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Protocol of Investigation on Sudden Death at Autopsy, Including Molecular, Genetic and Toxicology Testing

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Abstract

The role of the autopsy: 1) *Whether the death is ascribable to a natural or unnatural cause* and when natural, if cardiac or extra-cardiac; 2) *The nosology of the cardiac diseases and the mechanism of cardiac death*, whether arrhythmic or mechanical; 3) *If the cardiac disease is inherited*, screening and counselling of the next of kin is required. About 30% of sudden deaths is ascribable to genetically determined morbid entities, mostly transmissible with the autosomal dominant pattern of inheritance, so that 50% of the first degree relatives are genetically affected ("carriers") and exposed at risk; 4) *If toxic or illicit drug abuse was involved*.

Keywords

Family Screening, Genetics, Inheritance, Molecular Autopsy, Sudden Death, Toxicology

1. Introduction

The aim of the current paper is to publish the topic in a surgical journal to make aware also the cardiovascular surgeons on this important topic.

1.1. Clinico-Pathological Correlation

Autopsy is still unreplaceable to establish the cause of sudden death (SD) [1]-[6]. If so, it should become mandatory to have an explanation of this dramatic event, for the implications on the relatives.

Medical history:

- age, gender, lifestyle;

- family history, inherited cardiac diseases;
- personal history: syncope, chest pain, etc, previous surgery, pace-maker, implantable cardioverter defibrillator (ICD), etc., ECG, blood pressure, echo, laboratory findings;
- circumstances of death: witnessed, carbon monoxide, violence, trauma, electrical burst, etc;
- Final ECG tracing, if any.

1.2. External Examination

- Establish body weight and height;
- Check for recent intravenous access, intubation and traumatic lesions;
- ICD/pacemaker inplantation.

1.3. Diseases at Risk of Sudden Death

A full autopsy should be performed to rule out extra-cardiac causes of SD [1]:

- Cerebral (sub-arachnoid or intra-cerebral haemorrhage, etc.);
- Respiratory (allergic asthma, anaphylaxis, etc.);
- Haemorrhagic shock (ruptured aortic aneurysm, gastrointestinal haemorrhage, etc.);
- Septic shock (Waterhouse-Friderichsen syndrome, etc.).

1.4. Gross Examination of the Heart

The search for the cardiac cause should be accomplished by exploring: great arteries, coronary arteries, myocardium, valves, conduction system [6].

The standard gross examination of the heart should be carried out:

- Open the pericardium and explore the cavity to exclude cardiac tamponade.
- Transecting the great arteries, 3 cm above the valve commissures.
- Transect the pulmonary veins origin and the superior vena cava 2 cm above the sulcus terminalis, to preserve sinoatrial node. Transect the inferior vena cava, close to the diaphragm.
- Open the right atrium, from the inferior vena cava to the apex of the right atrial appendage.
- Open and inspect the left atrium, through the pulmonary veins and then open and inspect the left atrial appendage.
- Look at the inter-atrial septum and establish whether the foramen ovale is patent. Examine the mitral and tricuspid leaflets (or valve prostheses, if any) and check the status of the papillary muscles and chordae tendineae.
- Inspect the aorta and the pulmonary artery to exclude aortic dissection or pulmonary embolism and take a look at the aortic and pulmonary valves (or valve prostheses, if any).
- Then examine the coronary arteries:

- size, shape, position, number and patency of the coronary ostia (**Figure 1**);

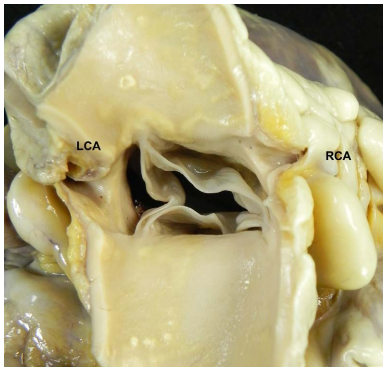

Figure 1. After a transection of the aorta 3 cm above the aortic valve, inspect the coronary ostia (LCA = left coronary artery; RCA = right coronary artery). From [6], with permission.

- establish the pattern of the course of the main subepicardial coronary arteries;
- make multiple transverse cuts at 3 mm intervals along the course of the epicardial arteries, branches included, and check patency, with special attention of the anterior descending coronary artery (the so called "artery of sudden death") (**Figure 2**);

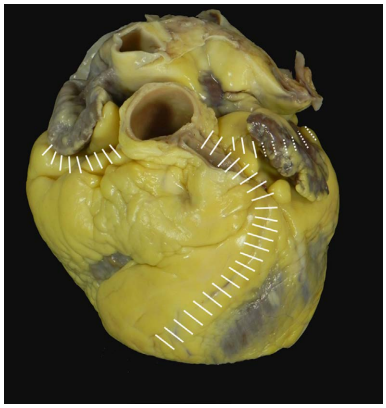

Figure 2. Serial cross sectioning of the right, left anterior descending and left circumflex coronary arteries. From [6], with permission.

- heavily calcified coronary arteries have to be opened with sharp scissors. If not possible, they should be removed intact, decalcified and then opened transversely;
- coronary artery segments with metallic stents should be referred to labs with the facility of diamond blade;
- coronary artery bypass should be carefully checked with transverse cuts and with proximal-distal anastomoses examination;
- make a short-axis cuts of the heart at the mid-ventricular level and parallel slices of ventricles up to the apex (**Figure 3**).

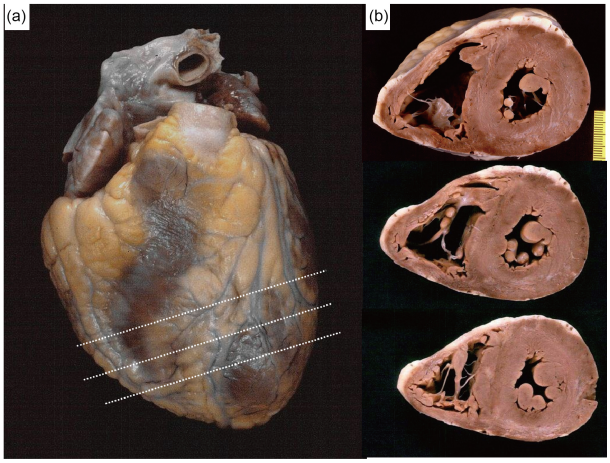

Figure 3. Short axis cross sectioning of the heart specimen from mid-ventricular to apical levels. (a): gross view of the specimen with cross sectioning. (b): transverse sections of the heart at three different levels. From [6], with permission.

- Empty the heart cavities of blood and then make the following:
- Total heart weight;
- Inspect endocardium, ventricular walls thickness by measuring thickness at mid free wall of the left and right ventricles (excluding trabeculae) and of the septum;
- Heart dimensions: the transverse size on the posterior aspect, from the obtuse to the acute margin in the posterior atrioventricular (AV) sulcus and longitudinal size (the distance between the crux cordis and the apex of the heart);
- Open the ventricular inflows and outflows. In case of ECG ventricular preexcitation, the AV rings should be maintained intact.

1.5. Histology and Electron Microscopy

- Subepicardial coronary arteries: the severe focal lesions should be sampled for histology in labelled blocks and stained routinely with Hematoxylin and Eosin (H & E) and a connective tissue stain (Elastic Weigert-van Gieson, Azan-Mallory).
- Myocardium: take labelled blocks from a transverse slice of the ventricles, the free wall of the LV (anterior, lateral and posterior), the ventricular septum (anterior and posterior), free wall of the RV (anterior, lateral and posterior) (**Figure 4**) and longitudinal RV outflow (**Figure 5**).
- H & E and a connective tissue stains are standard also in the myocardium. Moreover, immunohistochemistry should be performed if indicated.
- Other cardiac samples: valve leaflets, atria, pericardium and aorta.

In case of rare cardiomyopathies (mitochondrial, storage, infiltrative, etc.), a small sample (2 × 2 mm) of myocardium should be fixed in 2.5% glutaraldehyde for ultrastructural examination.

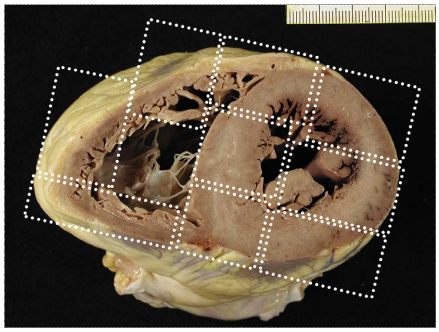

Figure 4. Sampling of the myocardium with several transmural blocks, along with the entire circumference from left ventricle, septum and right ventricle. From [6], with permission.

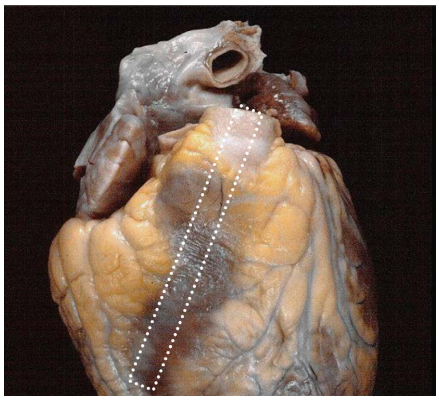

Figure 5. Longitudinal sampling of the right ventricular outflow. From [6], with permission.

1.6. Conduction System

If ECG tracing suggests disturbances of the origin and transmission of the electrical impulse, investigation of the conduction system should be carried out by serial sections technique, removing and including in paraffin two blocks of sinoatrial and av septal junctions [7] (**Figure 6**).

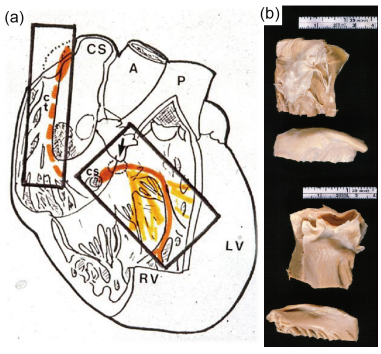

Figure 6. Sino-atrial and atrioventricular septal junctions are removed in single blocks, included in paraffin and submitted to serial sectioning for the study of the conduction system. (a): drawing of the blocks to be sampled: the sino-atrial and atrio-ventricular septal junctions. (b): removed blocks. From [6], with permission.

In case of Wolff-Parkinson-White syndrome, the serial sections investigation should be extended to the lateral left and right rings, in search of accessory pathways.

1.7. Molecular Autopsy

Molecular autopsy aims to study DNA/RNA in the setting of viral myocarditis or genetically determined heart disease [1] [5].

In both circumstances, EDTA blood and ventricular myocardium will be taken, frozen and stored at −80°C or alternatively stored in RNA later at 4˚C, with nucleic acid extraction accomplished through termocycler.

Gene sequencing in fresh tissue can be carried out with success up to 100% of cases [8].

From formalin fixed and paraffin embedded tissue, nucleic acid extraction and gene sequencing is successful up to 85% of cases, if amplicon length is less than 300 bp [8].

1.8. Toxicology

The toxicological investigation aims to understand if any xenobiotic was involved as a cause, contributing-cause or death mechanism [9]. Due to the extremely large panel of xenobiotics that may be responsible of toxic reactions, it is important to perform a systematic toxicological analysis in order to cover volatile substances, alcohol and congeners, basic, neutral and acidic drugs. The panel of drugs should include at least the most common illicit drugs, psychotropic medicinal drugs, doping substances, poisons of vegetal origin.

In case of suspicion, especially on unwitnessed SD or victims found dead at bed, peripheral blood from femoral veins (10 mL), heart blood (10 mL), urine (30 - 50 ml) or bile (20 - 30 ml) (if urine are not available), should be collected in tubes with sodium fluoride as a preservative and stored at −20˚C until toxicologic analysis are performed. Vitreous humor (from both eyes) can also be collected and stored at −20˚C.

One or more locks of hair (100 - 200 mg) should be cut and taken from the back head or from the pubis.

The toxicological analysis should be quantitative and performed in certified referral laboratories [9].

2. Epicrisis and Final Recommendations

A definitive cause of SD can be identified already at gross and histological investigations in the majority of cases.

However, different degrees of certainty exist in establishing the cause-effect relationship between the observed cardiovascular substrate and SD.

The causes of SD should be considered as certain, highly probable or uncertain (**Table 1**). Acute coronary occlusion by thrombosis, embolism or dissection, rupture of dissecting aortic aneurysm with hemopericardium and cardiac tamponade, pulmonary thromboembolism should be regarded certain. If coronary

Table 1. The diagnosis of sudden cardiac death at autopsy may be considered as certain, highly probable and uncertain, modified from [1].

Certain	Highly probable	Uncertain
Massive pulmonary embolism	Stable atherosclerotic plaque with luminal stenosis > 75%, with or without acute or healed myocardial infarction	Minor anomalies of the coronary arteries from the aorta (RCA from the left sinus, LCA from the right without inter-arterial course, high take-off from the tubular portion, LCx originating from the right sinus or RCA, with retroaortic course, coronary ostia plication, fibromuscular dysplasia, intramural small vessel disease)
Hemopericardium due to aortic or cardiac rupture	Anomalous origin of the LCA from the right sinus with intramural and inter-arterial course	Intra-myocardial course of a coronary artery (myocardial bridge)
Mitral valve papillary muscle or chordae tendineae rupture with acute mitral valve incompetence and pulmonary edema	Cardiomyopathies (hypertrophic, arrhythmogenic right ventricular, dilated, others)	Focal myocarditis, hypertensive heart disease, idiopathic left ventricular hypertrophy
Acute coronary occlusion due to thrombosis, dissection or embolism	Myxoid degeneration of the mitral valve with prolapse, ventricular fibrosis and intact chordae	Myxoid degeneration of the mitral valve with prolapse, without atrial dilatation or left ventricular/papillary muscles fibrosis and intact chordae
Anomalous origin of the coronary artery from the pulmonary trunk	Aortic stenosis with left ventricular hypertrophy	Dystrophic calcification of the membranous septum (±mitral annulus/aortic valve)
Neoplasm/thrombus obstructing the valve orifice	ECG documented ventricular pre-excitation (Wolff-Parkinson-White syndrome, Lown Ganong Levine syndrome)	Atrial septum lipomatosis
Thrombotic block of valve prosthesis	ECG documented sino-atrial or AV block	AV node cystic tumor without ECG evidence of AV block, conducting system pathology without ECG documentation
Laceration/dehiscence/poppet escape of the valve prosthesis with acute valve incompetence	Operated congenital heart diseases	
Massive acute myocarditis	Not-operated congenital heart diseases, with Eisenmenger syndrome	

atherosclerosis stenosis exceeds 75% degree, the cause may be considered highly probable.

Coronary artery congenital anomalies like left circumflex arterial branch from the right coronary artery-right aortic sinus with retroaortic course or myocardial bridge should be regarded as uncertain, since they may be variants of the normal. In these circumstances, arrhythmic diseases like ion channel mutations should be always excluded through molecular investigation and illicit substance should be ruled out by toxicological examination.

In the case of unlikely or uncertain causes, the case should be evaluated on in-

dividual level since the clinical history and the circumstances of death may help to achieve the final diagnosis.

Finally, there are "gray zones" in which the border between physiologic and pathologic changes is poorly defined, like fatty tissue of the right ventricular free wall vs arrhythmogenic cardiomyopathy, athlete's heart versus hypertrophic cardiomyopathy, focal inflammatory infiltrates versus overt myocarditis.

Finally, extensive photographic documentation should be carried out with precise indication where blocks were taken from. Moreover, send the entire heart to specialized centers or preserve the heart, for leaving the possibility of a second opinion.

3. Declarations Section

Ethical Approval and Consent to participate: In Italy autopsy procedure is considered a regular laboratory investigation to establish the cause of death, which does not require family consent and is left to discretion of the doctor. Moreover, in case of suspicion of unnatural death, the autopsy is ordered by judicial authority.

Human Ethics: the need of family screening, to identify genetic diseases, is clearly highlighted.

Consent for publication: Publication is a self-decision of the authors, to make public the results of research plans.

Availability of supporting data: The contents of the paper derived from a longstanding experience of our group on pathology of sudden death, dating back 1980.

Competing interests: The authors declare no conflict of interest.

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Authors' contributions:

1) conceived and designed the experiments (G. Thiene, D. Favretto);

2) performed the experiments (G. Thiene, D. Favretto);

3) analyzed and interpreted the data (G. Thiene, D. Favretto);

4) contributed reagents, materials, analysis tools or data (Department of Cardiac, Thoracic, Vascular Sciences and Public Health—University of Padua);

5) wrote the paper (G. Thiene, D. Favretto).

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Agreement to Conditions

All authors of the manuscript have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript.

The submitted article is original work that is not being considered or reviewed by any other publication and has not been published elsewhere in the same or a

similar form.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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