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Clinical Case Report

Sudden fetal death due to dualism of the sino-atrial node

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ABSTRACT

First, we report a sudden fetal death at 33^{+3} weeks due to sino-atrial node dualism. The female stillborn was delivered by induced labor. The postmortem examination of the cardiac conduction system revealed a dualism of the sino-atrial node, associated with fragmentation of the atrio-ventricular node and His bundle. These abnormalities of the cardiac conduction system represent the morphological substrate for the development of malignant arrhythmias. In particular, the dualism of the sino-atrial node can cause the dissociation of the longitudinal nodal impulse into two distinct ways of different pulse generation, resulting in supraventricular tachyarrhythmias. This observation suggests new avenues of research on the pathogenesis of the sudden unexpected fetal death. Moreover, our findings confirm the need for an accurate postmortem examination, including serial sectioning of the cardiac conduction system, in every case of unexplained fetal death, following standardized autoptic protocols.

The expression "sudden intrauterine unexplained death syndrome" (SIUDS) has been introduced to describe those fetuses that suddenly and unexpectedly die with no explained cause after the 25th gestational week, before complete expulsion or extraction of the fetus from the mother. This event results in a stillbirth that remains unexplained after a routinely postmortem examination, in a similar manner to that observed for the "sudden infant death syndrome" (SIDS) [1].

In developed countries, 1 out of 100–200 apparently problem-free near-term pregnancies ends in stillbirth, which has a six- to eightfold greater incidence than SIDS [2]. Such deaths remain unexplained in 40–80% of cases after a routine autopsy, including fetal adnexa examination [3,4]. The frequency of this form of death has not significantly decreased in the last 20 years, despite to the modern advances in maternal–infant care [4]. This is particularly due to the scarce and fragmentary anatomo-pathological research. SIUDS and SIDS represent a great enigma and one of the main open issues in the social and scientific setting of the modern medicine. The emotional consequences among victims' families are devastating, and the cost of psychological support programs and/or adaptation therapies is significantly heavy.

In fetuses, the cardiac conduction system, like other structures, can be abnormally developed, but so far, the study of the conducting tissue through serial sections has never been considered, except by the *Lino Rossi* Research Center of the University of Milan [5–7], according to the Italian Law n. 31 "*Regulations for Diagnostic Post-Mortem Investigation in victims of the Sudden Infant Death Syndrome (SIDS) and Unexpected Fetal Death*" [8]. This law states that all infants who suddenly died within the first year of age, suspected for SIDS, and all fetuses died after the 25th week of gestation, without any apparent cause, must undergo an in-depth anatomo-pathological examination, particularly of the cardiac conductions system and autonomic nervous system.

The Autonomous Province of Trento has been the first to understand its importance and to apply these guidelines, establishing in 2012 a convention with the *Lino Rossi* Research Center for the application of the Law n. 31 [9]. The case herein described precisely comes from the Autonomous Province of Trento and, to the best of our knowledge, represents the first reported observation of a sudden fetal death due to sino-atrial node (SAN) dualism.

A female fetus was found suddenly and unexpectedly dead in utero at the 33rd week of gestation plus 3 days. The mother, a 37-year-old woman, had been admitted to the emergency room for the absence of fetal movements. The diagnosis of intrauterine fetal death was confirmed, and the patient was transferred to the obstetric unit for delivery induction by prostaglandins. The pregnancy had been complicated by two episodes of threatened abortions at the 10th and 20th gestational weeks, resolved with antispasmodic treatment. Ultrasound showed a

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focal area of detachment of the chorionic plate from the underlying decidua, measuring 12×5.8 mm. The medical history of the mother included an elective abortion, a spontaneous abortion, and a previous uncomplicated delivery of a healthy firstborn child 5 years earlier.

A necropsy examination was requested because of the clinical suspicion toward SIUDS. After the general autopsy, the cause of death remained unknown. The case was so submitted, according to the aforementioned convention, to the *Lino Rossi* Research Center of the University of Milan, where more in-depth examinations were performed. Such examinations were implemented following the available investigation guidelines for unexpected infant and perinatal death [10], including, in particular, the deep assessment of the cardiac conduction system and brainstem centers, checking the vital functions. Moreover, a fresh specimen of the cerebral cortex was required for molecular analysis of the serotonin transporter gene (5-HTT) polymorphism.

A complete autoptic examination was performed including a systematic gross and microscopic evaluation of the body, placental disk, umbilical cord and membranes. Multiple samples of all organs were fixed in formaldehyde, processed and embedded in paraffin; 5-μm thick sections were stained with hematoxylin and eosin (H&E).

According to the protocol of the *Lino Rossi* Research Center, the heart was weighed and measured, and the values were compared with the normal values for fetuses of the same gestational age. After having grossly excluded cardiac malformations, the origins of the coronary arteries were carefully inspected. The heart structures were routinely examined for pathological changes in atria, septa, ventricles, pericardium, endocardium, and coronary arteries. Multiple samples of the myocardium and major coronary arteries (left main, left anterior descending, left circumflex, right main, right posterior descending and right marginal) were fixed in formaldehyde, embedded in paraffin, and stained with H&E and Heidenhain's trichrome (Azan stain). In particular, the histopathological observations were focused on the cardiac conduction system, which was removed in two blocks. The first block, or sino-atrial block, contained the junction of the superior vena cava and right atrium encompassing the entire area of the SAN. The second block, or atrio-ventricular junctional block, contained the atrio-ventricular node (AVN), the His bundle, its bifurcation, and the bundle branches located in the superior two thirds of the interventricular septum (VS). The sino-atrial block was serially sectioned in a plane parallel to the *crista terminalis*. The atrio-ventricular junctional block was serially sectioned in a plane parallel to the two atrio-ventricular valve rings. All sections were cut at intervals of 40 μm (levels). For each level, five sections were retained, mounted, and stained alternatively with H&E and Azan. All intervening sections were kept and stained as deemed necessary.

Normally, the examination of the brainstem, according to the guidelines, includes sampling of three blocks for the specific study of the main vital centers. The first specimen, ponto-mesencephalic, includes the upper third of the pons and the adjacent portion of the mesencephalon. The second extends from the upper portion of the medulla oblongata to the adjacent caudal portion of the pons. The third specimen extends 2–3 mm above and below the obex. However, this procedure was not applied in this case, given the advanced state of maceration of the brainstem.

The stage of the lung development was evaluated on the basis of a microscopic criterion that is the radial alveolar count (RAC) [11]. This parameter is obtained examining at least 10 high power fields to estimate the number of alveoli transected by a perpendicular line, drawn from the center of the most peripheral bronchiole to the pleura or the nearest interlobular septum [11].

According to Heils et al. [12], the 5-HTT polymorphism was genotyped using specific primers. Polymerase chain reaction (PCR) was carried out in a final volume of 50 μl consisting of approximately 50-ng genomic DNA. Amplification was performed using: 20 μl of genomic DNA, 1 × PCR Gold Buffer (Applied Biosystems, Foster City, CA, USA), 2-mM MgCl2, 0.4-mM dNTPs, 50 ρmol each of the required primers, and 2-U AmpliTaq Gold (Applied Biosystems). Temperature cycling was performed using an Applied Biosystems 2720 Thermal Cycler with 10 min at 95 °C, followed by 40 cycles at 95 °C for 1 min and 61 °C for 10 min, with a final extension for 7 min at 72 °C. PCR amplification products were electrophoresed through a 1.5% agarose gel and visualized by UV-light in the presence of ethidium bromide.

The fetus showed common changes of advanced maceration and autolysis, such as redness of the skin and dermo-epidermal slippage, due to retention in utero longer than 48 h after the death. The stillborn was described as a well-developed, well-nourished white female fetus, with a body weight of 1940 g, a crown-rump length of 34 cm, a rump-heel length of 18 cm, a head circumference of 30 cm, a chest circumference of 29 cm, and an abdominal circumference of 26 cm.

The macroscopic examination did not reveal malformations, organ malpositions, or alterations of the fetal adnexa. The heart weighed 14 g; the cardiac diameters were: longitudinal 3.2 cm, transverse 3 cm, antero–posterior 1.2 cm. The heart dissection showed a brownish and homogeneous appearance of the myocardium, resulting from congestion and stasis. The coronaries were within the norm in relation to the gestational age.

The complete examination of the cardiac conduction system, by using serial sections, pointed out the presence of two distinct SANs on separate sides of the right atrium (Fig. 1). The first SAN was located in the superior third of the *crista terminalis*, directed along its greater axis, in the back wall of the right atrium (Fig. 1A), while the second SAN was located close to the right auricula (Fig. 1C); both of them were centered by the SAN artery. The two SANs consisted of small and round normal nodal cells, assembled in groups, primarily located at the center of the node, with scarce myofibrils and cytoplasmic granules, and of slender transitional cells, mainly distributed in the peripheral half of the node with a longitudinal orientation (Fig. 1, B and D).

The AVN and the His bundle were fragmented and dispersed. Islands of junctional conduction tissue in the central fibrous (CF) body were also observed (Fig. 2). The bifurcation of the His bundle was normally formed and located on the left side of the septal crest. The bundle branches were unremarkable. The fetal brain was very soft, semiliquid, and friable. Wherever recognizable, the cerebral and cerebellar cortex presented normal level of maturity in relation to the gestational age. The left lung weighed 18 g, while the right 21 g. The microscopic examination disclosed a mild pulmonary hypoplasia with an RAC index of 3.0 (normal RAC for the gestational age>3.2). Subpleural petechiae were also observable, but no other significant pathological change was found.

The molecular analysis for the polymorphic region of the serotonin transporter gene demonstrated that the fetus was homozygous for the short allele (S) of the 5-HTT gene (S/S genotype).

The pathogenesis of SIUDS and SIDS is largely unknown; our previous investigations on the cardiac conduction system have discovered important anatomo-pathological findings, likely representing the morphological substrate of the unexpected death in infants and fetuses, such as impaired resorptive degeneration, AVN or His bundle dispersion or septation, accessory pathways, mainly Mahaim fibers, and cartilaginous metaplasia of the CF body [5,7]. However, the cardiac conduction system in fetuses has been still poorly examined from a morphological and functional point of view.

The abnormalities of the cardiac conduction system, described in the present case, represent the morphological substrate for the development of malignant arrhythmias. In particular, the SAN dualism can cause the dissociation of the longitudinal nodal impulse into two distinct ways of different pulse generation, resulting in supraventricular tachyarrhythmias (SVT). SVT is an abnormal heart rhythm arising from improper electrical activity of the heart and originating at or above the AVN. It can be due to two pathophysiological mechanisms: reentry and automaticity [13]. Reentry usually presents with an almost immediate increase in heart rate, which reverts to normal just as suddenly. In the automaticity type of SVT, a gradual increase and decrease in the heart rate is observed for the presence of another supraventricular area which generates its own electrical signal [13]. This is the same