E-publishing ahead of print is increasingly important for the rapid dissemination of science. The Early Access service lets users access peer-reviewed articles well before print / regular issue publication, significantly reducing the time it takes for critical findings to reach the research community.

These articles are searchable and citable by their DOI (Digital Object Identifier).

The Journal of Biological Research is, therefore, e-publishing PDF files of an early version of manuscripts that undergone a regular peer review and have been accepted for publication, but have not been through the typesetting, pagination and proofreading processes, which may lead to differences between this version and the final one.

The final version of the manuscript will then appear on a regular issue of the journal.

E-publishing of this PDF file has been approved by the authors.

To cite this Article:


©The Author(s), 2024
Licensee PAGEPress, Italy

Note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries should be directed to the corresponding author for the article.

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.
Sudden cardiac death in the young. From gross to molecular autopsy
Gaetano Thiene, Cristina Basso, Monica De Gaspari, Stefania Rizzo
Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Italy

Correspondence: Gaetano Thiene, Emeritus Professor, Via A. Gabelli 86, 35121 Padova, Italy. E-mail: gaetano.thiene@unipd.it

Key words: genetically determined cardiovascular diseases; molecular autopsy; sudden cardiac death.

Conflict of interest: the authors declare no conflict of interest.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Conference presentation: Lecture held in Trieste at the 95° SIBS Congress - April 12-15, 2023.

Acknowledgments: the investigation has been accomplished within the Regional Registry for Cardio-Cerebro-Vascular Pathology, Veneto - Italy. We thank Giulia Vangelista for the excellent support.

Abstract
Sudden Cardiac Death (SCD) may complicate diseases of the heart and great vessels. The cause is easily visible at the naked eye at autopsy in the presence of coronary thrombosis, aortic dissection, pulmonary thromboembolism, or at the microscope with histological anomalies (inflammation, necrosis, storage, fibrosis). However, there are cases of SCD in which the heart appears normal, both at gross and histological examination. They may present electrocardiogram (ECG) disorders of depolarization and repolarization of myocardial electrical activity (long and short QT, repolarization syndrome) or of electro-mechanical coupling (catecholaminergic ventricular tachycardia), due to
alterations of Na\(^+\), K\(^+\) or Ca\(^{++}\) flows, known as channelopathies. They are genetic, hereditary morbid entities transmitted at the time of conception. Molecular studies of SCD at autopsy include both the detection of viral genomes in inflammatory cardiomyopathies and gene mutations in either structural or nonstructural genetically determined heart diseases.

**Introduction**

Giovanni Battista Morgagni in 1712, at the age of only 29 years old, delivering the Commencement Lecture of the Theoretical Medicine Chair at the University of Padua, stated:\(^1\) “It is impossible to pursue the nature and cause of any disease without dissection of the respective bodies”. It was the era of autopsy limited to organ pathology, with the eyes as the only tool for investigation. Nowadays gross dissection associated with a light microscope is still an unreplaceable method to establish the cause of death and explain symptoms. However, there are cases of "sine materia", which require molecular investigation (so-called "molecular autopsy").\(^2,3\)

John A. MacWilliam (1857-1937), in his book “Cardiac Failure and sudden death”, published in 1889,\(^4\) wrote: “…sudden stoppage of the heart’s action has often been observed apart from the occurrence of gross structural lesions, associated with no very obvious or extensive alteration in the cardiac tissues…not infrequently the cardiac substance has exhibited no pronounced morbid change”.

Years later, in 1894 at a meeting held in Rome, entitled “Morgagni and the Anatomic Concept”, during a discussion with Karl von Rokitansky, Rudolph Virchow raised these questions: “…Any anatomic modification is material, but is any material modification anatomic? Why not molecular? Can a profound molecular modification occur in the setting of a normal structure? These modifications belong more to physiology than to anatomy, they are functional-dynamic… the method of investigation will never be morphological”.

The causes of “sine materia” SCD lie in the code of life: they are genetic diseases. Gregor Mendel (1822-1884),\(^5\) working on beans, discovered the principles of genetic heritage, summarized in the Mendel’s Laws with the concepts of dominant and recessive. In 1953, with the discovery of the DNA and double helix by Watson and Crick,\(^6\) the molecular nature of genes was finally established. The invention of Polymerase Chain Reaction, the tool to clone the genes made by Kary Mullis in 1986,\(^7\) opened new diagnostic avenues.

As far as sudden death, the role of autopsy is to establish:\(^8\) i) whether the death was natural, ascribable to cardiac or other diseases; ii) in case of cardiac death, whether it was arrhythmic or
iii) in the absence of gross and histological substrate, to establish if it was “functional”, thus requiring molecular investigation and, if genetic, screening the next of kin;\(^9,10\) iv) the existence of toxic and illicit drug abuse or concealed trauma (\textit{i.e.} cervical fracture with injury of brain stem, where the breathing pacemaker is located).\(^{11}\)

In early our experience of 300 consecutive cases of sudden death in the young (<35 years old), the cause was 91% cardiovascular, 5% cerebral, and 4% respiratory.\(^{12}\) As for the cerebral cause, rupture of a berry aneurysm of the Willis circle is usually the culprit, and, as for respiratory, allergic asthma. Concerning the mechanism of cardiac arrest in SCD, it was mechanical in 7% (pulmonary thromboembolism or rupture of aortic dissection with cardiac tamponade) or arrhythmic in the remaining 93%, mostly ventricular fibrillation.

The substrate of SCD should be searched in the aorta, coronary arteries, myocardium, valves, conduction system, and ion channels.\(^{13}\)

In our preliminary experience, aortic rupture accounted for 2% of cases, whereas coronary artery disease for 18%. Unlike adult and elderly, where the coronary artery disease is usually multivessels with occlusive thrombosis due to plaque rupture, in the young the disease is mostly single vessel (descending coronary artery) with vasospasm.\(^{14-16}\)

The atherosclerotic coronary artery disease increases with age as a cause of SCD and becomes the most frequent in the time interval 30-40 years old.\(^{17}\)

Also, hidden coronary artery anomalies may precipitate SCD during effort, particularly the origin from the wrong sinus,\(^{18}\) which accounts for 5% of SCD in the young.

Among cardiomyopathies, arrhythmogenic right ventricular cardiomyopathy (ARVC) is the “leader” with 10% (19, 20), followed by hypertrophic cardiomyopathy (HCM) with 9%.\(^{21,22}\) They are both genetically determined heart muscle diseases: ARVC a desmosomal disease\(^{23}\) and HCM a sarcomeric disease.\(^{24}\)

Among inflammatory cardiomyopathies, viral myocarditis comes first (12%; Figure 1).\(^{25,26}\)

Finally, there are sudden deaths “sine materia”, called channellopathies, in which electrical instability of the heart occurs in the absence of structural substrate (Figure 2).\(^{2,3,8-10}\)

Long (Figure 3) and short QT syndromes are due to “gain-of-function” mutations of potassium or sodium channel genes, with either dominant or sporadic forms.\(^{27}\) Brugada syndrome (nonischemic ST-segment elevation) in 20% of cases is a familial dominant or sporadic autosomal form, due to mutations of sodium channel gene SCN5A.\(^{27}\) Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)\(^{28}\) is due to mutations of the ryanodine receptor 2 (RyR2) gene\(^{29}\) in the dominant form and to calsequestrin 2 (CASQ2) gene in the recessive form.\(^{27}\)
These genes are in charge of calcium release from the smooth endoplasmic reticulum for electromechanical coupling. It is a quite long gene, however just a pin-point mutation of a nucleotide can change the printed amino acid which is enough to account for ventricular tachycardia and fibrillation.\textsuperscript{30}

Mutations of RyR2 are the most frequently found at molecular autopsy of SCD “\textit{sine materia}”.\textsuperscript{31} Early repolarization with J wave has been proven to be a risk factor for idiopathic ventricular fibrillation and has been ascribed to arrhythmogenicity of subendocardial Purkinje fibers.\textsuperscript{32} In our longstanding experience of SCD in the young, normal heart was found in only 17%, whereas other groups reported higher rates, up to 50\%,\textsuperscript{3} probably because only unexplained cases “\textit{sine materia}” were submitted to their reference center.

Anyhow, since one-third of SCD are genetic in origin, with or without structural substrate, genotyping the victim is mandatory. The finding of a disease-causing mutation will make necessary a rigorous screening of all relatives, to identify those who carry occultly the same mutation. This approach should now become part of the routine post-mortem study of unexplained cases of SCD.\textsuperscript{27}

Thus, the study of SCD is moving from the traditional gross pathology autopsy to the double helix. For this purpose, 10 mL of ethylenediaminetetraacetic acid (EDTA) blood and 5 g of heart and spleen tissues should be taken, frozen, and stored at -80°C. Alternatively, blood may be stored in RNA later at 4°C for up to 2 weeks.\textsuperscript{33}

Last but not least, it is mandatory to detect or rule out toxic or illicit drug abuse. The guidelines consider toxicology investigation of equal importance to genetic analysis.\textsuperscript{11} 3.1\% of SCDs were found to be related to cocaine abuse triggering coronary artery disease.\textsuperscript{34}

Shifting the attention to the aorta, aortic dissection accounts for 3\% of SCD in the young. The main culprit is the aortopathy associated with the bicuspid aortic valve with severe disruption of the elastic lamellar units of the tunica media by the release of metalloproteinases.\textsuperscript{35} Among other valve diseases, ventricular fibrillation is the mechanism of arrhythmic mitral valve prolapse (8\% of SCD).\textsuperscript{36} The congenital disjunction between the atrioventricular mitral annulus and leaflet insertion was discovered. After billions of systoles during life, fibrosis develops in the papillary muscles and postero-lateral left ventricular free wall, becoming an arrhythmic substrate. As far as the conduction system, 6\% of SCD in our experience was caused by ventricular preexcitation in the setting of Wolff-Parkinson-White syndrome. The “Kent fascicle”, abnormally connecting the atrial with the ventricular myocardium, is an ordinary myocardium without decremental properties. If paroxysmal atrial fibrillation occurs in these patients, every atrial wave may reach the ventricles, then transforming atrial fibrillation into ventricular fibrillation with cardiac arrest.\textsuperscript{37}
References


Figure 1. Molecular diagnosis of viral myocarditis at autopsy. The myocarditis rate as a cause of juvenile SCD is 12%.
Sudden Cardiac Death and Normal Heart: «mors sine materia»

a) Long QT  

b) Short QT  

c) Brugada syndrome  

d) Catecholaminergic Polymorphic VT  

![Pie chart showing 17% for Normal heart.]

Figure 2. SCDs in the young occurs in “normal hearts” (channelopathies with ECG phenotype) with a rate of 17% in our experience.
Figure 3. Sudden death in a 16 years-old boy affected by long QT. Gross and histology of the heart appear normal (“mors sine materia”).