THE ROLE OF MOLECULAR AUTOPSY IN 2014: FROM THE ANATOMICAL THEATRE TO THE DOUBLE HELIX

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The Role of the Autopsy in SD

To establish or consider:

- whether the death is attributable to a **cardiac disease or to other causes** of sudden death;
- the **nature of the cardiac** disease, and whether the mechanism was arrhythmic or mechanical;
- whether the cardiac condition causing sudden death may be **inherited**, requiring screening and counselling of the next of kin;
- the possibility of **toxic or illicit drug** abuse and other unnatural deaths.
1594, a permanent anatomical theater is built, first Lab in the history of medicine

William Harvey. 
*Exercitatio Anatomica De Motu Cordis et Sanguinis in Animalibus, 1628*
“We will state that it is impossible to pursue the nature and cause of any disease without dissection of the respective cadavers”

DAWN OF ORGAN PATHOLOGY
... And here, with the name of sudden death we mean the death that, expected or unexpected, kills the man abruptly...

Giovanni Battista Morgagni. De Sedibus et Causis Morborum per Anatomen Indagatis, 1761
The Autopsy Procedure

Full autopsy with sequential approach to the causes of SD

- Exclusion of non-cardiac causes of sudden death
- Any natural sudden death can be considered cardiac in origin after the exclusion of non-cardiac causes. Thus, a full autopsy with sequential approach should be always performed to exclude common and uncommon extra-cardiac causes of sudden death, especially:
  
  - Cerebral (e.g. sub-arachnoid or intra-cerebral haemorrhage, etc)
  - Respiratory (e.g. asthma, anaphylaxis, etc)
  - Acute haemorrhagic shock (e.g. ruptured aortic aneurysm, peptic ulcer, etc)
  - Septic shock (Waterhouse–Friderichsen syndrome)
Mechanisms of Sudden Death in 300 Consecutive Cases (≤35yrs)

- Cardiovascular: 91%
- Cerebral: 5%
- Respiratory: 4%

Legend:
- Yellow: Cardiovascular
- Orange: Cerebral
- Teal: Respiratory
Pathophysiology of Sudden Cardiac Death

- **93%** Arrhythmic
- **7%** Mechanical

**Typical sequence of electrical events:**
- Sinus rhythm
- Ventricular tachycardia
- Ventricular fibrillation
- Asystole
The Autopsy Procedure

Search for the Culprit

Leonardo, 1510
Sudden Cardiac Death in the Young
The substrate should be searched in the:

- Aorta
- Coronary arteries
- Myocardium
- Valves
- Conduction system
- Ion channels
Referral of hearts to specialised centres

Best practice is that the entire heart is retained and sent to specialized centres. The referring pathologist should complete steps 1–5 of the standard gross examination of the heart, make a transverse apical section of the heart and empty the heart of blood.

Tissues, blood and other fluids for toxicology and molecular pathology should be taken before fixing the heart in formalin 10% (see “Molecular pathology” below). If the heart cannot be retained, it is essential that extensive photographic documentation is made, indicating where individual blocks are taken.
Myocardial sample to be frozen -80°
Molecular studies of SCD include both detection of viral genomes in inflammatory cardiomyopathies, and gene mutational analysis in both structural and nonstructural genetically determined heart diseases.

For these purposes, **10 ml of EDTA blood and 5 g of heart and spleen tissues are:**

- either *frozen and stored at −80°C,*
- • or alternatively *stored in RNA later at 4°C* for up to 2 weeks
Toxicology Investigation

For the purpose of SD investigation, the following amounts are adapted from the Guidelines of the Society of Forensic toxicologists and the American Academy of Forensic Sciences: heart blood 25 ml, peripheral blood from femoral veins 10 ml, urine 30–50 ml, bile 20–30 ml (when urine is not available).

All samples are stored at 4°C.

A lock of hair (100–200 mg) should be cut from the back head (or from the pubic hair when head hair is not available). Toxicological analyses should be quantitative.
Genetic, Potentially Recurrent Cardiac Diseases at Risk of SD in the Young (<35 yrs)

- Arrhythmogenic RV cardiomyopathy
- Hypertrophic cardiomyopathy
- Dilated cardiomyopathy
- Marfan syndrome
- Supravalvular aortic stenosis
- Long and short QT syndrome
- Brugada syndrome
- Effort polymorphic ventricular tachycardia

~30-40%

**ARVC 10%**
Arrhythmogenic RV Cardiomyopathy: A DESMOSOMAL DISEASE

Basso C et al. Lancet 2009;373:1289-300
Sudden Cardiac Death and Normal Heart

- Long QT
- Short QT
- Brugada syndrome
- Catecholaminergic Polymorphic VT

Normal heart 17%
CARDIAC ION CHANNELS
Molecular Autopsy in SCD

• Fresh tissue RNA later
• Autoptic blood in EDTA
• Frozen tissue

Nucleic acid extraction up to 100%
Amplicon length >300bp
Molecular investigation good (PCR, dHPLC, Sequence)

Formalin fixed and Paraffin embedded tissue (FF-PET)

Nucleic acid extraction up to 85%
Amplicon length <300bp
Molecular investigation hard (PCR, dHPLC, Sequence)

UNEXPLAINED DEATH AND JUVENILE SCD

**RyR2**

14 yr old female
SD while swimming
*Clinical history: none*
*Family history: positive*
*Autopsy: normal heart*

**DNA extraction**
NEGATIVE

**FF-PET**
Unexplained SD in a 16 year old boy

**Ryr 2 Gene Mutations**

**CASE 2-04**

Frozen tissue DNA extraction POSITIVE

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Family 169

G→C Ala→Pro
Tester & Ackerman, J Am Coll Cardiol 2007
“...Given the observation that one-third of these cases are of genetic origin, it seems no longer justifiable to ignore genotyping in these victims of SD. To find a disease-causing mutation will enable a rapid screening of all relatives; this should identify those who carry the same mutation as the victim. This approach should now become part of the routine postmortem study of SUD cases”
...Any anatomic modification is material, but is any material modification anatomic? Why not molecular? Can a profound molecular modification occur in the setting of an apparently normal structure? These modifications belong more to physiology than to anatomy, they are functional-dynamic...

Many phenomena are merely functional in nature and when you try to explain them mechanistically, on the basis of subtle molecular changes, the method of investigation will never be morphological...

R. Virchow, *Morgagni and the Anatomic Concept, 1894*
Conclusions

• The study of SCD moved from classical postmortem dissection to molecular autopsy. However the game is still played in the anatomical theater, “the place where death enjoys to save lives”

• SCD prevention has to be approached by an interdisciplinary team (cardiologist, geneticist, pathologist), the Morgagni clinic-pathologic correlation being the polar star