

WORKSHOP
“Up-to-date revision of ALTE & SIDS
and
Techniques for autopsy in suspected SIDS”
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Preface

Apparent life threatening event (ALTE), sudden infant death syndrome (SIDS) and sudden intrauterine death (SIUD) during the 3rd trimester of pregnancy, are probably in great part the epiphenomenon of the same syndrome. Anatomic-pathological, genetical and clinical exams all contribute to the definition of each of these events and tend to find out a common cause, while excluding possible concealed diseases that can reveal themselves with the same acute outset in apparently normal babies or pregnancies. Important studies are reported on the anatomic-pathological and genetical findings. Epidemiological data on ALTE and home-monitoring as deriving from a regional webnet, and proposal of clinical protocols for the detection of the possible causes of ALTE and SIDS are discussed. Clinical cases of sudden deaths in infancy and technical aspects on how to conduct a correct necropsy in SIDS and SIUD are here offered. The aim is to create a new multidisciplinary and co-ordinate approach to this rare but impressive disease.

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Update and guidelines for the pathology examination of sudden infant death syndrome and sudden intrauterine unexpected death

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Key words: Pathology, SIDS, SIUD

Unexpected perinatal death and SIDS are different expressions of the same multifactorial disorder, which manifests in the form of the triggering of vagal reflexes, mostly of respiratory type, generally due to congenital abnormalities of the structure of the autonomic nervous system. Overall, these diseases are responsible for the death of approximately 5–7% of newborns (0.7–1% of them die of SIDS).

Moreover, from 50–80% of fetal deaths remain unexplained after the routine anatomopathology examination. In a case series including 135 cases of SIDS, 40 control infant deaths and 71 unexpected perinatal deaths (57 fetuses and 14 newborns), examination of the central and peripheral autonomic system and of the cardiac conduction system revealed alterations, most of which were of a congenital na-

ture, of the various structures examined only in victims of SIDS and stillborns. There was a high proportion of findings of hypoplasia of the arcuate nucleus, found to have the same incidence (approx. 57%) in both victims of SIDS and of late fetal death. This anomaly was frequently associated with hypoplasia of the respiratory reticular formation, pulmonary hypoplasia and chronic hypoxia. Congenital abnormalities were also present in other nuclei of the brain stem (the tractus solitarius, dorsal vagus and hypoglossus nuclei), either as an isolated finding or in association.

There was a high number of cases with hypoplasia of the parabrachial/Kölliker-Fuse complex, which could plausibly be the morphological substrate of neonatal respiratory deaths, and with defective maturation of the cerebellar cor-

tex and the dentate nucleus. Functional alterations of catecholamine synthesis were also demonstrated, especially of noradrenaline in the locus coeruleus and of somatostatin in the hypoglossus nucleus.

In approximately 5% of SIDS victims, acquired diseases of the structures of the brain stem were observed, consisting mainly of encephalitis of various nuclei and in one case, of hemangi endothelioma of the area postrema.

Notably, among the identified alterations of the peripheral nervous system, neuronal immaturity of the superior cervical ganglion was present in 9% of cases, and reactive hypoplasia of the mediastinal paraganglia in 25% of cases.

In addition, cardiac abnormalities were often found, likely the substrate of sudden unexpected death. These frequently consisted of congenital and/or acquired alterations of the cardiac conduction system. The latter, found in 30% of the examined cases, featured accessory fibers, mostly

Mahaim, causing sometimes lethal arrhythmia of junctional type. The acquired abnormalities were largely of neoplastic origin, and Purkinje cell tumours and cardiac fibroma were especially common.

Finally, it must be underlined that a smoking habit in the mother and/or father is a fundamental risk factor for these abnormalities. In fact, a highly significant statistical correlation has been demonstrated between maternal smoking and hypoplasia of the brain stem nuclei and/or functional alterations of the synthesis of the neurotransmitters.

The atherogenic effect of maternal cigarette smoking must also be stressed, which can cause the development of atherosclerotic lesions already during fetal life (after the 35th week of gestation) and their progression after birth, to the extent that true juvenile atherosclerotic plaques may already be observable by the first weeks of life.

Genetics and SIDS

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Key words: AFLP, molecular markers, serotonin

Introduction. A widely held perspective of SIDS shows that it is not a genetic disease and that the environmental factors are the main cause of death. However, since the first studies on genes involved in metabolic disorders, additional associations between DNA markers, risk factors and children SIDS have been demonstrated (1). The search of genetic factors in families with repetitive SIDS, generated speculations on the existence of an autosomal dominant gene with incomplete penetrance (2). Nowadays, researchers emphasize the importance of gene-environment interactions and the genetic component of sudden infant death is referred to 2 different categories: mutations involved in genetic disorders that are a cause of death themselves and polymorphisms that might potentially predispose to death in critical situations (3). In this paper, preliminary results obtained by the analysis of MCAD, SCN5A and 5-HTT genes are reported. Particular attention has been dedicated to the potential role of 5-HTT genotypes in the etiopathogenesis of SIDS. In addition, an innovative approach based on AFLP markers to search new candidate genes, is presented.

Materials and methods. DNA was extracted from autopsy samples of 8 SIDS children using standard techni-

ques. Controls were obtained from 5 healthy individuals of different ages. Screening for mutations was carried out by direct sequencing using specific primers for MCAD exon 11, SCN5A exon 16 and the promoter region of 5-HTT. AFLP markers were obtained following the protocol optimized at our laboratory. Both sequencing and detection of DNA fragments were carried out using the automatic sequencer CEQ 8000. AFLP data were elaborated by Genographer software.

Results and Discussion. Sequencing of 400 bp of MCAD exon 11 did not reveal mutation A985G in the 8 SIDS cases and in the controls. A novel polymorphism 1161A/G was detected but its translation resulted in a silent mutation for the same valin aminoacid (Fig. 1). Similar results were obtained after sequencing 400 bp of SCN5A exon 16. No mutation referred to long QT syndrome was detected (4). As a genetic factor, the polymorphisms of 5-HTT appear important for the etiology of SIDS (5). In this work, both S and L alleles of the promoter region were found. In particular, 5 homozygote genotypes L/L and 3 heterozygotes S/L were detected in the 8 investigated infants. The frequency of L allele was 81% in SIDS samples

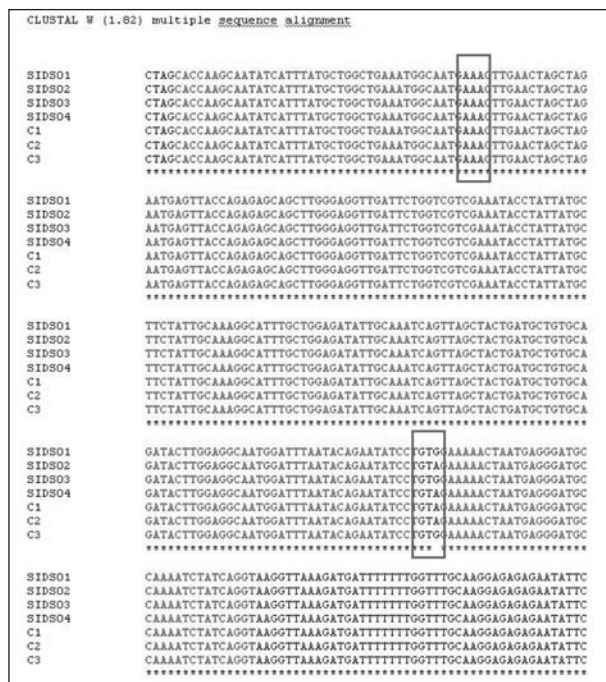


Figure 1. Sequences of 300 bp of MCAD exon 11 in 4 SIDS children and 3 controls. No mutation A985G was detected (top frame). A silent polymorphism G/A was evidenced (bottom frame)

and 30% in the controls. It should be remarked that the L/L genotype has been recently proposed as a SIDS risk factor. Ten different *EcoRI/TaqI* primer combinations were analyzed in the range 70–600 bp to obtain about 450 AFLP fragments in each individual. Duplicate analyses in 4 different tissues evidenced 16 polymorphic fragments in nervous system samples of one child and 2 in the medulla of a second child (Fig. 2). Most polymorphic combinations were E32/T32, E32/T33, E33/T37 and E40/T37.

Conclusions. Analysis of MCAD and SCN5A genes allowed the exclusion of fatty acids β -oxidation deficiency and long QT syndrome, while detection of the promoter L allele of 5-HTT gene resulted more frequent in SIDS infants than in the controls. Data on allelic frequencies of the serotonin transporter were in agreement with previous observations on Japanese (5) and American (6) populations and confirmed the role of L/L genotype as a risk factor for SIDS. Preliminary results on AFLPs evidenced particular polymorphisms detected in brainstem and cerebral cortex. Although obtained on a limited number of samples, AFLPs represent the first application of this methodology to SIDS and encourage to go on with the investigation on additional cases.

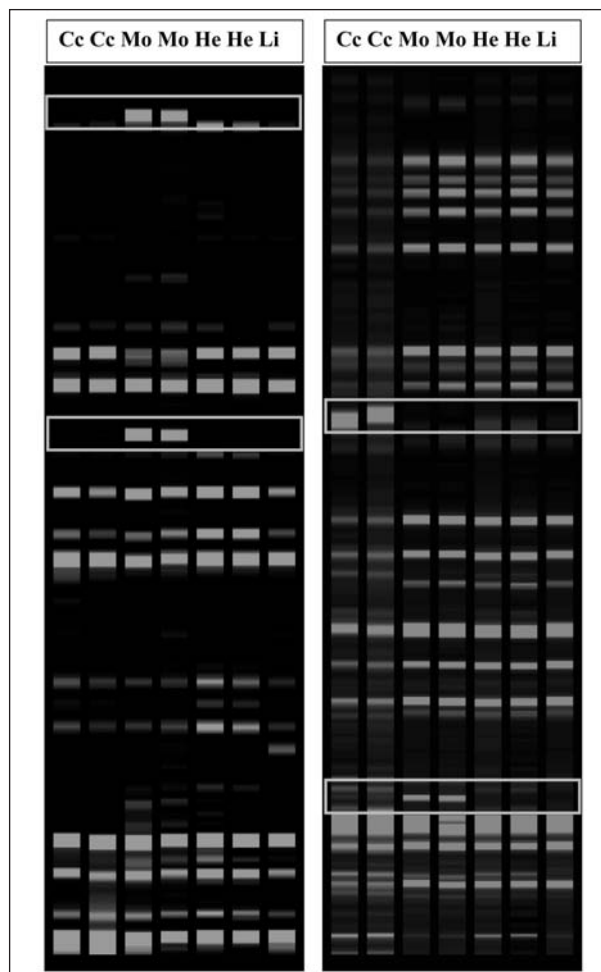


Figure 2. AFLP polymorphisms (inside frames) in different tissues of a SIDS child obtained with two 3-nt selective primer pairs. Analyses were carried out in duplicate in cerebral cortex (Cc), medulla oblongata (Mo), heart (He), liver (Li)

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Webnet survey in Emilia-Romagna Region on ALTE (Apparent Life Threatening Event)

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Key words: webnet, SIDS, ALTE, epidemiology

Introduction. Due to the lack of epidemiological data on SIDS and ALTE in Italy we are collecting the data of Emilia-Romagna Region via a dedicated webnet among the 27 pediatric centres of the region.

Results. In 24 months we have found an incidence of SIDS of 0.2 ‰ live births while ALTE was 1.2 ‰ live births. Only one case of ALTE was followed by SIDS. The frequency of the presenting symptoms is reported in table 1. The most frequent association of presenting symptoms was “change of colour + need of vigorous stimuli” (50%). Female were 57% and 74% of cases were less than 2 months old. The frequencies of some preventive measures for SIDS is reported in table 2. In 40% of cases of ALTE a diagnosis was made and hence a therapy started. The most frequent morbidity was related to gastroesophageal reflux (26%).

Discussion. We found that the incidence of ALTE is lower than awaited: this is probably due to the fact that the collection of data is not fully developed at all centres. Concerning the details of ALTE, the relatively high number of cases with the baby breast fed, or supine or on the right side is likely due to the spreading of the rules to prevent SIDS that, even if irregularly and intermittently, has been carried out in the previous years. The need of performing several exams is outlined by the high possibility of diagnosing a specific disease and starting a specific treatment.

Conclusion. The aforementioned data show how it is possible to define the incidence of ALTE, but also its characteristics and causes thanks to a web system involving all pediatric centres. The final aim is to reduce at a minimum the number of ALTE *sine causa*, thus possibly contributing to reduce the number of SIDS.

Table 1. Rate of presenting symptoms

Presenting symptom	%
Change of colour	85
Change of muscle tone	61
Apnoea	57
Upper airway obstruction	30
Loss of consciousness	26
Choking	17
Need of vigorous stimuli	73

Table 2. Frequencies of preventive measures

Preventive factors	Frequencies
Feeding	48% breast; 17% formula; 30% mixed
Position at ALTE	32% supine; 4.5% prone; 32% in parent's arm; 27% on right side; 4.5% sit
Parents' smoke	55%, 30 % of which only father and 15% both parents

Role of the placenta as a cause of sudden unexpected death during late pregnancy

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Key words: placenta, villus tree deficiency, immaturity

Introduction. Much of what occurs in the very early days of life is a direct consequence of something that happened in utero and we can draw an imaginary line linking all immaturity and dysmaturity events in utero with imma-

turity events in the very early period of life, including Sudden Infant Death Syndrome (SIDS), Apparent Life-Threatening Events (ALTE) and Sudden Intrauterine Unexplained Death (SIUD).

Patients and results. In our series of fetal-neonatal post-mortem investigations (Fig. 1), a peak is recorded around 39-40 weeks. It features late fetal deaths in normal pregnancies subject to monitoring, thus representing an unexpected event at the end of pregnancy. It should be pointed out that there is a difference between fetal deaths in the 3rd trimester with more or less documented growth retardation and SIUD at termination of pregnancy. A series of post-mortem investigations we presented in Turin in 2004 (1) showed that *placental causes* are at the root of 54.2% of fetal-perinatal deaths, whereas fetal and infection causes account for 25.5% and 20.3% of cases, respectively.

Discussion. This case series first of all shows that no post-mortem exam can be conducted on the fetus, without also examining the placenta. Further, we believe that placental examination not only explains the causes of fetal death, but also of poor outcomes in the neonatal period. A likely cause of SIUD can be found in *placental villous tree defects*. If we compare the structure of fetal cotyledonary villi with well branched trees, stem villi make up the tree trunk, mature intermediate villi (MIV) - namely those preceding the budding of terminal villi - make up the branches, out of which leaves will grow; then there is the role played by a third population of MIV, namely those that make the villous tree grow into branches, that are not functionally useful for gas exchange, but, from a structural point of view, anticipate and then guide the growth of the placenta. Finally, there are terminal villi, which, just like leaves, are used for

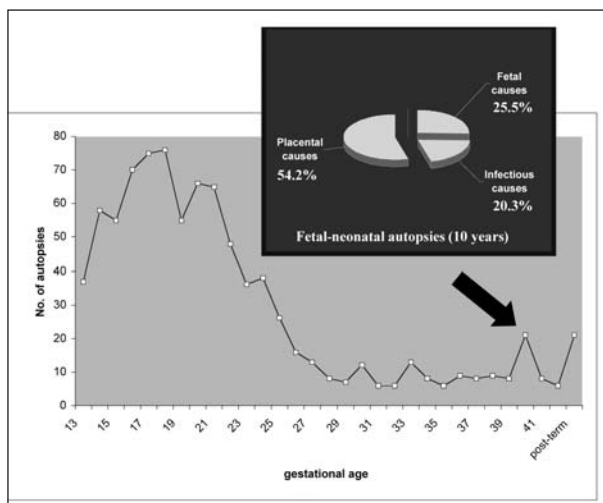


Figure 1. Cause of death from No. 886 foetal-neonatal autopsies (1983-2004) at University Hospital San Martino, Genova (meanly No. 2200 deliveries/yr). Peak at 39-41 weeks' gestation (arrow)

most mother-fetus exchange functions. A well known exemplary diagram by Benirschke-Kaufmann - known as "Villi Maturation Triangle" (2) - illustrates the relationship between the various villous components during placental growth and maturation. Normal maturation corresponds to the base of the triangle, whereas the upper and the bottom left angle correspond to pictures of extreme immaturity, with two possible pictures: 1) immature intermediate villi (IIV): severe dysmaturity with an excessive number of IIV, which prevail over the others and are responsible for the growth of a large, overweight placenta, made of non functioning villi only; or 2) severely hypo-branched villi with mostly MIV prevail: this is the result of partially inadequate and poor implantation, and it features rare, poorly branched, hyper-mature and atrophic villi, which look like stunted buds of a plant that fails to thrive into well grown branches and leaves; large stem villi or MIV prevail, out of which only some rare terminal villi will grow. In both cases, the picture is characterized by a low number of exchange terminal villi and is called villous tree deficiency (Fig. 2) unable to guarantee sufficient exchange functions to the fetus. Assuming that placental immaturity and dysmaturity may be the basis for fetal dysmaturity and immaturity, especially with regard to central nervous system and respiratory system growth, in 1995 we first expressed the idea of a correlation between heart and pulmonary disease in fetuses and severe placental immaturity (3) and, in 2000 between placental immaturity and ALTE (4).

Conclusion. When presented with an immature or dysmature placenta, it should be easy to speculate also the existence of circulation deficiencies impacting from the placenta to the fetus, and back from the fetus to the placenta. All this should be taken into account, when assuming a unique correlation between SIDS and SIUD events.

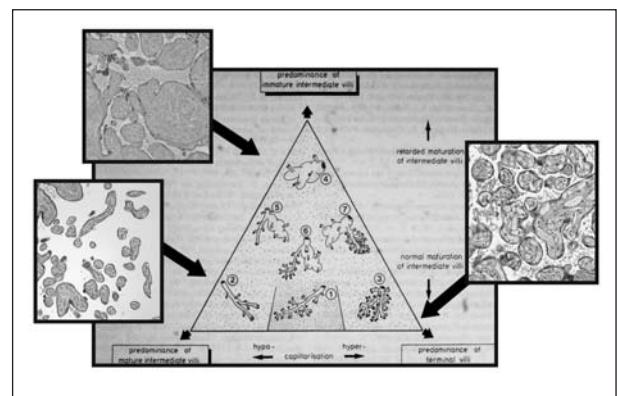


Figure 2. Villi Maturation Triangle (Modified from 2)

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A case of respiratory unexpected death

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Key words: SIDS, hypoglossus nucleus

Introduction. The borderline Sudden Infant Death Syndrome (SIDS) or «gray zone» cases have been described as those cases in which anatomic-pathological findings alone might not have accounted for the sudden deaths, if it had not been for the concomitant presence of additional abnormalities which could have had a triggering role (1).

Materials and methods. A female baby with a history of episodes of gastro-esophageal reflux and pneumonia was found unresponsive in her crib. At the age of one month she

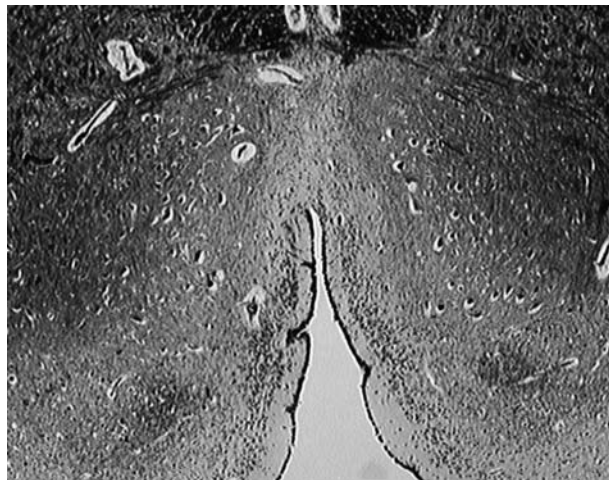


Figure 1. Bilateral hypoplasia and neuronal immaturity of the hypoglossus nucleus. Hematoxylin-Eosin, 25x.

presented an episode of apnea which required resuscitative maneuvers. The baby was given wide spectrum antibiotic therapy and was discharged six days later in good general conditions. The baby continued well until the day of her sudden death. A complete autopsy was performed, including examination on serial sections of the central and peripheral autonomic nervous structures (1).

Results. The postmortem study of the brainstem revealed hypoplasia and neuronal immaturity of the hypoglossus nucleus (Fig. 1).

Discussion. The congenital abnormalities of the hypoglossus nucleus seem to have been responsible for the baby's symptoms of deglutition impairments with subsequent episodes of recurrent pneumonia. Milk aspiration can be attributed to functional swallowing disturbances caused by congenital alteration of the hypoglossus nucleus.

Conclusions. Our case acquires an unique interest about the role the abnormalities of the hypoglossus nucleus might have played in the lethal outcome. A concomitant diagnosis of pneumonia does not exclude that of SIDS or borderline SIDS.

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A case of neonatal Sudden Infant Death Syndrome (SIDS)

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Key words: arcuate nucleus, Medium-chain acylCoA dehydrogenase (MCAD), Sudden Infant Death Syndrome (SIDS)

Introduction. Sudden Infant Death Syndrome (SIDS) has been defined as “the sudden death of an infant under one year of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history” (1). In this paper, we report a case of neonatal SIDS, with clinicopathological features and a genetic study.

Clinical History. A female newborn, born at 35 weeks' gestation, showed hypotonia, asystolia at the age of three days. Despite different attempts at resuscitation she died. Neither of the parents used tobacco and drugs. Her maternal history revealed death of a brother at the age of three days. Clinically, a SIDS was suspected and an autoptic study was required in the effort to explain the causes of death. *Autopsy* was performed according to the technique of Langlely (2). Thus, initially, malformations were excluded which can be responsible for SIDS such as anomalies of the coronary arteries (3, 4). Then, a histological examination of every organ was performed. Moreover, standardized techniques were used for the cardiac conduction system (CCS) and nervous centers, examination which are both involved in the cardiorespiratory control (5). A *genetic study*, useful to evidence medium-chain acyl-CoA dehydrogenase (MCAD) was performed on small hepatic fragments, frozen at -80° C. This anomaly represents an enzymatic mitochondrial defect involved in the fatty acids beta-oxidation cycle (MCAD, MIM #201450) and is recognized among the causes of SIDS (6). After DNA extraction the gene of MCAD was examined. The sequence was compared with that of control cases and the data present in a bank data.

Pathological findings. Histological sections of some organs revealed findings of acute hypoxia, such as congestion and lymphocytic depletion of the thymic cortex and pseudocysts of adrenal cortex. Microscopic examination of CCS revealed only islands of conduction tissue in the central fibrous body, designated as persistent fetal dispersion. These islands were separated from the atrio-ventricular node and His bundle. The main finding was however in brainstem with hypoplasia of the arcuate nucleus in its in-

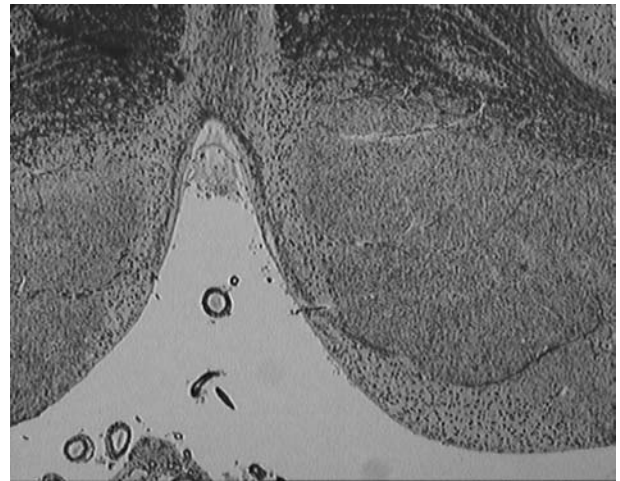


Figure 1. Hypoplasia of the arcuate nucleus of brainstem in its intermediate portion

termediate portion (Fig. 1). *Results of genetic analysis:* the most common mutation of DNA in the gene of MCAD (which is an A->G transistion at position 985, resulting in a change of amino acid residue 329 from lysine to glutamic acid) was absent.

Discussion and Conclusions. In conclusion, the present case reveals that neonatal SIDS can be related to hypoplasia of the arcuate nucleus in its intermediate portion. This anomaly does not seem to be caused by a defect of MCAD. Fetal dispersion of CCS can be a normal transient variation in life (7) but must not be considered the anatomic substrate for arrhythmias and SIDS. By the presentation of this case we suggest an autoptic diagnostic approach in cases of suspected SIDS relating autopsy findings and genetic analysis.

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Very-long-chain-acyl-CoA dehydrogenase (VLCAD) deficiency: report of a case

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Key words: VLCAD deficiency, FAO disorders, tandem mass spectrometry

Introduction. Studies report fatty acid oxidation disorders (FAOD) as a cause of sudden unexpected deaths in infancy. VLCAD catalyses the first step in β -oxidation.

Case report. A newborn girl showed hypoglycaemia (6 mg/dl), hypothermia and moderate metabolic acidosis at 26 hours. She improved after 10% dextrose, NaHCO₃ and antibiotics administration, but hypotonia persisted and creatinase rose. At 51 hours glycaemia fell and she died following cardio-respiratory arrest. The autopsy revealed degeneration of tubular renal cells, lung congestion and moderate desquamative pneumonia, vacuolated myocytes with lace-like appearance, and overall, diffuse hepatic degeneration with macro and medium vesicular steatosis (Fig. 1). Suspecting metabolic disease tandem mass spectrometry (MS/MS) in cultured fibroblasts was done and showed VLCAD deficiency (VLCADD).

Discussion. Three phenotypes of VLCADD are known: 1. severe, early-onset form, with cardiomyopathy and hepatopathy; 2. hepatic, whose outset is in infancy with recurrent hypoketotic hypoglycemia; 3. milder, later-onset, myopathic form, with episodic muscle weakness, myalgia, and myoglobinuria. Scientific studies support MS/MS-based newborn screening for FAOD (1). Recently the New England and German newborn screenings showed VLCADD rate of 1/42,500 - 1/125,000 births, higher than previously reported.



Figure 1. Diffuse hepatic macro and medium vesicular steatosis

Conclusion. Tight collaboration among pathologist, paediatrician and laboratory physician is important to prevent misdiagnosed SIDS.

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Per partum death at term pregnancy due to spontaneous cord hematoma

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Key words: umbilical cord, hematoma, fetal death

Introduction. Hematoma of the umbilical cord is rare (1/5500 deliveries) but the outcome is poor in half of the cases (1). It can determine a compression on the umbilical vessels with a consequent acute deficit in the haematic supply to the fetus (2).

Clinical cases. Three cases of fetal death as a result of an umbilical cord hematoma at term of pregnancy have come to our attention. During labor, CTG signs of acute fetal suffering were observed. The fetal autopsies were negative. Examination of the cord showed a hematoma with characteristics of acute hemorrhage. In two cases, a relative shortness of the umbilical cord was observed; in all three cases, the vein was shown as the damaged vessel. We observed in two cases parietal malformations in the affected tract and, in the third, a funisitis.

Discussion. The risk associated factors include infections, morphological anomalies, congenital alterations of the vascular wall, prolapse, traction on the short cord, post-mature pregnancies. Spontaneous cord hematoma can morphologically present in a different way so in all cases in which acute asphyxia is verified, it is necessary to proceed to an accurate study of the cord. In fact, we think that the diagnosis of spontaneous cord hematoma is underestimated because the study with multiple sections is not routinely performed.

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How to sample medulla oblongata and medulla spinalis in cases of Sudden Infant Death Syndrome (SIDS)

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Key words: medulla oblongata, medulla spinalis, Sudden Infant Death Syndrome

Introduction. Some cases of SIDS can be related to abnormalities in nervous centers of brain stem (BS) (1, 2) and spinal cord (SC) (3) involved in central cardiorespiratory control. Here I depict the anatomopathologic technique that can be adopted to study medulla oblongata (MO) and medulla spinalis (MS) in cases of SIDS.

Methods of sampling. To study MO, BS is removed by sectioning cerebellar peduncles and cutting some millimeters proximally beyond the border between the MO and the pons and caudally some millimeters below the pole of the olive. After fixation in 10% buffered formalin solution this specimen is sectioned in blocks. In infants BS is divided into 3 blocks. The first block extends from the border between

MO and pons, up to the upper pole of the olivary eminence. The second block corresponds to the submedian area of inferior olivary eminence and extends 2 to 3 millimeters above and below the obex. The third block corresponds to the lower pole of the inferior olivary eminences and adjacent to the lower portion of MO. Every block is sectioned in toto and serially, in order to allow a thorough examination of all nervous centers. The first and the second blocks are sectioned in a cranial-caudal direction. Instead, the third block is sectioned in caudal-cranial direction. In stillborn, BS is entirely included in paraffin and serially sectioned. It is important to evaluate both cervical and thoracic portions of MS. The main nervous center to be examined in the MO is

the arcuate nucleus. It is a cardiorespiratory center of the ventral surface of the MO shown to be hypoplastic in SIDS victims. Such hypoplasia can have a great morphologic variability and be associated with alterations of other nervous centers of MO (2). The exam of cervical portion of MS can reveal subarachnoid haemorrhage (consequence of an injury to the cervical spine and spinal cord and caused by dystocia) that can give SIDS compressing nervous centers of cervical MS and BS. The thoracic portion of BS can show anomalies of the nucleus paragigantocellularis lateralis that controls cardiovascular reflexes and respiration thanks to connections with the arcuate nucleus and other centers of BS such as the ambiguus and the tractus solitarius nuclei.

Conclusion. Right autoptic examination of MO and MS in SIDS can show changes of some nuclei, especially hypoplasia of the arcuate nucleus that has been related to specific genetic mutation (4).

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How to sample pons, mesencephalon and cerebellum

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Key words: Parabrachial/Kölliker-Fuse complex, cerebellar cortex, dentate nucleus

Cerebellar and pontine-mesencephalic structures are essential to control a number of vital activities, such as acquisition of locomotor skills, muscular contraction, maintenance of muscle tone and balance, and respiration. Hypoplasia of the pontine-mesencephalic Parabrachial/Kölliker-Fuse complex, reduced synthesis of noradrenaline in the locus coeruleus, various developmental defects of the cortex and deep nuclei of the cerebellum, particularly the dentate nucleus (such as neuronal immaturity, intense c-fos and apoptotic expression) have been reported. The pathologic exam must include the study of the entire brain stem (1-3), from the rostral portion of the pons to the caudal portion of the mesencephalon, containing the parabrachial and Kölliker-Fuse nuclei and the locus coeruleus, as well as the whole cerebellum, carefully examining cortex and deep nuclei. The cerebellum is excised from the brain stem by cutting through the cerebellar peduncles before proceeding to the horizontal sections. The cerebellar hemispheres are cut on the sagittal plane in serial parallel sections at 0.5 cm intervals, beginning at the vermis and then proceeding to the right and left of the midline. Brain stem and cerebellum samples are fixed in 10% phosphate-buffered formalin, then embedded

in paraffin. Transverse serial sections are made at intervals of 30 µm (levels). For each level, twelve 5 µm sections are obtained, two of which routinely stained for histological examination using alternately hematoxylin-eosin and Klüver-Barrera stains. Additional sections are subjected to immunohistochemistry, particularly for the study of the c-fos protein, apoptosis, TH and the glial fibrillary acidic protein.

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How to sample the cardiac conduction system

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Key words: cardiac conduction system, sampling technique, sudden unexpected death

In order to examine the cardiac conduction system, the heart is sampled in two blocks for paraffin embedding. The cardiac conduction system is removed in two blocks for paraffin embedding. It is important to "save the pacemaker" by avoiding the customary cut of the right heart margin by driving lengthwise the intercaval bridge. Indeed, by this technique one happens to slash diagonally the sino-atrial node, together with the *Crista Terminalis*. Block 1 consists of a portion of the right atrial wall including the lateral half of the funnel of the superior vena cava, sulcus and *crista terminalis*. This block includes the sino-atrial node, its atrial approaches, the *crista terminalis* (with the upper 2/3 of the posterior internodal tract, the proximal part of the middle and anterior tracts), and the ganglionated plexus of the sino-atrial node (Fig. 1). Block 2 consists of the lower portion of the atrial septum, the trigonum fibrosus, and anterior contour of the coronary sinus and the upper 2/3 of the ventricular septum. This second block contains the atrio-ventricular node, His bundle, bifurcation, and bundle branches (Fig. 1) (1). The two blocks are serially cut.

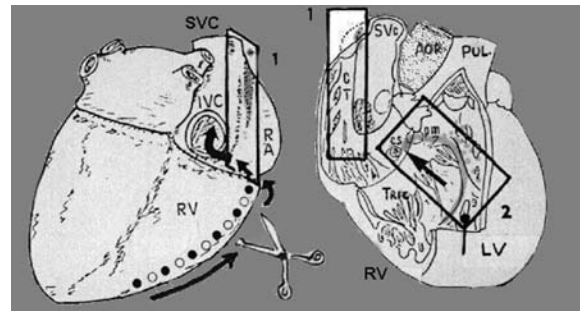


Figure 1. The procedure to remove the two blocks for the study of the cardiac conduction system. See text for details. SVC=superior vena cava; IVC=inferior vena cava; RA=right atrium; RV=right ventricle; LV=left ventricle; TRIC=tricuspid valve; CT=Crista terminalis; CS=coronary sinus; PM=pars membranacea septi

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Update on anatomo-pathological and medico-legal diagnosis of stillbirth and SIDS

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The pathogenetic mechanism of stillbirth and SIDS can be attributed to the triggering of vagal reflexes. This is due to an altered glossopharyngeal/vagal arc reflex, whose cross-point lies in the brain stem. The prevalent cause seems to be congenital abnormalities found in various nuclei, as previously described. To make a complete examination of the various nuclei throughout their extension, it is essential to perform serial analyses according to the following simplified procedure in which the brainstem is divided into four blocks. The first, cranial block extends from the border between the medulla oblongata and pons up to the upper pole of the olivary nucleus. The second, interme-

diante block, corresponding to the sub-median area of the inferior olivary nucleus, has the obex as the reference point and extends 2-3 mms above and below the obex itself. The third, caudal block, includes the lower pole of the inferior olivary nucleus and the lower adjacent area of the medulla oblongata. A fourth block contains the rostral portion of the pons and the adjacent caudal mesencephalon. Transverse serial sections are made through each block at intervals of 30 μm (levels). For each level, twelve 5 μm sections are obtained, three of which are routinely stained for histological examination using alternately hematoxylin-eosin and Klüver-Barrera stains, and the other 9 are immunostained for

the study of biological markers (TH, somatostatins, c-fos, apoptosis, etc.) or preserved for further investigations. The pertinent nuclei are outlined, namely the arcuate nucleus, the hypoglossus nucleus, the dorsal vagus motor nucleus, the tractus solitarii nucleus, the ambiguus nucleus, the trigeminal tractus and nucleus, and the ventrolateral reticular formation in the medulla oblongata; the locus coeruleus, the parabrachial/Kölliker-Fuse complex in the ponto-mesencephalic sections. In order to examine the intermediate lateral nucleus, or effector of the orthosympathetic reflexes, it is necessary to evaluate the spinal cord in the cervico-thoracic tract, in particular from T1 to T5.

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Observation of the histological preparations of nuclei of pons, mesencephalon and cerebellum

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Key words: Parabrachial/Kölliker-Fuse complex, cerebellar cortex

Introduction. The aim of the present study is to examine in histological sections the cytoarchitecture of the parabrachial/Kölliker-Fuse complex, a ponto-mesencephalic structure involved in the respiratory activity control, and the cerebellar cortex maturation.

Microscopic observations. Parabrachial/Kölliker-Fuse complex: this complex, extending from the rostral dorsolateral region of the pons to the caudal portion of the mesencephalon, is made up of the lateral parabrachial nucleus (IPB), located between the superior cerebellar peduncle (scp) and the lateral lemniscus, the medial parabrachial nucleus (mPB), medially to the scp, between the locus coeruleus and the ventral termination of the scp, and the Kölliker-Fuse nucleus (KF), a group of large neurons with an eccentric nucleus, abundant cytoplasm and Nissl substance, ventrally located to the IPB between the medial limit of the scp and the medial lemniscus. The KF includes the compactus and the dissipatus subnuclei (Fig. 1). Cerebellar cortex (CC): only from 30th gestational week, a four-layered structure is recognizable formed by the external granular layer (EGL), 6-8 rows of small round cells, the molecular layer (ML), that contains several migrating EGL cells, the Purkinje cell layer (PL) with 5-6 cell thick layer of round large neurons, and the internal granular layer (IGL) with numerous small round cells. From the 2nd postnatal month the EGL becomes progressively thinner and disappears from the 12th month of life. The PL is a sin-

gle layer of large cells. The CC shows the three-layered definitive structure (ML, PCL, IGL).

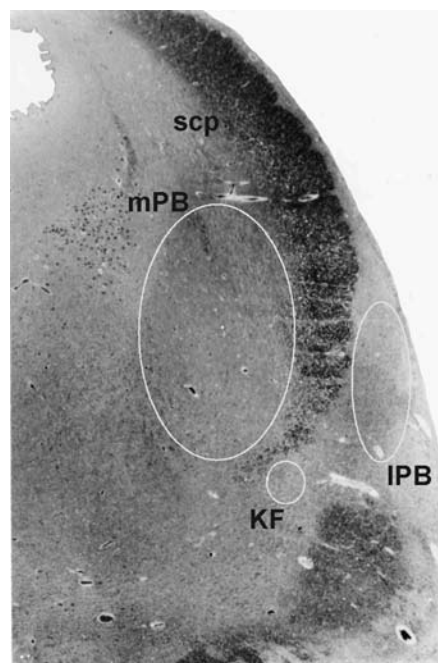


Figure 1. Kölliker-Fuse nucleus (KF), medial parabrachial nucleus (mPB), lateral parabrachial nucleus (IPB) and superior cerebellar peduncle.

Histological Observation of the cardiac conduction system in the diagnostics of sudden infant death syndrome (SIDS)

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Key words: cardiac conduction system, postnatal morphogenesis of the atrio-ventricular junction, SIDS

Introduction. SIDS cases do not present any abnormality of the ordinary myocardium, while the *core* of the heart, where cardiac rhythm arises and spreads, can be abnormal, representing an insight to the solution of the problem (1).

Materials and Methods. To examine the cardiac conduction system, two blocks for paraffin embedding are prepared. The first block contains the junction of superior vena cava and right atrium encompassing the entire area of the sinus node; the second block contains the atrio-ventricular node, His bundle down to bifurcation and bundle branches. The serial section is a must in the conduction system investigation (1).

Results. Areas of resorptive degeneration were detected in 97% of the SIDS cases and in 76% of the controls. The accessory pathways were mainly Mahaim fibers (Fig. 1), detected in 22.5% of SIDS and in 8% of controls. Dispersion or septation of the atrio-ventricular node and/or His bundle, was observed in 41% of SIDS cases and in 16% of control cases. Cartilaginous meta-hyperplasia of the central fibrous body was present in 6% of SIDS and in 4% of controls. Dualism of the atrio-ventricular node and/or His bundle was present in 3% of our SIDS cases, and absent in controls.

Discussion. Frequent alterations are the accessory atrio-ventricular pathways, mainly of Mahaim type (Fig. 1). These fibers can play an important role as pathogenic background of a significant number of arrhythmogenic SIDS (1).

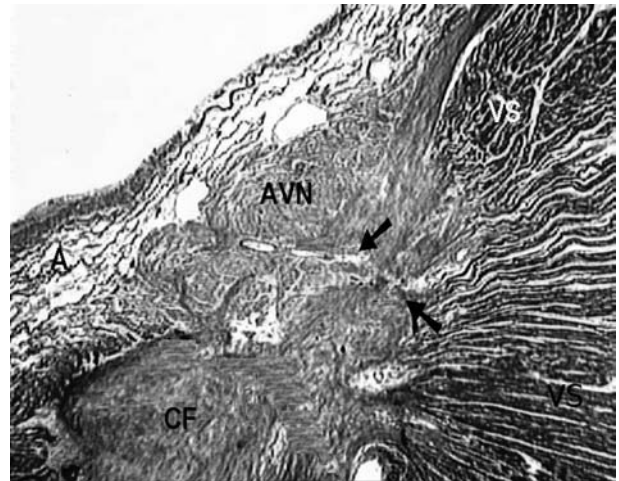


Figure 1. Mahaim fiber (arrows) in a SIDS case, bridging the atrio-ventricular node (AVN) with the ventricular septum (VS), through the central fibrous body (CF). Trichromic Heidenhain, 25x

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Inclusion, cut, staining and immuno-histochemical techniques of the drawn samples

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Key words: cardiac conduction system, Klüver-Barrera, somatostatin

The procedure for the accurate pathological study of each case of SIDS and perinatal death includes the following autopsy samples (Tab. 1):

The samples of the point A) are autopsy samples so

that they are treated with the usual histologic techniques. After fixation, the samples of the point B) are manually processed, embedded in paraffin with metallic moulds because they are bigger or taller than the routine samples. Using a

Table 1.

<p>A) System samples</p> <ul style="list-style-type: none"> • Respiratory (lung, trachea, bronchi, larynx, vocal cords) • Digestive (tongue, oesophagus, stomach, liver, pancreas, small and large intestine) • Endocrine (adrenal gland, thyroid, epiphysis cerebry) • Immune (spleen, thymus...) • Skeletal apparatus (rib...) • Genito-urinary (kidney, bladder, gonads...) • Central and Peripheral nervous (brain, midbrain, cerebellum, spinal cord) • Cardiovascular system 	<p>B) Serial samples</p> <ul style="list-style-type: none"> • Conduction system (Atrio-Ventricular node; Sino-Atrial node) Mediastinal Ganglionic plexuses: <ul style="list-style-type: none"> • Coronaric • Intertruncal Intercarotid neuroreceptors • Cardiac Nervous system <ul style="list-style-type: none"> Carotid bifurcations: <ul style="list-style-type: none"> - on the right - on the left Cervical sympathetic ganglion: <ul style="list-style-type: none"> • Right stellate • Left stellate • Spinal cord • Brain stem
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normal manual sledge microtome, adapted for tall specimens, each block is entirely cut in series to ensure a detailed examination. The obtained slides are stained at predetermined intervals to leave the most remaining part of the blank slides in archive for eventual investigation follow up. We employ histochemical stains, such as AZAN for cardiac conduction system and Klüver-Barrera for brainstem, also utilized for morphometric analysis. Besides immuno-histoche-

mical reactions on silanized blank slides, such as the neurohormone Somatostatin and the enzyme TH that regulate the respiratory and cardiovascular activity are performed. Our studies on the brainstem include the use of the gene C-FOS that activates in presence of hypoxia and the homeogene EN-2, expressed during arcuate nucleus envelopment. Lastly, we employ the method for Apoptosis to study the programmed cell death following a definite genetic program.

Acute metabolic failure in infancy

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Key words: acute onset inborn errors of metabolism, infant acute metabolic failure

Introduction. Inborn errors of metabolism (IEM) are rare conditions individually, but collectively numerous. Experience in dealing with IEM is usually limited, diagnosis requires unusual exams, and treatments also are unusual and often complex. Most IEM present in the neonatal period or during infancy, especially those with acute onset.

To suspect IEM. The outset of IEM is unspecific and often mimics sepsis, but some clues allow to suspect the diagnosis. The first step is an accurate collection of history, including obstetric (previous miscarriage or still birth, fetal movements and maternal health during index pregnancy) and family history (parental consanguinity, previous neonatal or sudden infant deaths, any similarly affected children within relatives, or known IEM). Other clues are symptom free interval, type of feeding, correlation between food ingestion and symptoms as well as between fasting and symp-

toms, symptom progression.

Presentation. Infant onset IEM may be due to 1) *problems in the synthesis of complex molecules* usually resulting in dysmorphic features at birth due to altered embryogenesis such as in peroxisomal biogenesis disorders {e.g. Zellweger syndrome, block in cholesterol synthesis, block in glycosylation [e.g. carbohydrate deficient glycoprotein (CDG) Ia]}; 2) *failure in breaking complex molecules* resulting in their storage as they are not cleared. Storage disorders do not usually present over the first year. This category includes mucopolysaccharidoses (MPS), gangliosidoses, glycolipidoses; 3) IEM caused by *intoxication* begin with initially healthy babies, but as feeding proceeds and the toxic metabolite accumulates, they become sick with poor feeding and eventually encephalopathy, usually within the first 72 hours. Sepsis as well as CNS or cardiac problems should always be ruled out.



The initial symptom free period is a major clinical clue. Examples are: urea cycle defects due to failure in ammonia clearance, organic acidaemias (OAs) due to amino acid breakdown failure, galactosaemia, due to a block in galactose metabolism; 4) symptom free period is not present in *energy insufficiency* IEM and they may present with immediate onset such as in congenital lactic acidoses (CLAs), even if with individual range of severity. Symptoms appear earlier than in intoxication IEM, but can require longer time to fully decompensate. Energy production may also fail if the fuel supply (feeds) is interrupted for several causes or energy requirements increased. CLAs include respiratory chain disorders (RCDs) and pyruvate metabolism disorders. IEM due to energy supply failure are fat oxidation defects (FAOs), glycogen storage disorders (GSD) and gluconeogenesis defects. *Many IEM have no specific clinical findings*, including respiratory distress, hypotonia, poor sucking reflex, jaundice, hepatomegaly, liver failure, vomiting, diarrhoea, dehydration, lethargy, myopathy, cardiomyopathy and seizures, sometimes resistant to the usual anti-convulsant drugs (e.g.: seizures of non-ketotic hyperglycinaemia respond to dextromethorphan and sodium benzoate, biotinidase deficiency responds to 10 mg biotin daily, and can be started whilst awaiting the results, serine is effective in controlling the seizures of 3-phosphoglycerate dehydrogenase deficiency). Sodium valproate should be avoided when a metabolic disease is suspected. Symptom progression often leads to coma. Many IEM have *ocular findings*, as corneal clou-

ding (mucopolysaccharidoses, cystinosis, tyrosinaemia type II, I-cell disease), cherry red spot (Tay Sachs, Niemann Pick A,C&D, GM1), and pigmentary retinopathy (RCDs, FAOs, peroxisomal disorders, CDG). Cataracts may be present in the neonatal period (galactosaemia, peroxisomal disorders, RCDs) and may be easily missed. *Cardiac findings*: cardiomyopathy may be associated with RCDs, FAOs, OAs, CDG and storage disorders. Hypertrophic cardiomyopathy is more usual. See Table for exams.

Samples to be collected in case of sudden death.

Blood 10 ml (lithium heparin) for red cells (store 4°C), plasma (store -20°C), blood spots on Guthrie card, blood 5 ml (EDTA) stored for DNA, urine 10-20 ml (plain tube, store -20°C), skin biopsy (store in culture medium or saline 4°C) and muscle & liver biopsy (snap frozen in liquid nitrogen within 1 h of death) for enzymology/histochemistry.

Emergency management. Before diagnosis is made, unspecific emergency treatment should be started to avoid further intoxication (stop feeds), promote anabolism (high energy intake: i.v. dextrose and, if needed, insulin), correct electrolyte imbalance and dehydration. Specific vitamin co-factors, such as biotin (10-20 mg/day), riboflavin (20-40 mg/day), cobalamin (1-2 mg/day), thiamine (10-50 mg/day) may help. In case of severe hyperammonaemia and/or OAs, elimination of toxic metabolites is urgent: sodium benzoate or phenylbutyrate for ammonia, carnitine for organic acids and/or peritoneal dialysis (for neonates), haemodialysis or continuous haemofiltration.

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A proposed guideline for metabolic investigation in case of suspected Sudden Infant Death Syndrome (SIDS)

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Key words: sudden infant death syndrome, tandem mass spectrometry, fatty acid β -oxidation disorders

Introduction. SIDS represents a dramatic event for families and paediatricians. A multidisciplinary approach is required in the attempt to make a causal diagnosis and doctors who are involved in post-mortem evaluation should apply a standardised diagnostic protocol and identify a physician responsible to co-ordinate investigations and family counselling. Metabolic investigations (MI) (1), recently renamed “metabolic autopsy”, should be routinely performed in addition to clinical and family history, physical examination, death scene investigation and autopsy (Fig. 1).

Metabolic investigations in case of SIDS. The first diagnosis of an inborn error of metabolism (medium-chain acyl-CoA dehydrogenase deficiency) in an infant initially classified as SIDS was made in 1984. Subsequently several cases of SIDS have been reported to be associated with fatty acid β -oxidation disorders (FAOD), confirming the opportunity of MI in SIDS for a definitive diagnosis. In addition to FAOD, other metabolic diseases are now recognised to be responsible for sudden death through an acute metabolic crisis. Respiratory chain disorders, congenital lactic acidosis, ketogenesis defects and biotinidase deficiency may present with an unexpected death without any preceding symptom. Organic acidurias, urea cycle and carbohydrate disorders generally have a latency period with intoxication or storage symptoms so that, if unrecognised, may sometimes dramatically progress to death. Although the frequency of inborn errors of metabolism among unexpected deaths has been reported to be approximately 5%, post-mortem MI has not yet been routinely performed for the complexity of the testing procedures. New techniques, particularly tandem mass spectrometry (TMS) (2, 3), coupled with the continuous improvement of genetic analysis, have completely changed the approach to the diagnosis of metabolic disorders, facilitating the identification of a wide range of inborn errors of metabolism using tiny samples of blood, plasma and bile, and performing specific enzyme assay or DNA mutational analysis on cultured skin fibroblasts, and muscle or liver biopsy. Therefore in case of SIDS it is mandatory to promptly collect appropriate biological samples, to keep and send them correctly to specialised laboratories for MI. How to collect

samples and which MIs are required for SIDS is shown in the table 1. It is important to stress that: a) samples of blood, urine and cerebral spinal fluid must be collected immediately post-mortem (if necessary bladder catheterization, supra-pubic puncture and heart puncture are required); b) muscle biopsy should be performed within 4 hours from death, otherwise the evaluation of activity of respiratory chain enzymes will not be reliable; c) skin biopsy can be performed soon after death or during autopsy, but in the latter case fibroblast culture may not be successful; d) liver biopsy and bile sample should be collected during autopsy. Finally it is recommended to get back neonatal screening cards from regional centre in order to compare the results.

Conclusion. This brief report summarises a proposed guideline for MI suspected SIDS. Timely collection and appropriate handling of the biological materials are of para-

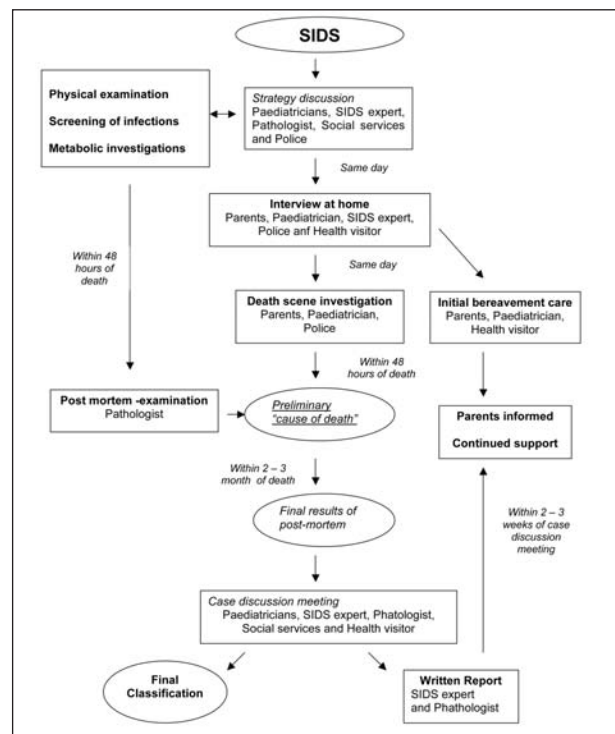


Figure 1. Flow-chart of SIDS management

Table 1. How to collect samples for metabolic autopsy

Sample		Handling	Defect / Disorder
Urine	10 ml	Sterile vial; freeze and store at - 20°C	Carboxylic acids*
Blood heparinized	10 ml	Plasma; freeze and store at -20°C	Amino acids** and Acylcarnitine*** profile
Blood spot	drops	Filter paper, dry at room temperature, do not put into plastic bag	Amino acids** and Acylcarnitine*** profile
Blood on EDTA	5 ml	Freeze and store at - 20°C	DNA analysis
Whole Blood	2 ml	Serum, freeze and store at - 20°C	Intermediate metabolites
Cerebrospinal fluid	3 ml	Freeze and store at -20°C	Aminoacid profile** (glycine)
Skin biopsy	3 x 3 mm	Sterile medium or saline; store at 4°C and send for fibroblast culture	Enzyme assay
Muscle biopsy	100-250 mg	Snap freeze at liquid nitrogen and store at - 70°C	Enzyme assay, respiratory chain complexes, DNA analysis
Liver biopsy	10-20 mg	Snap freeze at liquid nitrogen and store at -70°C	Enzyme assay, DNA analysis
Bile spot	Drops	Filter paper, dry at room temperature, do not put into plastic bag	Amino acids** and Acylcarnitine*** profile Amino acids** and Acylcarnitine*** profile
Bile	2 ml	Freeze and store at - 20°C	

* Organic acidurias (methylmalonic aciduria, propionic acidemia, isovaleric acidemia)

** Urea cycle disorders (i.e. ornithine transcarbamylase def.)

*** Fatty acid β -oxidation deficiency (i.e. medium chain acyl-CoA dehydrogenase def.)

DNA analysis or enzyme assay: Diagnostic confirmation of suspected disease; Respiratory chain disorders, Botinidase deficiency; Congenital lactic acidosis (i.e. pyruvate dehydrogenase deficiency); Carbohydrate disorders (i.e. galactosemia, hereditary fructose intolerance)

mount importance for the accurate diagnosis of inherited metabolic disorders. Parents should be informed of the used approach, and the time required (usually months). If data confirm a metabolic defect then genetic counselling is mandatory.

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ALTE management in a large city area

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Key words: ALTE, epidemiology

Applying the data of the incidence of ALTE provided by the literature to a medium-sized city like Bologna (2,943 residents < 12 months old, 3,044 newborns alive, over 5,000 entries of infants >12 months old in the emergency unit – Data referred to 2004), one expects 15-30 cases of ALTE every year. In Bologna, the cases of ALTE are treated in different care units: the University Clinic (emergency unit,

neonatology, pediatric intensive care, infant ward), the pediatric department of a general hospital (emergency unit and pediatric ward) and by over 40 family pediatricians. These events are not frequent and are scattered among several healthcarers.

The pending problems are of epidemiological and caring nature:

- To obtain complete records of all cases, it is necessary to implement a network of all healthcarers that may be involved with children with ALTE. The data can't be retrieved afterwards based on the hospital data, since no ICDM9 code is available for ALTE.

- Setting up a common protocol for diagnosis and follow up would help to treat more uniformly children with previous ALTE.

- It would be advisable to establish definite criteria for monitoring the infants at home, and one reference point only to supply and check the monitoring device.

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Who should home monitors be given to?

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Key words: SIDS, home monitors, ALTE

Home apnea monitors have been prescribed since the early '60s for "preventing the Sudden Infant Death Syndrome (SIDS)", even though the effectiveness of such intervention has not been demonstrated.

A recent Policy Statement¹ indicates that home monitors are useful in the event of apnea, respiratory failure, airway obstruction, interruption of supplemental oxygen supply, or failure of mechanical respiratory support.

Infants with a history of apparent life-threatening events (ALTE), former preterms (symptomatic and/or affected by pulmonary bronchodysplasia), technology-dependent children, and infants with tracheostomies may be susceptible to the above mentioned events and are therefore potential candidates for this intervention.

Home monitoring has changed profile over the years, moving from a generic prevention measure to a more targeted strategy, adoptable in infants exposed to hypoxemic events which are potentially life-threatening or harmful for the integrity of the central nervous system.

New brands of home memory monitors incorporating both pulse oximetry and trans-thoracic impedance are suitable for this electronic surveillance.

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Who must manage the monitor?

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Key words: monitor, ALTE, SIDS

Introduction. When an infant with apparent life threatening event (ALTE) or newly born infant sibling of SIDS is discharged from hospital, he/she needs a daily monitoring.

In such cases who must manage the home-monitor? (1)

The actors and their actions

- Hospital:
 - trains the parents to use the monitor, teaching

- them how to switch it on and off, to position electrodes, to recognize wrong alarms, to take reports of all the data;
- trains the district operators (doctors and nurses) by discussing all the problems with them before the patient's discharge, listing all the nursing items that can be necessary at home and explaining them the use of the monitor;
- takes care of the software, checking data every 15-30 days and evaluating all the registered alarms (2).
- Family:
 - Carries out the practical management of the monitor;
 - takes records of all the events;
 - notifies the hospital every little machine default.
- Primary care department:
 - provides home nursing care that can be useful for parents' support, monitor check, and data records;

- family paediatrician is responsible for the therapeutic part, and active or not the home nursing care.

Conclusions. The integration among the above cited actors is the guarantee of a safe home monitoring (3). A right flow of informations about all cases of ALTE or SIDS would improve the system.

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The voice of the scientific societies

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Key words: scientific societies, ALTE

Introduction. Scientific societies are the guarantors not only of the solidity of the scientific bases but also of the formative events and also of their pedagogical quality and effectiveness (agreement between the Italian government and the Conference of the Scientific Societies, 2003).

Discussion. In particular ALTE is defined, by the National Institutes of Health, as an episode that is frightening to the observer and is characterized of apnoea, colour change (cyanotic or pallid) marked change in muscle tone, choking or gagging. The infant, admitted to hospital, through the Emergency Ward, may need resuscitation or may appear, following a crisis, to be in a stable clinical condition. The ESPID, the SIP, the AFP and other societies have elaborated the ALTE management guidelines. The most important part of the evaluation

is the medical history taken from parents or caregivers but it is also important to examine the infant in detail and to make a diagnostic evaluation based on the results of investigations and laboratory checks of first level and, if necessary, more specifically, of second level. Up to 50% of all ALTEs are unexplained. In these cases or cases in which there is a high risk of repetitive crisis it is useful to apply the cardiorespiratory home monitoring preferably with ECG, oxygenation and event memory. However it must not be used as a strategy to prevent SIDS (AAP 2005).

Conclusion. A systematic diagnostic evaluation of infants with an ALTE, together with a comprehensive treatment programme, should increase survival and quality of life for most affected infants (ESPID 2003).

Effect of home monitoring (HM) on the parents' emotional stress

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Introduction. HM is given to infants who had an Apparent Life Threatening Event (ALTE), to siblings of SIDS, to infants with recurrent apnoea (1). The parents' emotive impact of HM is up to now controversial: some researches have suggested that HM increases emotional stress while others report support and comfort (2).

Results. Between Sep 1994 - Sep 2004 HM was started at our SIDS centre in 58 cases, 28/58 for ALTE *sine causa*. We carried out a survey on the emotional experiences of these 28 families: parents answered to Self-Rating Anxiety Scale and a questionnaire. Ninety-five percent were *less anxious* and *more peaceful* after HM than during HM, even if their levels of anxiety were on the average. All parents outlined the advantages of HM, and 30% of them were also worried about it. Differences were shown in the perceived benefits reported by mothers and fathers: 37% of mothers indicated that "*with monitor they were able to sleep again*"; 90% of fathers found in HM a possibility to intervene in time. Anxiety also showed different levels: 20%

of mothers evidenced that "*to stop the monitoring was difficult*" instead 30% of fathers was disturbed by false alarms.

Discussion. Both parents felt stress and anxiety during HM after ALTE *sine causa*. They all valued HM as positive, but for different reasons. Mothers appeared more satisfied and benefited by HM than fathers, probably because they spent more time alone with the infant.

Conclusion. To propose HM after ALTE should take into account the different approaches to mothers and fathers.

References

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