W. G. Stevenson. "Sudden arrhythmic death syndrome". In: *Heart* 93.5 (2007), pp. 547–548. DOI: 10.1136/hrt.2006.108134.

[6] J. Tfelt-Hansen, B. G. Winkel, M. Grunnet, et al. "Cardiac channelopathies and sudden infant death syndrome". In: *Cardiology* 119.1 (2011), pp. 21–33. DOI: 10.1159/000329047.

[7] B. H. Tan, K. N. Pundi, D. W. Van Norstrand, et al. "Sudden infant death syndrome-associated mutations in the sodium channel beta subunits". In: *Heart Rhythm.* 7.6 (2010), pp. 771–778. DOI: 10.1016/j.hrthm.2010.01.032.

[8] S. G. Priori, A. A. Wilde, M. Horie, et al. "Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes". In: *Heart Rhythm.* 10.12 (2013), e85–e108. DOI: 10.1016/j.hrthm.2013.07.021.

[9] L. C. Wong, F. Roses-Noguer, J. A. Till, et al. "Cardiac evaluation of pediatric relatives in sudden arrhythmic death syndrome: A 2-center experience". In: *Circ. Arrhythmia Electrophysiol.* 7.5 (2014), pp. 800–806. DOI: 10.1161/CIRCEP.114.001818.

[10] M. Wilhelm, S. A. Bolliger, C. Bartsch, et al. "Sudden cardiac death in forensic medicine – Swiss recommendations for a multidisciplinary approach". In: *Swiss Med. Wkly.* 145 (2015), w14129. DOI: 10.4414/smw.2015.14129.

[11] K. Braysh, R. Zerbe, R. J. Zein, et al. "The advancement of postmortem investigations in sudden cardiac death". In: *Post Mortem Examination and Autopsy - Current Issues From Death to Laboratory Analysis*. InTech, 2018. DOI: 10.5772/intechopen.71555. URL: https://doi.org/10.5772/intechopen.71555.

[12] S. K. Suvarna. *Cardiac Pathology: A Guide to Current Practice*. 2nd ed. Springer Nature, 2019.

[13] V. Castiglione, M. Modena, A. Aimo, et al. "Molecular autopsy of sudden cardiac death in the genomics era". In: *Diagnostics* 11.8 (2021), p. 1378. DOI: 10.3390/diagnostics11081378. URL: https://doi.org/10.3390/diagnostics11081378.

[14] C. Semsarian, J. Ingles, and A. A. Wilde. "Sudden cardiac death in the young: The molecular autopsy and a practical approach to surviving relatives". In: *Eur. Heart J.* 36.21 (2015), pp. 1290–1296. DOI: 10.1093/eurheartj/ehv063. URL: https://doi.org/10.1093/eurheartj/ehv063.

[15] A. L. George Jr. "Molecular and genetic basis of sudden cardiac death". In: *The J. Clin. Investig.* 123.1 (2013), pp. 75–83. DOI: 10.1172/JCI62928. URL: https://doi.org/10.1172/JCI62928.

[16] A. M. Tsiatsiuyeu, V. V. Siamionau, and O. A. Yudina. "Technique of heart autopsy during forensic examination of adult bodies in cases of sudden death". In: *Forensic Expert. Belarus* 1.14 (2022). [In Russian], pp. 49–55.