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# Unexplained stillbirth versus SIDS: Common congenital diseases of the autonomic nervous system—pathology and nosology

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ABSTRACT

*Objective:* To contribute to a more balanced assessment of the morphological substrates underlying unexplained perinatal death and SIDS.
*Methods:* In-depth histological, immunohistochemical and genetic examinations were performed on the autonomic nervous and cardiac conduction systems in 95 unexpected perinatal deaths, 140 SIDS and 78 controls (44 infants and 34 perinatal death victims).
*Results:* The study revealed the localization and the nature of a variety of specific congenital abnormalities of the autonomic nervous system, central and peripheral, and of the cardiac conduction system that represent the morphological substrates of the pathophysiological mechanism of sudden fetal death and SIDS.
*Conclusions:* The observation of similar anomalies of the autonomic nervous and the cardiac conduction systems in both unexplained perinatal deaths and SIDS indicates their common congenital nature. Therefore, the definitions of these deaths, currently nosographically distinct, should be unified.


## 1. Introduction

Perinatal loss and the Sudden Infant Death Syndrome (SIDS) are still an unresolved, major social and health problem today [1].

This paper will tackle unexplained stillbirth and early neonatal deaths, as well as the SIDS, which are apparently accounted for by exclusively or preeminently neurovegetative abnormalities which were not suspected during prenatal clinical examinations. So far, only a few studies have made a close examination of the nervous system, although abnormalities of this system are obviously extremely relevant to any analysis aiming to gain a better understanding of unexplained death during gestation and in early infancy. Thus, today's basic information in this field is still inadequate [2–5].

To contribute to a more balanced assessment of the morphological substrates underlying unexplained perinatal death and SIDS, the present article will focus upon the multifaceted involvement of the central and peripheral autonomic nervous system, as well as the cardiac conduction system, subject to autonomic nervous system control.

The results presented herein, obtained from in-depth histological examinations of the autonomic nervous and cardiac conduction systems in a very wide sample of unexpected perinatal deaths (65 stillbirths and 30 early neonatal deaths), SIDS (140 victims) and 78 controls (44 infants and 34 perinatal death victims), show similar alterations in unexplained death victims, indicating their common congenital nature and then that unexplained fetal and early neonatal death should not be regarded as distinct from the SIDS.

## 2. Materials and methods

### 2.1. Study subjects

The study included 313 subjects. This was a selected set of cases, sent to our Research Center according to the application of the guidelines recognized by Italian law n.31 "Regulations for Diagnostic Post Mortem Investigation in Victims of SIDS and Unexpected Fetal Death" over a 9-year period (2000–2009). This law decrees that all infants suspected of SIDS who died suddenly in Italian regions within the first year of age, as well as all fresh fetuses who died after the 25th week of gestation without any apparent cause, must undergo an in-depth anatomo-pathological examination. The autopsy was performed in all cases according to the International Standardised Autopsy Protocol (ISAP) of the Global Strategy Task Force of SIDS International [6], and the neuropathologic protocol developed at the Authors' research center [7,8]. These guidelines include all the methodologies for the study of the central and peripheral autonomic nervous system and of the cardiovascular system.

Below we briefly summarize the protocol for the examination of the brainstem, the spinal cord and the cerebellum, where the main structures participating in control of the vital functions (cardiorespiratory, arousal,

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upper digestive tract, etc.) are located. The other methodologies are available in the above-mentioned references [7,8].

Fresh specimens were firstly collected from the brainstem, near the obex, and conserved in ethanol or in RNA-later reagent (AMBION, Inc; Austin, TX) for genetic studies of the serotonin transporter polymorphism that has been widely associated to SIDS [9], and of the PHOX2B gene, whose mutation causes a large decrease in the central chemoreflex responsible for the Congenital Central Hypoventilation Syndrome (CCHS) [10].

After fixation in 10% phosphate-buffered formalin, the brainstem, the spinal cord and cerebellum were processed and paraffin-embedded. Transverse serial sections of the midbrain, pons, medulla oblongata, thoracic spinal cord and cerebellar hemispheres were made at intervals of 50–60 μm. For each level, serial 5 μm sections were obtained, two of which were routinely stained for histological examination using hematoxylin–eosin and Klüver–Barrera and the remaining sections were submitted to immunohistochemical study of neurotransmitters such as somatostatin, serotonin, tyrosine-hydroxylase, or specific visualization of apoptotic cells and reactive astrocytes through glial fibrillar acidic protein (GFAP) method.

The routine histological evaluation of the brainstem was focused on the locus coeruleus and the parabrachial/Kölliker–Fuse complex in the rostral pons/caudal mesencephalon, on the retrotrapezoid nucleus, the superior olivary complex and the facial/parafacial complex in the caudal pons; on the hypoglossus, the dorsal motor vagal, the tractus solitarius, the ambiguus, the pre-Bötzinger, the inferior olivary, the raphé and the arcuate nuclei in the medulla oblongata. In the thoracic spinal cord the intermediolateral nucleus was the subject of analysis. In the cerebellum, the cortex layers (external granular layer, molecular layer, Purkinje cell layer and internal granular layer) and the medullary deep nuclei (the dentate nucleus, the fastigial nucleus, the globose nucleus and the emboliform nucleus) were examined.

In 235 cases, after the in-depth anatomopathological examination, the death remained totally unexplained. A diagnosis of "unexplained perinatal death" was established for 65 stillbirth cases (30 females and 35 males, aged 24-40 gestational weeks; median age: 38 weeks; 49 ante-partum deaths and 16 intra-partum deaths) and 30 early neonatal death victims (16 females and 14 males, who died in the first days of life) and of "SIDS" for 140 infants, 66 females and 74 males, aged from 1 to 10 postnatal months (median age: 3.3 months) or more precisely, from 35 to 73 postconceptional weeks (median age: 46 postconceptional weeks).

In the remaining 78 cases, 44 infants (16 females, 23 males; aged from 2 to 8 postnatal months, median age: 3 months) and 34 perinatal death victims (15 females, 19 males aged from 25 gestational weeks to 7 days of postnatal life; median age: 36 gestational weeks), a precise cause of death was formulated at autopsy (adnexa pathologies and cardiomyopathies in perinatal deaths; cardiomyopathies and pneumonia in infant deaths). These cases were used as "Controls."

The histological analyses were carried out by two independent and blinded observers. Comparison among diagnoses was performed employing Kappa statistics (Kappa Index—KI) to evaluate the inter-observer reproducibility. The inter-observer reproducibility assessed throughout the study was very satisfactory (KI = 0.85). Moreover, in case of disagreement between the investigators, the slides were reviewed and discussed until the same results were obtained.

### 2.2. Statistical analysis

The statistical significance of direct comparisons between groups of victims (unexplained perinatal and infant deaths) was determined using the Levene test, one way analysis of variance (ANOVA) and Student's *t*-test. The selected threshold level for statistical significance was $p<0.05$.

For each case, all available information about pregnancy, fetal development and delivery and, in cases of infant death, about the environmental and familial situation where the death occurred, besides information related to the potential risk factors (such as maternal smoking, maternal obesity, type of milk feeding, position the baby was last left in), were collected and categorised during post-mortem family interviews.

All the information sheets were recorded in the registry of a dedicated data bank, administered by the Health Government of the Lombardy Region, and established under two subsections: one for perinatal loss (unexplained stillbirth, early neonatal death and perinatal controls) and another for the SIDS [11].

## 3. Results

We firstly analyzed the distribution of different information extracted from the data bank related to 129 perinatal deaths (65 sudden fetal deaths, 30 sudden early neonatal deaths and 34 control perinatal deaths) and 184 infant deaths (140 SIDS and 44 controls) to evaluate the potential risk factors. Tables 1 and 2 display the rates and percentage distributions of these variables related to perinatal and infant deaths, respectively. Significant correlations ($p<0.05$) were observed between maternal smoking, maternal obesity, brain weight below the normal value and sudden unexplained perinatal deaths (Table 1), and between prematurity, maternal smoking and the SIDS (Table 2). Data related to maternal abuse of alcohol, drugs, or sedative drugs were not available.

All the pathologic results revealed by in-depth anatomopathological examinations are summarized in Table 3. A more in-depth description of the neuropathologic findings and the related illustrations can be found in our previous works [12–18].

**Table 1**
Distribution of potential risk factors for unexplained perinatal deaths (65 stillbirths and 30 early neonatal deaths) and controls (34 cases) — (total number of victims: 129).

| | Unexplained perinatal deaths | Explained perinatal deaths |
|---|---|---|
| Total number | 95 (rate) | 34 (rate) |
| Age | | |
| ≤35 gestational weeks | 35 (36.8%) | 16 (47.0%) |
| >35 gestational weeks | 60 (63.2%) | 18 (53.0%) |
| Sex | | |
| Male | 49 (51.6%) | 19 (55.9%) |
| Female | 46 (48.4%) | 15 (44.1%) |
| Race | | |
| White | 73 (76.8%) | 28 (82.3%) |
| Others | 22 (23.2%) | 6 (17.7%) |
| Weight | | |
| Normal value for age | 36 (37.9%) | 18 (52.9%) |
| Below normal value for age | 59 (62.1%) | 16 (47.1%) |
| Brain weight (*) | | |
| Normal value for age | 32 (33.7%) | 24 (70.6%) |
| Below normal value for age | 63 (66.3%) | 10 (29.4%) |
| Adnexa (placenta-umbilical cord) | | |
| Normal structure | 75 (71.2%) | 22 (64.7%) |
| Pathological structure | 20 (28.8%) | 12 (35.3%) |
| Maternal age | | |
| ≤30 years | 33 (34.7%) | 14 (41.1%) |
| >30 years | 62 (65.3%) | 20 (58.9%) |
| Maternal weight (*) | | |
| Normal value for age | 50 (52.6%) | 28 (82.3%) |
| Above normal value for age (Obesity) | 45 (47.4%) | 6 (17.7%) |
| Maternal smoking (*) | | |
| No | 50 (52.6%) | 29 (85.3%) |
| Yes | 45 (47.4%) | 5 (14.7%) |

(*) When comparing unexplained perinatal deaths with the control group, $p<0.05$.