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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, $p<0.05$.

Table 2
Distribution of potential risk factors for SIDS (140 cases) and controls (44 cases) — (total number of victims: 184).

	Unexplained infant deaths (SIDS)	Explained infant deaths
Total number	140 (rate)	44 (rate)
Postnatal age		
≤4 months	115 (82.1%)	37 (84.1%)
>4 months	25 (17.9%)	7 (15.9%)
Postconceptional age		
≤52 weeks	123 (87.8%)	39 (88.6%)
>52 weeks	17 (12.2%)	5 (11.4%)
Gestational age at birth (*)		
≤37 weeks (prematurity)	76 (54.2%)	6 (13.6%)
> 37 weeks	64 (45.8%)	38 (86.4%)
Sex		
Male	74 (52.8%)	26 (59.0%)
Female	66 (47.2%)	18 (41.0%)
Birth weight		
normal value for gestational age	56 (40%)	14 (31.8%)
below normal value for gestational age	84 (60%)	30 (68.2%)
Brain weight		
normal value for age	94 (67.1%)	26 (59.0%)
below normal value for age	46 (32.9%)	18 (41.0%)
Race		
White	112 (80.0%)	33 (75.0%)
Others	28 (20.0%)	11 (25.0%)
Maternal smoking (*)		
no	81 (57.8%)	34 (77.3%)
yes	59 (42.2%)	9 (22.7%)
Feeding		
human milk	71 (50.7%)	19 (43.2%)
formula/mixed	69 (49.3%)	25 (56.8%)
Position last left		
prone	75 (53.6%)	25 (56.8%)
supine	65 (46.4%)	19 (43.2%)

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

3.1. Neuropathologic findings in stillbirth versus SIDS

3.1.1. Autonomic central nervous system

- Morphological alterations

In the brainstem the main structural alterations were hypodevelopment of the different nuclei and structures.

In the medulla oblongata hypoplasia of the arcuate nucleus was present in over 50% of the sudden perinatal deaths and SIDS victims. In detail, in 36% of the cases the hypoplasia was bilateral, extending throughout all the nucleus; in 28% there was partial hypoplasia, generally confined to the inferior two thirds and in 12% the hypoplasia was unilateral, involving the right portion. In addition, agenesis of the arcuate nucleus was diagnosed in 14% of the cases, and defective neuronal maturation in 10% of the victims, all SIDS.

Hypoplasia of the pre-Bötzinger nucleus, with a decreased neuronal number and/or dendritic hypodevelopment of the reticular formation, was found in 25% of unexplained perinatal deaths and in 8% of the SIDS.

Hypoplasia of one or more nuclei of the raphe system (obscurus, pallidus, median, magnum, caudal linear raphe nuclei) was seen in 57% of the SIDS and in 67% of perinatal victims. Hypodevelopment of other nuclei, such as the hypoglossus, dorsal vagal and tractus solitarii nuclei, was also occasionally found.

In the rostral pons and caudal mesencephalon of 35% of perinatal deaths, particularly intra-partum deaths, hypoplasia was observed, with a few immature neurons or agenesis of the parabrachial/Kölliker–Fuse complex.

Hypoplasia of the parafacial/facial complex, with a decreased neuronal density and area, was diagnosed in 60% of unexplained stillbirths. Neuronal cell bodies of the hypoplastic nuclei were small and lengthened, with a flattened nucleus.

Besides, hypodevelopment of other nuclei (hypoglossal, dorsal vagal, tractus solitarii, inferior olivary nuclei) was occasionally observed.

In the cerebellum, histological examination showed an immature structure of the cerebellar cortex, uniformly made up of small, round cells without the usual four-layered shape, in 20% of late unexplained perinatal deaths and in 26% of SIDS victims.

In the spinal cord, various degrees of hypodevelopment (neuronal immaturity in a normal structure/hypoplasia/agenesis) of the intermediolateral nucleus were found in 45% of the victims of unexplained death.

Fig. 1 summarizes the incidence of the main developmental alterations.

- Functional alterations

Using immmunohistochemical methods with specific antibodies for the different neurotransmitters, an altered expression of somatostatin, that is widely present in brainstem nuclei during fetal life but scarcely expressed after birth, was highlighted in the hypoglossus nucleus. Despite a normal structure, this nucleus was in fact somatostatin-immunonegative in 27% of the unexplained fetal deaths and, on the contrary, immunopositive in 44% of SIDS victims.

A decreased serotonin synthesis in the neuronal cell bodies and fibers of the raphe nuclei was observed in 30% of the victims.

Negative expression of tyrosine-hydroxylase (TH), an essential enzyme in catecholamine biosynthesis, was detectable despite a well-structured locus coeruleus (the major brainstem producer of noradrenaline), in 57% of the perinatal and infant sudden deaths.

Table 3
Number of cases with the different alterations.

Pathological results	Unexplained perinatal death victims ($n=95$)	Control perinatal death victims ($n=34$)	SIDS ($n=140$)	Control infant deaths ($n=44$)
ANS alterations (hypoplasia/agenesis/delayed maturation)—Total number				
Brainstem				
Arcuate nucleus	49	8	41	10
Pre-Bötzinger complex	24	–	11	–
Raphè nuclei	64	1	78	–
Parafacial complex	57	–	–	–
Parabrachial/Kölliker–Fuse complex	33	–	4	–
Other nuclei (hypoglossal, dorsal vagal, tractus solitarii, inferior olivary)	15	–	18	1
Cerebellar cortex	19	–	36	3
Spinal cord				
Intermediolateral nucleus	43		63	–
CCS alterations (Resorptive degeneration, Mahaim fibers, cartilaginous metaplasia, islands of conduction tissue in the CF)	18	4	8	9
Atherosclerotic alterations	51	1	88	3

ANS = autonomic nervous system.
CCS = cardiac conductions system.
Individual victims may display any combination of these pathological alterations.

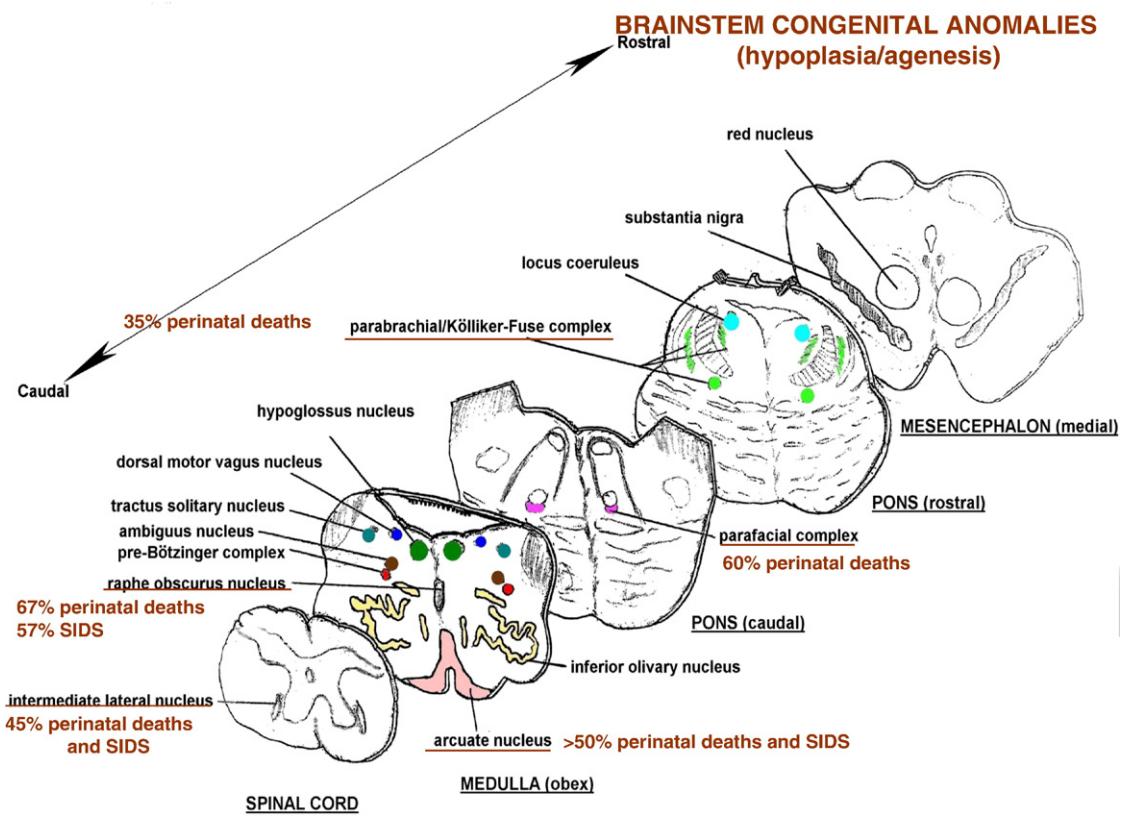

Fig. 1. Drawing of histological sections obtained from the brainstem and spinal cord showing the localization and incidence of the main developmental alterations observed in unexplained stillbirths and SIDS.

Unusual apoptosis of the Purkinje cells and of the internal granular layer in the cerebellar cortex was found in 18% of SIDS victims. A high apoptotic index was observed in the dentate nucleus of 34% of SIDS cases.

Application of the GFAP method showed the presence of numerous reactive astrocytes in the brainstem and cerebellum of both sudden death victims and controls.

Frequently, above all in fetal deaths, two or more morphological and/or functional alterations were simultaneously present in the same case, such as hypoplasia of the arcuate, pre-Bötzinger and parafacial nuclei and negative somatostatin expression in the hypoglossus nucleus.

- Genetic alterations

Investigation of the polymorphisms of the serotonin transporter (5-HTT) gene, the major determinant of serotoninergic function through the modification of gene transcription and transporter expression, showed that the frequency of the L/L genotype and L allele was 4-fold higher in unexplained deaths than in controls.

Specifically, the L/L homozygote and S/L heterozygote genotypes were detected in 39% and 47% of SIDS cases, respectively. Likewise, the L/L and S/L genotypes were detected in 30% and 50% of stillbirths, respectively. The L/L and S/L genotype frequencies in controls were 12% and 47%, respectively. The S/S genotype was detected in 14% of SIDS, in 20% of SIUD and in 41% of control cases, respectively. Therefore, the frequency of the L allele was 62% in SIDS, 55% in SIUD and 35% in controls.

Comparative analysis of the genetic and morphological results indicates a correlation between genotypic serotonin polymorphisms and hypoplasia of the raphe nuclei in the brainstem of unexplained fetal deaths and SIDS victims.

The DNA from all SIDS, SIUD cases and Controls submitted to the homeobox transcription factor PHOX2B testing, showed a PHOX2B 20/20 genotype in all cases, indicating the normal number of 20 alanines on both alleles. These results confirm that none of the victims of sudden death or controls had a mutation compatible with Congenital Central Hypoventilation Syndrome [10].

3.1.2. Autonomic peripheral nervous system

Abnormalities were detected in the superior cervical ganglia in 39% of cases of unexpected perinatal loss and in 22% of the SIDS. In these, there were SIF (small intensely fluorescent) immature neurons, unipolar or with very poor processes, large juxtaposed bodies and sometimes anastomosed with each other, lacking a satellite-cell mantel. Interneuronal cells, paraganglionic in nature, laden with argentophilic Grimelius positive granules (aminic neurotransmitter) were numerous, occasionally gathered around clusters of immature neurons.

Similar glomus cells were also observed in the capsule of the ganglium nodosum. The mediastinal paraganglia were grossly hypertrophic, with an uneven depletion of the chief-cells neurotransmitter granuli in 24% of SIDS cases.

3.2. Neuropathologic findings in controls

Only hypoplasia of the arcuate nucleus was observed in 23% of cases, rarely associated to minor hypodevelopment of other brainstem nuclei and delayed maturation of cerebellar and cerebral cortex structures.

3.3. Cardiovascular pathologic findings in stillbirth versus SIDS

- Cardiac conduction system

Accessory AV pathways, mainly Mahaim fibers, were detected in 39% of unexplained perinatal deaths and in 23% of SIDS victims.

Accessory AV communications were uncommon, both of James type, detected in 2 stillbirths, and of Kent type, that is the substrate of Wolf–Parkinson–White ventricular pre-excitation,, detected in 1 stillbirth

and 2 SIDS victims. Cartilage metaplasia of the fibrous body was detected in 19% of unexplained perinatal deaths and in 6% of the SIDS.

- Arterial walls
  In 54% of fetuses and in 63% of neonatal deaths and SIDS victims, structural lesions of the arterial wall were evident from the 35th week of gestation. The development of atherosclerosis in fetuses is characterized by foci of gross subversion of the tunica media with fragmentation of the smooth muscle cells (SMCs), perpendicularly oriented and infiltrating the intima. After birth there was progressive myointimal thickening, caused by lipids and glycosaminoglycans deposits synthesized by SMCs and by the infiltration of monocytes. These lesions progressed to the formation of common atherosclerotic plaques, already recognizable in infants after few weeks of life.
  These atherosclerotic alterations were systemic; they were frequently observed in coronary arteries, mainly in the anterior descending branch, in the carotid, cerebral arteries, aorta and also in medium-sized arteries, including the arteries supplying the cardiac conduction system, and in the arterial walls of the fetal adnexa. Fibromuscular hyperplasia of the pulmonary arteries was also a frequent finding.

4. Discussion

The problem of unexpected perinatal loss and the SIDS at post-mortem examination is a Gordian knot that needs to be untied, not cut. Pathologic data on the SIDS, beginning from the so-called thymus-death, a possible lethal thymus-lymphatic state marked by gland enlargement, occasionally related to stenotic respiratory diseases, has been accumulating since 1800. From the 1970s, inflammatory respiratory diseases, namely pneumonia, bronchiolitis, tracheo-bronchitis, from bacterial or viral causes, and myocarditis, particularly viral, are described among the possible causes and concauses of the SIDS [19].

A relevant growth of knowledge on the anatomical substrates of sudden perinatal loss and the SIDS has been gained from studies of the autonomic nervous system.

Early studies on neuropathology were conducted by Naeye in 1976 [20], who described the presence of brainstem astrogliosis in half of his SIDS cases. This alteration was again emphasized by Kinney et al. [21] in 22% of the SIDS victims examined.

Subcortical leukomalacia in the white matter [22], developmental delay of dendritic spines and synapses [23], a decreased number of myelinated vagal fibers [24], increased apoptosis in brainstem nuclei [25] and abnormalities in neurotransmitters [26] have all been reported in the SIDS in subsequent research.

All these lesions, albeit non-specific, can be attributed to chronic or repeated hypoxia complicating sleep apnea and alveolar hypoventilation, suggesting the possibility that abnormal development of the neuronal circuitry between the brainstem centers that regulate rhythmic breathing and arousal gives rise to cardiorespiratory instability. This concept provides an insight into the specific mechanisms leading to unexpected death.

A variety of abnormalities is also found in the brains of stillborns, the most common being brainstem and spinal cord necrosis, periventricular leukomalacia, gliosis, intraventricular hemorrhage, cerebral infarcts [2].

No cause of death was instead identified after autopsy in unexpected fetal death [27].

5. Neuropathology of stillbirth versus SIDS

While the data reported in the literature in unexpected perinatal loss and SIDS refer to non-specific alterations of the autonomic nervous system, our investigations in this field have contributed to reveal the localization and the nature of a variety of specific neuronal congenital anomalies, particularly of the brainstem, spinal cord and cerebellum.

From the overall analysis of the neuropathologic results, the following prominent data emerged:

- in 92% of sudden perinatal and infant deaths one or more congenital morphological and/or functional abnormalities of the autonomic nervous system are present. These alterations are more numerous in unexplained antepartum deaths as compared with intra-partum, neonatal deaths and the SIDS;
- ante-partum deaths are characterized by the association of many developmental alterations of the brainstem, cerebellum and spinal cord structures; an exclusive alteration in these deaths is hypoplasia of the facial/parafacial complex;
- intra-partum deaths are characterized by a decreased number of nuclei shown to be involved in developmental disturbances, and by the high frequency of hypoplasia of the parabrachial/Kölliker–Fuse complex;
- in SIDS victims the main alteration is hypoplasia of the arcuate nucleus, present in over 50% of victims, sometimes associated to hypoplasia of other nuclei such as the hypoglossus, the pre-Bötzinger, the tractus solitarii, the inferior olivary nuclei;
- a frequent finding in both unexpected perinatal death and the SIDS is defective synthesis of neurotransmitters (catecholamines in the locus coeruleus, serotonin in the raphe nuclei, somatostatin in the hypoglossus nucleus);
- there is a high incidence of genetic polymorphisms, such as the serotonin L/L genotype, present in 39% of SIDS cases and in 30% of sudden fetal deaths, frequently related to morphological hypodevelopment of one or more nuclei of the raphe complex.

A common finding in both unexplained perinatal death and the SIDS was hypoplasia of the arcuate nucleus, a component of the ventral surface of the medulla oblongata which participates in sleep-related homeostatic responses including chemoreception, thermoregulation, respiratory drive and breathing, arousal, reflex regulation of blood pressure and cardiovascular responses [12,13,28].

Arcuate nucleus hypoplasia is frequently associated to hypoplasia of the pre-Bötzinger nucleus, defined as a group of neurons of the ventrolateral medulla, that are essential for generating the respiratory rhythm, as well as for modulating eupneic breathing [14,29]. Instead, the Kölliker–Fuse nucleus was found to be hypodeveloped particularly in sudden intra-partum deaths; it has an important function during intrauterine life, inhibiting the response of central and peripheral chemoreceptors (which are already fully formed and potentially functional in the last weeks of pregnancy) and therefore any respiratory reflex. After birth, the Kölliker–Fuse abruptly reduces its inhibitory effects and becomes active as a respiratory center able to coordinate the pulmonary motor responses to hematic oscillations of pO_2, pCO_2 and pH [15].

In addition, exclusively in unexplained stillbirths, hypoplasia of the parafacial nucleus, consisting of “pre-inspiratory” neurons that periodically trigger the inspiratory neurons of the pre-Bötzinger complex, was very frequent. Therefore, the function of hierarchical modulation of the breathing circuitry can be ascribed to the parafacial nucleus [16].

Among the functional alterations, defective expression of neurotransmitters was diagnosed in the brainstem, particularly of catecholamines in the locus coeruleus, serotonin in the raphe complex and somatostatin in the hypoglossus nucleus, besides altered apoptotic programs in the cerebellum, and precisely in the Purkinje and internal granular layers of the cerebellar cortex as well as in the dentate nucleus.

A notable finding in this study was the increased frequency of the serotonin L allele not only in SIDS cases, in agreement with previously published data [9], but also in unexplained stillbirths, never considered in relation to serotonin network dysfunctions [30]. An additional observation was the significant correlation between the L allele and hypoplasia of the raphe nuclei. In fact, a high percentage of sudden fetal and infant deaths with the L/L or L/S genotype showed hypoplasia of one or more nuclei of both the superior and inferior raphe groups,

suggesting involvement of a serotonin polymorphism in hypodevelopment of the raphe system. It is known, in fact, that the serotonin system plays a trophic role during neuronal development in the fetal brain. A dysfunction in serotonergic transmission during intrauterine life could lead to hypodevelopment of the neuronal structures checking vital functions, with a fatal outcome.

6. Cardiovascular pathology of stillbirth versus SIDS

Unexpected perinatal loss and the SIDS are also significantly associated with developmental disturbances of the cardiovascular system.

Sudden death can occur due to changes in the cardiac action, mostly manifesting with arrhythmias, that may be caused by microscopic malformations of the conduction system. The finding of accessory AV communications, particularly nodo-fascicular ventricular bundles (Mahaim fibers) is quite frequent in perinatal unexplained loss, as also in SIDS (but a clinicopathologic assessment of their lethal arrhythmogenic potential is often impossible). These congenital abnormalities, under particular conditions and/or neurovegetative stimuli, are liable to provoke electrical dyshomogeneity, instability and desynchronization, raising the risk of malignant functional arrhythmias.

In our study, accessory AV pathways, mainly Mahaim fibers, were detected in 39% of unexpected perinatal deaths and in 23% of SIDS victims. Accessory AV communications of James and Kent type the substrate of Wolf-Parkinson-White pre-excitation, were uncommon, being detected in only 2 unexpected perinatal deaths and in 2 SIDS victims, respectively.

These lesions have been attributed to the variable outcome of a "resorptive degeneration" process that normally "reshapes" the functional pathways in the fetal and early neonatal period; theoretically, if this process is defective, anomalous AV connection may persist, whereas, if it is exaggerated, it could result in blocking disruption. Clinically, this abnormality can present without hemodynamic impairment, while manifesting with high-risk reciprocating arrhythmias.

In addition, the central cardiac structure "supporting" the cardiac system could possibly interface with the conduction of impulses, as in the cases of cartilaginous metaplasia of the fibrous body.

The most frequent observations in the vascular system of sudden perinatal deaths and SIDS are arterial atherosclerotic lesions observed in 54% of fetuses from the 35th gestational week and in 63% of neonatal deaths and SIDS victims. The atherosclerosis is systemic in nature and in the prenatal period, involvement of the fetal adnexa arteries is common. By thickening the arterial wall, the atherosclerotic lesions can reduce the placental flow and reduce fetal oxygenation. Finally, serious alterations of the pulmonary arteries were observed in 7% of the sudden perinatal deaths and the SIDS.

7. Exogenous-environmental risk factors

Among the causes which trigger and/or promote unexplained death, exogenous risk factors that alter the intrauterine environment are relevant,such as infections (pulmonary virosis with respiratory impairment), familial tobacco smoking, maternal drug abuse, maternal alcoholism and likely atmospheric pollution.

In particular, exposure to tobacco smoke in utero is the most important preventable risk factor. Smoking, by affecting fetal oxygenation, causes vasoconstriction and reduces the fetal blood flood, as well as inducing placental atherosclerosis

In addition, air pollution, particularly in the Lombardy Region, where our studies have been prevalently conducted, which features high rates of both gases (carbon monoxide, nitrogen dioxide, ozone, sulphur dioxide) and particulate matter (above all PM_{10}, with a median diameter of $<10\,\mu m$), could have an important influence in determining sudden unexplained perinatal and infant death. We postulate that gas pollutants in particular, like cigarette smoke, can cross the placenta during pregnancy through the maternal blood, and lead to a hypoxic status responsible for structural and/or functional impairments of the central nervous system.

Finally we point out that maternal and antenatal risk factors could yield phenotypes susceptible to sudden and unexpected death in perinatal and infant life in the presence of protein products produced by a specific polymorphism and/or the mutation of genes affecting brainstem autonomic control, i.e., the same risk factors could have very different effects in subjects with a different genetic constitution. All the morphological and/or functional alterations reported up to now can be interpreted as the consequence of these interactions.

8. Nosology

The observation of similar anomalies of the autonomic nervous system and of the cardiac conduction system in both unexplained perinatal deaths and SIDS indicates their common congenital nature and supports the statement in the National Institute of Child Health and Development SIDS Strategic Plan 2000 that "SIDS is a developmental disorder. Its origins are during fetal development" [31]. These observations supported a new holistic approach to the SIDS, analogically linked with unexpected perinatal loss.

The developmental anomalies and genetic substrates common to unexpected stillbirth, early neonatal death and SIDS suggest that the definitions of these deaths, currently nosographically distinct according to the International classification of disease (ICD) [32], should be unified.

The need to perform a complete autopsy of all victims of these unexpected deaths is unanimously recognized, since prevention of these diseases will be based mostly on a better recognition of the abnormalities found in various organs, as well as on the individuation of the basic underlying mechanisms. Since this problem is highly complex, it is extremely important to obtain an exhaustive diagnosis of each case, together with full details of the familial and circumstantial background to the demise. The scientific advantages derived from a better understanding of perinatal loss and SIDS are difficult to evaluate, but are certainly extremely significant. Such an understanding would encompass the prenatal period, which is now regarded as the background to many responses to disease in infants, adults and even elderly patients.

Conflict of interest statement

All Authors declare that they have no conflicts of interest, financial or otherwise to declare.

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