Latest Research on Forensic Science

Chapter 1

Negative Autopsy: Post-Mortem Genetic Diagnosis of Inherited Arrhythmogenic Syndromes

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Abstract

Negative autopsy is a complete post-mortem examination without a definite cause of death. A low percentage of autopsies remain as negative but most part of these cases usually occurs in infants and young population who died suddenly and unexpectedly. In these concrete situations, an arrhythmogenic event is highly suspected as most probable cause of sudden death. Therefore, sudden death is a natural and unexpected decease that occurs in apparently healthy individuals, or whose disease was not severe enough to expect a fatal outcome. This lethal event can be due to several pathologies, usually malignant cardiac arrhythmogenic (sudden cardiac death). Nowadays, a 20% of all sudden cardiac death is of genetic origin, being a result of an inherited arrhythmogenic disease. Therefore, a comprehensive genetic analysis (molecular autopsy) is highly recommended despite no regularly performed. Unfortunately, sudden cardiac death could be the first manifestation of any of these arrhythmogenic diseases. Early identification of a genetic alteration associated with the disease may help to adopt preventive personalized measures to reduce risk of malignant arrhythmias and sudden death. In recent years, technological improve in genetic diagnosis has allowed a comprehensive and cost-effective genetic diagnosis, helping to unravel origin of arrhythmogenic diseases. Nowadays, the main challenge is the interpretation of the large amount of data originating after a genetic analysis. This chapter focuses on molecular autopsy using new genetic technology in malignant inherited arrhythmogenic syndromes.

Keywords: Post-mortem; Sudden Cardiac Death; Genetics; Molecular Autopsy

1. Introduction

An autopsy can be defined as a post-mortem examination of a deceased person to unravel the time and cause of death. Concerning cause of death, the result is not conclusive (negative autopsy) in nearly 5% of all autopsies after a comprehensive analysis of all data recompiled [1]. The sudden death of an apparently healthy individual occurred within an hour of symptom onset or within 24 hours from the last time the person has been seen alive is defined "*sudden unexplained death*" if at the autopsy no cause of death is found [2]. In these concrete circumstances, an arrhythmogenic inherited disease is the most plausible cause of death [3], mainly in young and infant population [4]. In recent years use of massive genetic sequencing technologies has allowed cost-effectives analysis of large number of genes in a reduced time. These genetic approaches have identified nearly 25% of deleterious alterations in genes

encoding ion channels or associated proteins responsible for proper electrical transmission through myocardium [5]. Current clinical guidelines recommend molecular autopsy in these cases [6]. Despite this fact, post-mortem genetic testing is not widely performed in most of the countries, mainly due to economic reasons or lack of protocol implementation in collection of post-mortem samples due to currently legal restrictions involved with the sampling and storage of DNA [7]. Genetic analysis and personalized genetic interpretation of rare variants identified may help to unravel cause of death but also identify relatives at risk, who can be asymptomatic. Unfortunately the first manifestation of any of inherited arrhythmogenic syndromes may be the sudden death thus early identification help to adopt measures focused on prevention of lethal episodes.

2. Inherited Arrhythmogenic Syndromes

Currently, nearly 85% of all sudden deaths are of cardiac origin (sudden cardiac death, SCD) being responsible for around 30-200/100.000 every year [8]. In population > 50 years old, nearly 80% of all SCD cases are consequence of ischemic/coronary disease [9], but in the population <35 years old, the main cause of SCD is inherited arrhythmias of genetic origin [10]. Inherited arrhythmias can be divided in two groups of diseases: cardiomyopathies which are characterized structural heart alterations and caused by genetic alterations in genes encoding structural proteins; second group are channelopathies or purely electrical disease, characterized by a normal heart and caused by genetic alterations in genes encoding ion channels or associated proteins [11]. These both groups of arrhythmogenic diseases predispose to disruption of electrical activity, leading to ventricular fibrillation, syncope and SCD.

In cardiomyopathies, the lethal arrhythmia is induced by structural abnormalities. These structural alterations may be macroscopic or microscopic due to cardiomyopathies are progressive diseases and structural alterations are first at microscopic level and progress to gross heart alterations. Thus, it is not rare that a comprehensive autopsy may identify alterations at microscopic level but not in anatomic examination. Curiously, both in infants and young population died suddenly, some studies identified rare genetic alterations associated with any of cardiomyopathies but not showing cardiac alterations, neither macroscopic nor microscopic [12]. It could be explained because of the first structural alterations occurs at ultramicroscopic level (identified using only electronic microscopic, not included in current protocols of autopsy). The electrical disturbance prior to malignant arrhythmia could be induced by these ultra-structural alterations. Hence, negative autopsy of infant and young population carrying alterations in genes encoding cardiomyopathies should not be discarding without a comprehensive analysis [13].

Concerning group of purely arrhythmogenic diseases without any heart defect (channelopathies), four main diseases are responsible of most part of SCD: Brugada syndrome

-BrS-, Long QT syndrome -LQTS-, Short QT syndrome -SQTS- and Catecholaminergic Polymorphic Ventricular Tachycardia -CPVT- [14]. Here we will focus mainly in these four arrhythmogenic diseases due to main cause of cases classified as negative after a comprehensive medico-legal autopsy.

3. Long QT Syndrome

This arrhythmogenic disease is characterized by prolongation of the QT interval on 12-lead ECG (QTc >460ms in women and >450ms in men). Clinical manifestations in patients may range from asymptomatic to syncope or cardiac arrest due to *torsade de pointes* (TdP) in a structural normal heart. Unfortunately SCD can be the first manifestation of the disease, especially in infants and young population. Clinical events may be precipitated by specific triggers, including exercise, swimming, emotional stress or sudden loud noises [15]. A negative autopsy of a case died suddenly at young age could be caused by this arrhythmogenic entity. Concerning treatment, administration of beta-blocker is highly recommended because it decreases the risk of SCD although do not provide full protection [6]. Implantation of an ICD is mandatory for those patients who are survivors of an aborted SCD or in patients with a diagnosis of LQTS who experience recurrent syncopal events while on β-blocker therapy [16]. Left cardiac sympathetic denervation (LCSD) should be considered in high-risk patients with symptomatic LQTS in whom β-blockers are ineffective or not tolerated and in patients with recurrent appropriate ICD shocks despite maximum tolerated doses of β-blockers [17]. Currently, more than 1000 genetic alterations have been identified in 25 genes (**Figure 1**). An exhaustive genetic analysis of all reported genes identifies the cause of the disease in nearly 85% of cases. However, 80% of cases carry the genetic alteration in 3 main genes: *KCNQ1, KCNH2*, and *SCN5A* [18]. Hence, current guidelines recommend perform a genetic analysis of these 3 main genes in LQTS cases as the most cost-effective approach [6].

4. Brugada Syndrome

This inherited arrhythmogenic entity is characterized by a coved-type ST-segment elevation in atypical right-bundle branch block in leads V1 to V3 (often referred to as type-1 Brugada pattern) in the ECG [6]. The ECG pattern can be baseline or intermittent, and it can be unmasked during a drug test using class IC sodium channel-blockers. It is considered a disorder involving mainly young male adults (about 40 years old), and SCD typically occurring during sleep or at rest. Patients with BrS usually remain asymptomatic and modulating factors such as fever, exercise or drugs may play a major role in the dynamic nature of the ECG pattern, increasing risk for SCD resulting from episodes of polymorphic ventricular tachyarrhythmias [19]. After surviving a cardiac arrest or the occurrence of syncope, the only treatment having any proven effect on the prevention of SCD is the ICD [20]. However, ICD implantation in symptomatic is not free from controversy, especially in children [21]. A negative autopsy of a

young man died at night could be caused by this arrhythmogenic entity. Nowadays, 28 genes have been potentially associated to BrS (Figure 1) but pathogenic or likely pathogenic variants have been reported only in *SCN5A* or associated sodium proteins [22]. A comprehensive genetic analysis identifies the cause of disease in a 35% of cases, and nearly 30% of patients carry the genetic alteration in *SCN5A*. Hence, current guidelines recommend perform a genetic analysis of only *SCN5A* in BrS cases as the most cost-effective approach [6].

5. Short QT Syndrome

This is a highly lethal arrhythmogenic disease characterized by a structural normal heart with a shortened QT interval (QTc < 340 ms), tall and peaked T waves, and poor rate adaptation of the QT interval. In addition, this entity can be also diagnosed with a QTc interval of <360ms with at least one of: (a) a definite pathogenic alteration; (b) a family history of SQTS; (c) a family history of SCD at age <40 years old, and (d) survival from a VT/VF episode in structural normal heart[6]. Clinical manifestations may range from lack of symptoms to syncope and even SCD, sometimes the first symptom of the disease. SQTS usually occurs in young population and even infants, being considered main cause of death in first year of life (Sudden Infant Death Syndrome, SIDS) [23]. A negative autopsy of an infant case died suddenly could be caused by this malignant entity. An ICD is used to prevent SCD in high risk patients. In patients with relatively benign phenotype, an individualized pharmacological measure may be use if ICD is not implanted, such as Quinidine or Hydroxyquinidine [24]. Until now, no more than 50 pathogenic or likely pathogenic alterations have been associated with SQTS. These alterations are located in 8 genes (*KCNQ1, KCNJ2, KCNH2, CACNA1C, CACNB2, CACNA2D1, SLC4A3,* and *SCN5A*) (**Figure 1**), mainly following an autosomal dominant pattern of inheritance [25]. A comprehensive genetic analysis identifies the genetic alteration in 45% of clinically diagnosed cases, being most part located in 3 genes encoding potassium channels: *KCNQ1, KCNJ2*, and *KCNH2* [26]. Hence, current guidelines recommend perform a genetic analysis of these 3 genes in SQTS cases as the most cost-effective approach [6].

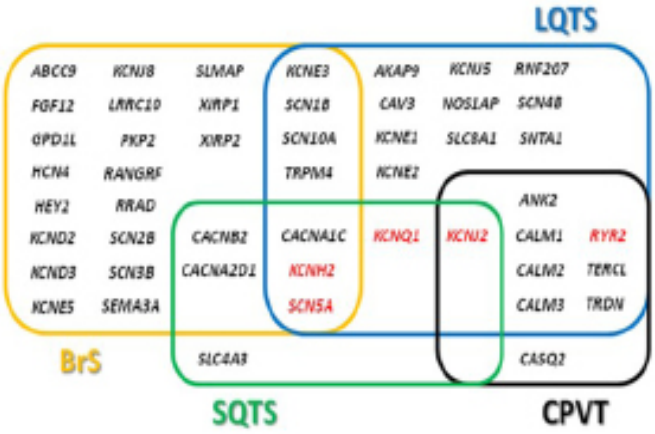

Figure 1: Genes currently associated with channelopathies. In red colour, five main genes associated with inherited channelopathies. BrS: Brugada Syndrome; CPVT: Catecholaminergic Polymorphic Ventricular Tachycardia; LQTS: Long QY Syndrome; SQTS: Short QT Syndrome.

6. Catecholaminergic Polymorphic Ventricular Tachycardia

This is a lethal inherited entity characterized by a 2-way polymorphic ventricular tachycardia in a structural normal heart [27]. The ECG is normal at rest (infrequently with bradycardia and U waves), and triggered exclusively by adrenergic stimulus, mainly exercise, excessive stress or emotion. CPVT is a main cause of unexplained SCD in young population (<40 years old and predominately males) [28], mainly in adolescents and children before 10 years old, and not rarely the first symptom of the disease [29]. Hence, a negative autopsy in a young patient died suddenly during adrenergic situation could be due to CPVT. Administration of beta-blockers is the first therapeutic approach and ICD is indicated for patients with aborted SCD or incompletely controlled CPVT despite a high dose of medications [30]. Concerning genetics, more than 200 pathogenic or likely pathogenic alterations have been reported so far in 9 genes (*ANK2, CALM1, CALM2, CALM3, CASQ2, KCNJ2, RyR2, TERCL* and *TRDN*) (Figure 1) mainly following an autosomic dominant pattern of inheritance. A comprehensive genetic analysis of all reported genes explains 65% of CPVT cases but nearly 55% are due to genetic alterations located in the *RyR2* gene [31]. Hence, current guidelines recommend perform a genetic analysis of only this gene in CPVT cases as the most cost-effective approach [6].

7. Medico-Legal Implications

When a person of any age suddenly dies, autopsy is a crucial step of clinical and forensic investigations both when it allows to macroscopically or/and microscopically identify the cause of the death (e.g. a myocardial infarction) and when it is completely or substantially negative [32]. In the latter scenario, the absence of relevant findings at the autopsy indicates in the first instance the need of toxicological testing to exclude common causes of death that are not usually associated to specific macroscopic or microscopic findings (e.g. opioid overdose).

When neither toxicological result is indicative, the pathologist should consider the possibility of a molecular autopsy to evaluate the presence of genetic variants considered pathogenic for arrhythmogenic syndromes. This process is conceptually extremely similar to the diagnostic algorithms used by clinicians to determine if a specific somatic complaint is more probably caused by an organic or a psychiatric disorder (the so-called "*diagnosis per exclusionem*").

In details, when can a molecular autopsy be considered if the autopsy fails to identify a reliable cause of death? This question is extremely relevant: it is complex to exactly define when an autopsy can be considered "negative" and thus when a molecular autopsy could be a valuable option. Indeed, anomalies of uncertain significance are often found (e.g. myocardial bridging): in these cases, the indication to molecular autopsy is still debated. However, when signs typical (but non diagnostic) of cardiomyopathies are found, molecular autopsy should be

carefully considered, because, as said in the previous paragraph, in children the macroscopic and microscopic signs of these diseases often are fewer and less evident and in athletes reliably differentiating at the autopsy the so-called "*athlete's heart*" from a mild hypertrophic cardiomyopathy is not always easy/possible [33] . Moreover, when absolutely no anomalies are found, post-mortem genetic testing is certainly a valid choice, because, for example, channelopathies-caused deaths are associated with no microscopic/macroscopic finding (in the future, if recent pioneering evidence will be confirmed, we should add to this sentence: with the potential exception of BrS) [34]. Molecular autopsy can be also recommended in the sudden unexplained deaths of epileptic patients, especially if they presented risk factors for SUDEP (Sudden Unexpected Death in Epilepsy) [35-37].

Why is the correct indication of molecular autopsy considered a key issue? In the first place, because is strategically for public health [38]. Indeed, if a variant pathogenic for arrhythmogenic syndromes found at post-mortem genetic testing is proved to be also carried by victim live relatives, in these patients early preventive measures can be started, making possible to reduce the risk of impairments and deaths (and thus relevant costs for the national health services). Moreover, molecular autopsy is often crucial in criminal/legal investigations [39]. For example, sudden deaths of professional athletes is a well-known issue, and in these cases it is not rare that cardiologists of sports clubs are claimed to be liable for missed diagnoses (medical negligence) [40]. When it happens, molecular autopsy is often the only way to demonstrate that the athlete suffered from an arrhythmogenic syndrome. Obviously, for the clinical issues cited in the previous paragraphs, in legal terms, proving the presence of these diseases does not automatically mean that it would have certainly been possible to make diagnosis of the disease before the athlete death.

8. Genetic Analysis

Sanger technology opened the field of genetic sequencing nearly 45 years ago. Nowadays Sanger sequencing is used due to high fidelity but is slow and expensive. Fifteen years ago, massively parallel sequencing (also called Next Generation Sequencing, NGS) arrived into research laboratories. The NGS technologies allowed massively parallel sequencing in a cost-effectives way in comparison to traditional Sanger sequencing. In last 10 years, NGS has been progressively incorporated to clinical diagnosis. Sanger sequencing continue to be used as a complementary strategy to sequence complex genomic regions and also to validate new NGS findings. Despite NGS technology continuously improve fidelity of genetic variants identified, nowadays Sanger help to unravel a percentage of false positive variants detected by NGS, mainly insertion/deletion sequences (*indels*).

Current massive sequencing approaches, such as personalized panels of genes associated with a specific disease, whole exome sequencing (WES) and even whole genome sequencing

(WGS) are increasingly being used more and more in genetic field. For genetic diagnosis, only panels have been used regularly. Both WES and WGS approaches are used mainly for research proposes. It is important to remark that some hospitals and centres of genetic diagnosis use WES approach but final report is only performed focused on a list of concrete genes associate with the suspected or diagnosed disease, in the same way that occurs using a panel. Nowadays, the cost of a personalized panel of genes, WES and WGS is similar (nearly 1.000 Euros). The current challenge is interpretation of data obtained after genetic analysis. Most part of alterations remains as ambiguous significance and an exhaustive analysis of each variant should be performed before translation into clinical practice [41-43]. Before clinical translation, a group of experts in each area should discuss the role of variants included in the final report due to it may alter the personalized therapeutic measures adopted in each patient. It is crucial for unravel cause of disease or death in a patient but if inherited disease is suspected, also genetic analysis help clinicians to adopt preventive measures in relatives at risk. Concerning family, all relatives of unexplained sudden death individual should undergo assessment by a multidisciplinary team [44] because of the investigation of an unexplained decease is extremely complicated in the families of the victims, mainly when the victim is a child or infant [45,46].

The NGS technology has been also incorporated in forensics in last 5-10 years, allowing a comprehensive post-mortem genetic analysis (called molecular autopsy). Recent studies showed that molecular autopsy using NGS has identified the cause of the death in a large part of cases with a no conclusive cause of death after comprehensive autopsy [47]. Current arrhythmogenic guidelines recommend use of molecular autopsy in young cases classified as negative after a complete autopsy [6]. Current NGS protocols require a suitable DNA quality and quantity. The best approach is obtaining DNA from post-mortem blood (no more than 48 hours after death), and conserved at 4ºC for no more than 4-5 days. If more days until extraction are necessary, the best approach is freezing at -20ºC; defrosting should be done progressively before DNA extraction (from -20ºC to 4ºC and finally room temperature). This process could break DNA if it is done too quickly. DNA from post-mortem tissue can be also an option but the tissue should be frozen immediately after extraction (at -80ºC or even liquid nitrogen –LN_2- if proper storage is available) and should be store at least at -20ºC. In this situation, defrosting should be also done progressively before DNA extraction. In current protocols of autopsy, at least a formalin-fixed, paraffin-embedded (FFPE) of tissue is obtained for histological examination. Currently, DNA from FFPE is not yet an option due to formalin treatment breaks DNA and no proper amplification is possible for NGS technology. However, DNA from FFPE can be used for amplification of a limited number of exons using Sanger sequencing. New NGS protocols allows perform a massive genetic analysis using minimum quantity of DNA with certain degradation,

9. Conclusions

Sudden death remains as a main problem due to the first manifestation of the disease can be the decease. After a comprehensive autopsy, large part remains classified as negative. In infant and young population, this lethal event usually is due to cardiac arrhythmias with a genetic origin. Therefore, family members can be at risk. Early identification may help to adopt preventive measures. However, molecular autopsy is not performed in all forensic centers and large percentage of cases remains without a definite cause of death. Use of new genetic technologies allows a comprehensive cost-effective analysis of these families. Current challenge is proper interpretation of genetic data. This fact requires sustained and close collaboration between research labs and clinical practitioners for before clinical translation.

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