

I SESSION: Nutrition and growth

Chairman: C. Fabris

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NEWS ON NUTRITION

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Survival of preterm infants has markedly improved during the last years. Early nutritional support of pre-term infants has a major impact on long-term health outcome. Current nutritional policies are based on the needs of healthy preterm infants and aim to sustain a postnatal growth (i.e. nutrient retention) similar to the intrauterine growth of a healthy fetus. The needs of protein and minerals are still discussed. In the few first weeks of life a protein deficit develops, so additional protein intake is necessary for early catch-up growth. Protein intake and the protein: energy ratio seem to be the major determinants of weight gain. An increase of the protein-energy ratio will improve the lean body mass accretion and limit fat deposition. Some studies suggest that protein intake should be adapted for post-conceptional age (PCA) instead of gestational age (GA) or birth weight.

The proteins have effects beyond the simple provision of substrate for protein synthesis. During the process of protein digestion biologically active peptides are released, which have regulatory effects on metabolism, gut function, and protein synthesis.

After birth, there are important physiological changes in bone metabolism resulting mainly from reduction in mineral supply and changes in hormonal environment. The remodeling process leads to an increase in bone resorption and a decrease in bone density. Mineral intake must allow an adequate mineralization, lowering the risk of fractures and clinical symptoms of osteopenia.

Fetal programming is the phenomenon whereby alterations in fetal growth and development in response to the prenatal milieu have long term or permanent effects. Evidence for fetal programming of body composition and musculoskeletal development rises from epidemiological studies and research dealing with the role of early undernutrition. Low birth weight and prenatal undernutrition are associated with changes in body composition (altered fat distribution, reduced muscle mass and strength, and low bone mineral content). The underlying mechanisms include a direct effect on cell number, altered stem cell function and new regulations of hormonal axes.

The factors that restrict fetal growth or cause low birth weight can also alter lung development and may lead to long term effects on lung function and respiratory health. Experimental studies show that the environmental insults may alter lung structure and function, increasing the risk of respiratory illness. Furthermore, early nutrition may influence (program) cardiovascular

health. Accumulated evidence shows the long-term effects of overfeeding in animals, and in human newborns is now well-recognized the beneficial effect of breast-feeding on the major components of the metabolic syndrome (obesity, blood pressure, cholesterol metabolism, and insulin resistance).

The study of changes in body composition may help to understand the nutritional needs for the infants, as well as the outcome of nutritional management in terms of neonatal programming of the adult morbidity.

Growth monitoring is necessary to adapt the nutritional options to the needs of Infants and to prevent under- or overfeeding.

Infant formulae are alternatives to breast milk for infants unable to continue breastfeeding through the first year of life. Ideally, the formulae should be designed to allow infants to achieve a growth and development close to breastfed infants. Recently, bioactive (functional) nutrients have been added to formulae: long-chain polyunsaturated fatty acids, probiotics and prebiotics, and nucleotides. Long-term follow-up data are needed in infants fed the newer formulae, to understand the role of functional nutrients. Oral insulin supplementation may have beneficial effects on intestinal maturation. Insulin is present in human milk and links to specific receptor on enterocytes. Oral insulin enhances the epithelial proliferation in the intestinal mucosa, the maturation of enzymatic system, and has some systemic effect.

Until recently, nutritional research focused on nutrient deficiencies and impairment of health nutritional genomics is considered one of the next frontiers in nutritional field. "Nutrigenomics" acts as a junction between health, diet, and genomics, and investigates the interface between the nutritional environment and cellular/genetic processes. It aims, through the introduction of certain nutrients, to reverse or modify the alterations of gene expression that may alter a normal, healthy phenotype.

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NUTRITION OF VLBW INFANTS

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Intrauterine growth is still the current reference standard for the growth of the preterm infant (1). Anyway, most of the very low birth weight (VLBW) infants, and especially the extremely low birth weight (ELBW) infants, accrue a significant growth failure during hospital stay. The consequences of this poor growth are still controversial, but it seems reasonable that preventing an excessive growth failure in the early post-natal period reduces the need of an intense and not-physiologic catch-up growth after discharge.

Preterm infants cannot be grouped in a single category, as they have very different nutritional needs in relation to their birth weight. As calculated by factorial and empirical approaches ELBW infants require a protein intake of 4 g/kg/day at least and an energy intake ranging from 105 to 120 Kcal/kg/day (2,3). These high nutritional needs are often not satisfied because of concurrent illnesses (necessity of fluid restriction, metabolic instability, drug therapy, surgical procedures), feeding intolerance or, merely, because of lack of information on this peculiar aspect of neonatal care. As a consequence, nutritional practices vary dramatically among different intensive care units. For instance, many clinicians are still reluctant to provide a sufficiently high parenteral amino acid supply from the first few days of life, although it is well known that even an intake of 3g/kg/day from the first day of life is well tolerated and allows a positive protein balance without a metabolic stress (4). Things become more complicated when enteral nutrition is established. NEC is still a matter of concern and is responsible of delayed and repeatedly withheld enteral feeds. Human milk, and specifically own mother's milk, provides several extra-nutritional advantages (immune protection, bifidogenicity etc.) but does not fit the huge nutritional needs of the ELBW infants. Even after fortification donor milk does not allow the growth rate of the reference foetus.

Another matter of serious concern pertains to the composition of current preterm formulas, which is not adequate to satisfy the high protein requirements of these growing infants. In fact, only in the last few years a pre-term formula, specifically designed for the nutritional needs of the ELBW infants, has become available in Italy. This formula is characterized by a protein content of 2.64 g/100 ml and a protein/energy ratio of 3.2 g/100 kcal. The protein requirement of 4 g/kg/day can be satisfactorily supplied with a volume intake of 151 ml/kg/day and a caloric intake of 124 Kcal/kg/day (5). On the contrary, using "old" preterm formulas with a lower protein/energy ratio (for instance 2.6-2.8 g/100 kcal) it would be necessary, in order to provide an intake of 4 g/kg/day of proteins, to feed greater milk volumes (up to 180 ml/kg/day) with a disproportionately high energy supply (150 kcal/kg/day). Recent data show that a better weight gain without evidence of metabolic stress can be obtained with an experimental preterm formula with a protein content of 3.6 g/100 kcal (6). The usefulness of hydrolyzed preterm formulas both in improving early feeding tolerance and preventing allergic disorders is still unclear (7). Controversial data still exist on the possible benefits of LcPUFAs on visual acuity and neurocognitive development of these infants (8,9); even though no precise institutional recommendation on the necessity to supplement preterm formulas with long chain polyunsaturated fatty acids is available, all the current preterm formulas are supplemented with LcPUFAs.

At discharge from NICU most of the VLBW and ELBW infants are growth restricted. Therefore post-hospital nutrition is crucial to sustain the catch-up growth and to improve the nutritional deficits acquired during hospital stay. Several dietary attempts have been proposed to achieve this goal, using either preterm or specific post-discharge formulas for a variable period after term (10). Beneficial effects have been obtained on growth, especially in male and SGA infants, with different results on weight, length and head circumference (10). Little or no effect has been obtained on bone mineralization and neurocognitive development and no data exist on long term effects (growth, metabolic and cardiovascular health) (11).

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PROGNOSIS OF SMALL FOR GESTATIONAL AGE CHILDREN (SGA) AND THEIR GH TREATMENT

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The international literature has been enriched in these late years with many studies about intrauterine growth retardation (IUGR), often improperly assimilated to small for gestational age children. For several reasons it isn't always possible to identify a cause for an intrauterine growth retardation, as it would be expected from a correct IUGR definition; in fact, in the group of IUGR children are included subjects where the phenotype is due to genetic disorders or different ethnical or environmental background (altitude for example), placental suffering, maternal deprivations, maternal pathologies, maternal habits (smoke abuse, alcohol), fetal pathologies, drugs...

It is clear that when the pathology has a genetic cause or a fetal beginning, the subject's destiny will depend on the disease and its therapeutic possibilities. It is difficult to formulate an aetiological diagnosis for many subjects, since we only can certify a low weight or length or both. It is essential to have reference para-

meters and a cut off below which there is this condition and upon which we can find normal children. This is for some aspects arbitrary and it is between the 3th and the 5th percentile for some authors (1), - 2 SDS for others (2,3), and below the 10th percentile for most of them (2,4). Since it isn't always possible to compare the weight of children different for ethnic features and geographical conditions, the reference parameters are often considered with criticism. It is suitable to use national standards to better compare the values, as we luckily do in Italy (6).

As concerns term newborns, in a recent study the Sweden Goteborg group evaluated the small for gestational age total rate about 5,4 % of living newborns, and these children according low weight and length were so classified: 1,5 % was both low weight and length, 1,6 % was only low weight and 2,4 % was only low length (7). We evaluated the incidence of these children using last 10 years data (about 600000 newborns) we get from Hospitals of Veneto, Friuli Venezia Giulia and independent Trento and Bolzano provinces, which follow common neonatal screening programs. The incidence was between 5,88 % and 7,1 % and for the most part they were term children, so our SGA number was higher than the Swedish one (5,5 vs 3,1). Actually our data are only about weight and we have to examine length data. Moreover Swedish data refer about 20 years ago when treatments were very different. These data are interesting and have to be carefully examined, because of the pool's consistency and numerosness, though the survey is made by different operators. There are also many post-term newborns whose weight is between appropriate and inappropriate, and this data must be examined with greater attention to verify if it is due to a wrong gestational age or to pathological conditions not identified during pregnancy. Many authors report a higher rate of preterm SGA than term SGA (8) and a greater mortality and morbidity (9) of those children that are both low weight and low gestational age. An effective prevention is hard because intrauterine echographic diagnosis is difficult and echography's accuracy is about 50 % (10), though recently Doppler echography and the functional haemodynamic valuation of fetal-placental unit allowed many diagnostic improvements (2).

Most investigations are been carried out by 3rd level centres and also in our experience there is a contrast between values obtained from total population and those from our newborns, though data about mortality have to be completely reviewed. During pregnancy, prevention of respiratory diseases by cortisone, of infections by antibiotics administered to running risk women, and above all therapeutic possibilities from surfactant's use for hyaline membrane disease reduced to less than one sixth total mortality of both appropriate and inappropriate for gestational age weight preterms.

It is a confirmed data that IUGR and also SGA children have a short stature when adults, so that they repre-

sent 20-25 % of all subjects with final short stature (7). The future of most of them (87 %) is turning out well by the first months of life or at the latest by the end of the first year. Small for gestational age term newborns have as temporal limit the first two years of life by which they have good possibilities to catch up children born at normal size. They have good probabilities to have a - 0,7 SDS final stature if they were low birth length babies and a - 0,5 SDS final stature if they were low birth weight. A few of them don't catch up the others by the end of the first two years (13 %), and so they probably could have a -1,8 SDS final stature if they were low birth length babies and a -1,7 SDS if they were low birth weight babies (11). The risk of having a short final height seems to be 5 times higher for children with a low birth weight and 7 times higher for those with a low birth length in comparison with children with a normal birth size (11). A recent French study showed that statural lack could be quantified as 8 cm for males and 10 cm for females. A constant of these children seems to be a shorter puberal spurt (12). Not all newborns both preterm and low birth weight catch up children born at normal size, and when they do (between 72 and 80 %), this happens later in time, usually by the end of the 4th year of life, without sexual differences and with a similar trend for low weight or low length children or both low weight/low length children (13,14). According to some authors, breastfeeding could give to these subjects a better growth, particularly during the first year of life (15). However it is difficult that this effect could spread further in time and lead these children to a normal final stature, so for term children that didn't catch up growth during the two first years of life and for preterms that didn't catch up growth during the first four years new treatments had been evaluated and particularly the possibility of GH treatment. There are evidences that the levels of insulin-like growth factor (IGF1) and IGF1-binding protein-3 (IGF-Bp3), both depending on GH production, are reduced in these subjects during fetal life compared with the reference values, and they don't always increase later in life (2,16,17). Moreover, there are evidences that subjects who don't catch up growth still maintain reduced IGF1 and IGF-Bp3 levels during prepuberal time and that this condition can be modified by GH treatment (18). Many treatment programs have been carried out and all agree about the short-term efficiency of GH therapy for these subjects; there are perhaps some doubts about therapeutic dose to employ, if it would be better a medium low dose (3 UI/m²) with a catch up growth during the first 4-6 years of treatment or a high dose (33-67 µg/K die), alternating if necessary treatment's periods with periods without treatment (19). Few studies can be found in literature about final stature of GH treated SGA children's and these studies are all based on national (20) or international (21) registers where neither doses nor therapeutic practices have been, obviously, controlled. Consequently, the results are contradictory: slightly pessimistic (20) or enthusiastic (21), and particularly

they don't elucidate the role of puberty that, as we have remembered, could play an important role, and it isn't clear if a possible lack in growth up at puberty could be offset by a more rapid growth during prepuberal time. At this time, the prescription is allowed in France only for patients with a growth less or equal to -3 SDS, and the first results seem to be hopeful. We have studied short-time results and they are optimistic (some patients were studied for 5 years). In a short time almost all major studies about final stature will ending, and all main researchers agree with the necessity to use higher doses of GH compared with that used now for GHD treatment and with the necessity to begin GH treatment as soon as possible, if necessary before the 4th year of age.

However, the major interest is shifting, after Barker studies and others, from difficulties to catch up a final stature within normal range, to possible correlations between low birth weight and pathologies with adult onset. In 1986 Barker showed a significant correlation between mortality for cardiovascular accidents and infant mortality and this correlation was true for both males and females and for the different geographic areas examined. These observations were extended to a further period of time and to social-economical conditions of the considered population and it was clear that the improved conditions of life and scientific discoveries (as for example antibiotics) had reduced mortality due to poverty, but highlighted that due to ischaemic heart disease in the same areas (22). Later, 16000 men and women born between 1911 and 1930 were followed up from birth to 1995 and it was observed a drastic mortality reduction for ischaemic heart diseases when birth weight shifted from 2500 g to 4310 g (23). The theories about possible effects due to a subject's adjustment to hard life conditions, during a time when different organs and systems are programmed and developing, don't seem very convincing. These studies also evidenced that, consensually to cardiovascular diseases, the frequency rate of non insulin dependent diabetes mellitus and glycaemic intolerance increased (24). The rate of subjects with type 2 diabetes mellitus and glycaemic intolerance was examined among 364 males of 64 years old living in Hertfordshire (Britain). The 40 % of subjects who had a birth weight less or equal to 2500 g, had the one or the other pathology, and this percentage decreased to 14 % in subjects with a birth weight about 4310 g or more (24). The same trend was observed in low weight subjects during the first year of life (24). The patients' age caused some interpretative problems because of the lapse between the deprivation time and the time when the pathology was manifested. Younger subjects were studied and there was evidence that a wrong insulin and glycaemic response was already present in 20-21 years old low birth weight subjects both males and females (25,26). It was found that a higher birth weight negatively related both with systolic and diastolic blood pressure in 30, 50 and 64 years old males and females and it was assumed that changes due to

nutrients deprivation during fetal life could interfere with vascular system maturation (27). The same subjects seemed to have pathological changes in factor Vlllo, fibrinogen and cholesterol levels, as they had a long-term hepatic involvement due to adaptation mechanisms established during fetal life to contrast nutrients' deprivation (28,29).

It seems that non insulin dependent diabetes mellitus and hypertension often are present in the same subjects and these subjects often have high insulin, high triglycerid and reduced HDL circulating levels. They are often frankly obese or overweight subjects with a high BMI index. These symptoms are now assembled together and they define a new pathology known as the X-syndrome (30). These symptoms frequently recur singularly or together in low birth weight subjects; so, a group of 64 years old man was examined to establish a relationship between low birth weight and the X-syndrome and, as expected, it was found that it was present in 22 % of examined subjects and it was found that the index risk was 10 times higher for low birth weight subjects compared with normal birth weight subjects (31). A reason behind these phenomena could be fetal stress and, as consequence, an adrenal gland hyperactivity. A constant adrenal gland hyperactivity may have effects both on glucose metabolism and on total glycaemic balance and as consequence on insulin secretion. Recently an inverse correlation has been showed in a 64 years old group of subjects between low birth weight and basal cortisol levels (32). Of course, as for the X-syndrome, the longitudinal observations will prove the consistency of these data. Moreover, the basis for a proper prevention will be set. Similarly, the same considerations have to be made about the possible association between low birth weight and SIDS incidence (33), or between low birth weight and impaired development of the male and female gonads (34,35). In addition, the cause for low birth weight or reduced birth length is not unique but multiple, and therefore there could be among these subjects individuals with associated various pathologies with adult onset.

Recently, a study on the adults survived during Leningrad siege reported data in contrast to Barker's hypothesis (36). However, the extreme conditions of that historical time led to an increase of spontaneous abortions for the risky gestations. Studies were also published about long-term effects due to low birth weight for maternal deprivations in Gambia. These studies compared two adult groups, the one born during the dry season, the other during the pluvius one. The first had an early death, and a life expectation more reduced compared with the other (37).

Pregnancy conditions obviously influence subject's life and they could be more or less important according to maternal diet, ethnic group and finally genotype both of the mother and of the unborn baby.

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II SESSION: Focus on...

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PERINATAL INFECTIONS AND WHITE MATTER BRAIN INJURY: A NEW MARKER

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Introduction

Perinatal infections complicate 1-5 % living newborns and constitute one of the major factors associated with perinatal mortality and morbidity (1). Among dramatic complications that can occur due to perinatal infections bacterial meningitis (BM) is responsible for one third of permanent neurological sequelae (2). Although several clinical, laboratory and biochemical markers have been studied for early prevention of post-natal complications, to date no reliable tool has been found (3). Therefore, the measurement of brain constituents able to diagnose sub-clinical lesions at this stage could be especially useful.

S100B is a dimeric calcium-binding protein (21 kD) primarily present in nervous tissue; it is eliminated or metabolized by kidney. Increased S100B in biological fluids has been shown to be a marker of brain damage both in adults and during the perinatal period (4). The aims of our study was to investigate whether S100B protein is a sensitive marker to monitor the occurrence of brain damage:

- a) in a animal model;
- b) in infants at risk of BM and encephalitis (BME).

Material and methods

Experimental Model

Twenty-one fetal sheep were chronically instrumented at a mean gestational age of 107 ± 1 d as previously described (5). The experiments were performed 3 d after the surgical procedure. The study group was treated either by 100 (n=9), 500 (n=5) or 2500 ng (n=1) LPS i.v. from one batch (derived from *E. Coli*; O127:B8, Sigma-Aldrich). Control group (n=6) received 2 mL 0.9% saline i.v. Eight predetermined monitoring timepoints (-1h before, at 1, 3, 6, 12, 24, 48, and 72h from LPS administration) were chosen for laboratory assessment and S100B maternal and fetal measurements.

Clinical Study

A case-control study was conducted in 44 patients with BM matched for gestational age at sampling with 44 patients without BM. Causative bacteria were: *Streptococcus agalactiae* (n=19), unidentified Gram-positive cocci (n=12) and Gram-negative rods

(*Haemophilus influenzae*, *E. coli*: n=13). Clinical and laboratory criteria (septicemia: 15/44 unidentified Gram-positive cocci, 22/44 *Staphylococcus epidermidis*, 7/44 *Staphylococcus* species). Controls recruitment criteria were identical to BM group except for CSF results, which were within normal ranges, and for the absence of US and/or CT patterns suggestive of encephalitis or CNS diseases. US was assessed at the time of CSF sampling and S100B assessment, at 72-hours from admission and on discharge from hospital. CT was performed for the detection of the presence and extension of encephalitis when US was already suggestive of brain lesion.

Animal and Human protocols were approved by respective Ethical Committee.

Results

Experimental Model

In controls there was no evidence of structural abnormalities or signs of axonal injury in the periventricular white matter and basal ganglia. LPS treatment increased the periventricular cell count, most prominently in the white matter dorsal to the lateral ventricles. In this area, two out eight LPS animals showed cystic lesions. LPS treatment caused inflammatory infiltrates in the periventricular white matter and basal ganglia, particularly the thalamus. S100B protein blood levels in the fetal descending aorta were significantly higher in the LPS group at 1h after LPS injection [LPS: 7.52 (1.24) µg/L versus control 2.15 (1.52) µg/L; P<0.01], peaking at 3h and returning to baseline between 12 and 72 h. The highest S100B levels have been shown in the three fetuses who died in the first 12h from LPS injection due to cardiovascular failure (15.9, 13.1 and 12.1 µg/L, respectively). These fetuses developed severe fetal hypoxia and metabolic acidosis in response to endotoxemia from which they did not recover.

Clinical Study

S100B was significantly higher in the infants who later developed encephalitis (BME) as compared with those without encephalitis (BM) or controls at a stage when standard monitoring procedures were unable to indicate which infants would develop encephalitis. The concentrations of S100B in CSF were significantly higher in the total BM group (median, 1.34 µg/L; 25th percentile, 0.84 µg/L; 75th percentile, 1.78 µg/L) than in the controls (median, 0.16 µg/L; 25th percentile, 0.11 µg/L; 75th percentile, 0.33 µg/L; P<0.001). CSF S100B concentrations were significantly higher in the infants of the BME group than in the BM group and the controls (P<0.001). The difference between the BM group and controls was also significant (P<0.01). Multiple logistic regression analysis showed a positive correlation only between CSF S100B and the occurrence of BME (P<0.001; odds ratio, 7.0). ROC curve analysis showed that higher S100B values were diagnostic for early BME detection, with a S100B cutoff of 1.0 µg/L, a sensitivity of 91% (CI95%: 71–98.6%), a specificity

of 82% (C.I.95%, 70–91.4%), and an AUC 0.918.

Discussion

Experimental Model

In the animal model, we report that intrauterine exposure to endotoxemia, induced by systemically applied LPS, results in periventricular brain white matter injury, inflammatory infiltration of basal ganglia and an increase of both the rate of apoptosis in the subcortical white matter and release of S100B protein into the fetal-maternal bloodstream. It is reasonable to assume that increased S100B levels in LPS-treated group are an expression of occurring damage in the nervous tissue due to endotoxin. The release into the blood of a brain constituent such as S100B offers a direct indicator of active cell damage and, at the same time, a measurable parameter of the extension of the lesion. Elevated maternal S100B concentrations support the notion, as previously demonstrated in humans (6), that under severe conditions a part of fetal S100B amount can passively transported in the maternal district. The finding is also supported by the absence of any maternal CNS damage. Among several factors responsible of the rise of S100B the exaggerated activation of glial cells, might be in part responsible of a increased concentration of the protein in the extracellular compartment and might have a deleterious effect on the brain. In this respect, the possibility that at least part of the S100B measured in the blood of LPS-treated fetal sheep derives from this process and participates in the pathologic cascade of events accompanying parenchymal damage is consistent. Finally, the hypothesis that part of S100B found in fetal-maternal blood might be released from placental tissue cannot be ruled out.

Clinical Study

We showed the potential usefulness of S100B measurements in CSF as a possible tool for the early detection of infants at risk of brain lesions from BM. Increased S100B has been reported in CSF, cord blood, amniotic fluid, and urine in preterm/term infants with cerebral bleeding, in high-risk fetuses, in asphyxiated newborns, and in children subjected to extracorporeal membrane oxygenation and cardiopulmonary by-pass (5). In these conditions, S100B concentrations have been shown to detect individuals at high risk when routine monitoring procedures are silent. Elevated S100B concentrations may be due to activation of proinflammatory cytokines, triggering into the brain an exaggerated activation of glial cells, which produce S100B. High extracellular concentrations of S100B have been shown to be neurotoxic, leading to apoptosis and neuronal death via a nitric oxide-mediated pathway (7).

The present findings support the notion that assessment of S100B in CSF may provide additional information to physicians (8). Measurement of a brain constituent could be particularly useful for monitoring the

effectiveness of therapeutic strategies. It is possible that S100B assessment in other biological fluids may help in the early diagnosis of BM and encephalitis in infants with infectious diseases. The availability of longitudinal S100B measurements (half-life of 1h) in other biological fluids (e.g., peripheral blood and urine) has been already reported (5). The correlation between S100B and polymorphonuclear leukocytes offers additional support to the notion that a more severe of infection is associated with a higher risk of neurological sequelae. This concept is supported by the correlation between S100B and other CSF variables.

Conclusion

The present investigation provides a new perspective for the clinical study of S100B, with special reference to neurologic sequelae after bacterial infections. Although CSF remains the biological fluid of choice, further investigations are needed in other biological fluids to improve the care of newborns.

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METABOLIC DISEASES: PRESENT AND PERSPECTIVES OF NEONATAL SCREENING

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Newborn screening began in the early 1960s with a test for phenylketonuria (PKU).

During the 1960s and 1970s, several States in US began to screen for other conditions for which it was believed that severe outcomes could be avoided. Screening for several metabolic diseases, including galactosemia, maple syrup urine disease, and homocystinuria, was initiated in a number of States by the late 1960s to prevent deaths during the neonatal period. Beginning in the late 1970s, screening and early treatment for congenital hypothyroidism was adopted to prevent intellectual disability and, like PKU, was found to be cost-saving.

In 1968 the World Health Organization articulated criteria for population-screening programs that focuses on public health benefits. In 1975, the National Research Council issued a report that concluded that mandated screening could be justified only if there was evidence that it would prevent death or other serious harm to the affected individual.

A draft report with a recommended core panel based primarily on the survey results, as well as condition summaries prepared by disease experts, was "accepted" by the Secretary's Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children in September 2004, and the HRSA released the report for public comment in March 2005.

Although newborn screening has been a public health activity for >40 years, technological advances are beginning to reshape these programs. In particular, States have begun to use tandem mass spectrometry (MS/MS), a technology that measures metabolic analytes that allow for the detection of many disorders.

A new report from the American College of medical

Genetics commissioned by the Maternal and Child Health Bureau of the Health Resources and Services Administration (HRSA) has proposed that 29 conditions be included in a uniform newborn-screening condition panel, including nonmetabolic disorders.

Also in Italy there are newborn screening programs with MS/MS in some regions (Toscana, Lazio, Liguria, see technical report SISN year 2004). In Toscana this screening is performed from 2002 and from 2004 is obligatory by regional law.

In Sardinia in the last 12 months epidemiological data show 6 newborn affected by inborn errors of metabolism of aminoacids or organic acids. Among these six inborn errors four have good prognosis if treated early. In particular these data show that in Sardinia the incidence of inborn errors of metabolism susceptible of newborn screening is 1/3250. These data justify newborn screening in Sardinia with MS/MS.

In addition to screening, parental permission may be required for inclusion of children in database to track long-term outcomes or for the retention of dried-blood-spot specimens for use in research.

When the balance between benefits and risks is not as dramatic as it is for congenital hypothyroidism and PKU, the argument for consent becomes more compelling.

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Global incidence of inborn errors of metabolism (aminoacids and organic acids) in Sardinia from 1990 to 2005

PKU	15	Methylmalonic acidemia with homocystinuria	2
Tyrosinaemia	1	Ketotiolase defect	3
MSUD	1	Glycogenesis type Ib	2
Homocystinuria	2	Glutaric acidemia type I	1
Canavan disease	1	Isovaleric acidemia	3
OTC	4	Glutaric acidemia type II	2
Citrullinaemia	1	Holocarboxylase synthetase defect	1
Argininosuccinic acidemia	3	Methylglutaconic acidemia	1
Iperprolinaemia	1	CDG	2
LCHAD	3	Adrenoleukodystrophy	2
Methylmalonic acidemia	1	Lactic acidemia	9
CPT2	2		

POST-DISCHARGE FOLLOW UP OF INFANTS WITH BRONCHOPULMONARY DYSPLASIA

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Advances in perinatal-neonatal care including antenatal use of steroids, introduction of surfactant therapy, new technology and new ventilation strategies have improved survival of the very low birth weight infants (VLBW, 1500g) and extremely low birth weight (ELBW, 1000g), considerably. Today over 90% of the 1001-1500g, 85% of the 751-1000g and close to 50% of the 500-750g VLBW infants survive the neonatal period. This has resulted in ever growing numbers of infants who after their discharge from the neonatal intensive care units (NICUs), face often serious ongoing health problems. Pediatricians and other health care providers should be aware of the very special post-discharge care and needs of the high risk premature infants and with the collaboration of the multi-disciplinary follow up team of the tertiary care NICU(1), enhanced care of "small preemies" can be achieved. Problems after discharge that often lead to re-hospitalization relate mainly to respiratory morbidity due to bronchopulmonary dysplasia (BPD, chronic lung disease). For these medically very fragile infants, prevention, early diagnosis and proper intervention are extremely important especially during the first year of life. Fortunately most of their problems can resolve over the first years if properly monitored and taken care of. However it is known that sequelae of the NICU graduates may not be evident at the time of discharge (eg, chronic lung disease of prematurity or bronchopulmonary dysplasia, retinopathy of prematurity-ROP) but may be identified later in infancy (eg, cerebral palsy, spastic diplegia) or even later in childhood (eg, behavioural problems, learning disabilities) and their special medical care needs continue for years after their discharge.

Post-discharge respiratory illness is common among very preterm infants with BPD.

Bronchopulmonary dysplasia was first described by Northway in 1967 in premature infants with respiratory distress syndrome (RDS) who developed chronic lung disease after being treated with positive pressure ventilation and oxygen supplementation. Today with the advances in perinatal-neonatal care, the "classic" BPD has been replaced by a less severe form the "new" BPD with considerably different features reflecting the changes in the degree of immaturity (very small and immature premature infants, <1000g) but also modifications in the management of the premature infant today.

The incidence of BPD is difficult to assess since there is no universally accepted definition (need for supplemental oxygen at 28 days of life or at 36 weeks corrected age). From recent data, using oxygen dependency at 36 weeks postconceptional age, the incidence of

BPD in infants with birth weight 501-750g is 52%, 34% in infants 751-1000g, 15% in infants 1001-1250g, and 7% in infants 1251-1500g (2). Although BPD is not a frequent adverse outcome for premature infants >30 weeks gestation and with birth weights >1200g, it still remains the most common form of chronic lung disease in infancy (3). The prevalence of VLBW survivors with BPD reaching adulthood is close to 3-4 per 1000, greater than that for many childhood diseases affecting the respiratory system, such as cystic fibrosis (4).

BPD is the result of dynamic processes involving inflammation, injury, growth, repair growth and maturation, occurring concurrently or sequentially in the lung. Major risk factors involved in the pathogenesis of BPD are: lung immaturity, mechanical ventilation (barotrauma-volutrauma), oxygen toxicity, infection / inflammation.

Pathological findings of the lungs of infants with "new" BPD are strikingly different from those in "classic" BPD. Histological changes in the lungs of the very small premature infants represent injury that occurs at a very earlier phase of lung development (the canalicular phase, 16-27 weeks of gestation) with most characteristic finding an arrest in alveolar development (5). This results in decreased number of alveoli, enlargement of the airspaces and a consequent decrease in the total gas exchange area. Recent research data suggest that alveolarization and lung angiogenesis go in parallel during lung development and reduction in alveolar number is coinciding with altered angiogenesis (6). Histological findings from lung biopsies suggest that the structural changes persist throughout life. Infants with BPD have a high risk of rehospitalization during the first year of life, with rates between 42% and 63%, higher than the rates for VLBW infants without BPD (7). Common causes of acute respiratory distress that contribute to the high rates of readmission to the hospital are: bacterial pneumonia, aspiration pneumonia (gastroesophageal reflux, sucking and swallowing difficulties), reactive airway disease and respiratory syncytial virus infection. The high rate of readmissions generally declines during the second year with few hospitalizations in the third year. The majority of reports on long-term respiratory outcomes of children with BPD include children with classic BPD in the pre-surfactant, pre-steroid era and thus the results may not apply to the preemies in the NICUs today. Few recent data suggest that the present population of BPD patients at preschool age also suffer chronic lung morbidity with troublesome respiratory symptoms such as wheezing and cough particularly with viral infections (8). Pulmonary hypertension and cor pulmonale may complicate the course of infants with moderate-severe BPD, a condition associated with high mortality. Infants with severe BPD need to be carefully monitored with frequent Echocardiograms and if there is evidence of pulmonary hypertension oxygen saturation should be maintained above 94%. Several studies evaluating the effects of prematurity and neonatal lung disease on Lung function testing (infancy, early child-

hood, early adulthood) have demonstrated a number of changes in pulmonary function: lower forced expiratory volume in the first second (FEV1), lower forced vital capacity (FVC), higher residual volume (RV) /total lung capacity (TLC), suggesting that significant obstructive airway disease with air trapping occurs (9). Airway hyperresponsiveness is also reported and seems to be unrelated to atopic status. No increase prevalence of atopy was found in BPD children. Asthma-like symptoms and airway hyper-responsiveness reported in very preterm infants with BPD, differ from typical childhood asthma and mechanisms underlying these respiratory symptoms are structural sequelae in the airways or in the interstitium rather than active inflammation (10).

The respiratory outcomes of children with BPD generally improve after early childhood with improvement of pulmonary function measures, decreasing rates of wheezing, pneumonia and readmissions but some 25% of adolescents and young adults with former BPD still continue to demonstrate respiratory symptoms.

Adequate nutrition and growth is extremely important for BPD infants who frequently (>65%) have moderate to severe growth failure at the time of discharge. Delay in somatic growth can delay lung growth and development and it is reported to be associated with prolonged lung dysfunction, while accelerated growth results in improvement of respiratory symptoms. Nutrient enriched formulas have demonstrated early benefits for growth and accretion of bone mass for infants recovering from BPD. The role of the pediatrician is very important to monitor infant's growth (plot growth curves according to 'corrected age' ie, chronological age minus number of weeks of prematurity), assure the use of proper formula and support the family. Catch-up growth mostly occurs within the first 1-2 years of life, may continue into adolescence, but despite this mean growth measurements of children and young adults remain lower than their term-born peers.

Further special care and follow up from the pediatrician is needed during the management of these infants for:

1) Frequent neurological assessments in view of the increased risk for cerebral palsy and neurodevelopmental delay, so that early detection and intervention will be achieved.

2) Careful adjustments of the medication they are on (bronchodilators, diuretics, inhaled steroids...)

3) Strict follow up of passive and active immunization policies (Synagis for RSV, influenza vaccine, DTaP, IPV, Hib...). Immunization should start according to chronological age, not 'corrected' age.

4) Regular testing for vision and hearing problems (strabismus, myopia, neurosensory and conductive hearing loss...)

In conclusion infants with BPD survive with significant respiratory morbidity that continues beyond infancy and requires special individualized medical care and intervention.

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SIDS TODAY

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Sudden Infant Death Syndrome (SIDS) is still a phenomenon of unknown cause and, although strongly reduced in rates over the last decades, remains in the developed world one the major causes of death for infants between the ages of 1 month and 1 year (1).

The SIDS incidence rate has dramatically declined since the AAP's 1992 policy statement, when the SIDS rate was 1.2 death per 1000 live births. By 2001, that rate had fallen to 0.56 death, a 53% reduction suggesting almost 3000 fewer infant deaths per year.

Definition and epidemiological entity of SIDS are not fully clarified.

The current generally accepted definition of this entity, according to the 1991 expert panel convened by the National Institute of Child Health and Human Development (2), is 'the sudden death of an infant under 1 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'.

As mentioned by the definition herein adopted, a full forensic analysis has to be performed, comprehensive of an overview of the death scene and of a toxicological screening on body fluids, to state a SIDS diagnosis. To fill the epidemiological information's gap observed in Italy, a bill (n. 31/2006) has been recently approved by the Parliament (3) to encourage a in depth investigation concerning the true incidence of SIDS in our Country and the pathological features of this syndrome.

The international collaborative efforts have identified in the last decades several independent risk factors, including prone sleep position, sleeping on a sofa surface, maternal smoking during pregnancy, overheating, late or no prenatal care, young maternal age, pre-term birth and /or low birth weight and male gender.

A tight control of few of these factors have been implemented in a surveillance program by health care givers, and the major goals achieved have been reached by the Back to sleep campaign (1).

By the means of widespread educational programs, a substantial reduction of death rate has been achieved although the initial rationale of the nonprone position statement has slipped – with the knowledge development – towards the back position.

A similar approach has been today proposed to avoid the use of soft bedding or pillows and to discourage the bed-sharing attitude, but this latter suggestions

have raised concerns by several authors focusing on the positive effects of bed sharing on breastfeeding and enhancement of maternal-infant bonding.

The advent of the genetic era has boosted the insight into the genetic mechanisms underlying the pathogenesis of the cumbersome topic of sudden cardiac death in infants.

Up to now, a piece of evidence has been achieved concerning several genetic syndromes related to SIDS or to SUD (sudden cardiac death): Brugada, LQTS (Long QT syndrome), SQTS (Short QT syndrome), CPVT (Chatecolamine-induced Polymorphic Ventricular Tachycardia) and the wider channelopathies family.

Although these syndromes account for not more than 10% of all the SIDS causes, a special effort to unravelling their biological basis is actually one of the hotspots of forensic genetics.

As affirmed by the 2005 AAP's Policy Statement (1), 'the level of suspicion of foul play should be increased on the recurrence of SIDS within a family unit'.

Carpenter et coworkers (4) in a recent article underlined that the recurrence risks for SIDS within a family in which 1 infant previously died of SIDS range from 2% to 6% while the occurrence of an homicide is between 6% to 10%.

A genetic analysis of the siblings focused on the aforementioned syndromes could help the forensic pathologist to partially discriminate among natural vs culpable death.

Table 1

Data to be collected and procedure to be performed in all forensic analysis of SIDS/ SUD cases

Full autopsy	Volume and weight of all organs has to be reported	All major macroscopically detected lesions have to be clearly documented
Full heart dissection	At least 10 sections have to be analyzed by histology	Ischemia histochemical markers
Full dissection of proximal and distal branches of pulmonary arteries	Diffuse sampling for histological analysis if thrombi have been observed	
Histology	all the organs	Gram, Grocott, PAS
Cytology	Pericardic, pleural, abdominal fluids and CSF	Gram, PAS
Neuropathology	Brain fixed (3-4 weeks in formalin solution)	Histology, Immunohistochemistry
Biochemical screening	Vitreous humour and pericardic fluid	Troponin and glucose concentration
Microbiological screening	Blood, fluids, inner organs if septic lesions are observed	HIV, HBV, PCR for the identification of viral proteins
Molecular Genetic	Blood and Heart samples	Screening for major mutations according to familial evidences
Total body radiological examination	If bone lesions are identified, specimen from the broken/repared bones have to be collected for histological examination	

To prevent errors, the AAP's Policy Statement strongly suggests that all cases of SIDS have to be considered as a criminal item including the study of the death scene, being necessary to perform not only a complete autopsy (as an average anatomopathological one) but also toxicological, histopathological and genetic analyses have to be carried out, as reported in table 1. But this task seems too tough to be addressed by the forensic specialist in his solitude, being necessary a multidisciplinary effort of cardiology, neonatology, medical genetic and electrophysiology experts.

The goal to be reached in the next future is a 'cardiac channel molecular autopsy for SUD/SIDS' (5).

A recent molecular autopsy series of SUD identified pathogenic mutations in Long QT syndrome and Catecholaminergic Polymorphic Ventricular Tachycardia associated genes in over one-third of cases while the same approach has identified in SIDS cases mutations ranging from 5 to 10% of them.

An accurate diagnosis, derived from a molecular autopsy, will guide the appropriate initiation of preemptive strategies in hopes of preventing future tragedies among those left behind (5).

The recent Bill dismissed by the Italian Parliament should represent the first step towards the creation of a repository of all the data – circumstantial, epidemiological, clinical, familiar, genetic and autoptical – to better elucidate the cause of SIDS and to clearly identify the main risk factors to be addressed in a health communication campaign.

As final products, national guidelines for SIDS prevention based on sound scientific evidence will be promulgated by the Health Ministry.

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The way we were

Chairman: V. Piras

THE NEWBORN IN THE "ISTITUTO LUCE" ARCHIVES

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LUCE is an acronym for "l'unione cinematografica educativa" (Institute of educational films) founded in Rome in 1924 and purchased the following year by the State. The "LUCE newspaper" started up in 1927 representing a means to report news and propaganda. Newsreels were made right up to the early 80's. It is a known fact that research and historical studies must be based solely on documents. The newsreels and documentary evidence found in LUCE archives form a fundamental documentary source. Through the contents and images, important data can be acquired to provide a framework for Italian history. In the case of neonatology and paediatrics, they supply a description of contents, priorities and methodology which is applied to the care throughout the age of development. By analysing film material some information can be drawn:

- L'ONMI, founded in 1925, encouraged actions by ensuring

* a nutritionally balanced diet for expectant mothers and women in childbirth and

* children, in particular

* encouraging breastfeeding and

* encouraging hygiene and proper bottle-feeding.

* Ensuring proper standards of hygiene during pregnancy and childbirth,

* giving value to the role of the mid-wife and

* ensuring paediatric and obstetric care within the area.

_ Films show that the social preventative measures in paediatrics prevail.

_ The introduction of antibiotics had a significant effect on the reduction of infant mortality hence,

*interest in the quality of food in neonatology has increased since the 50's

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The way we'll be

Chairman: G. Rondini

A EUROPEAN DREAM FOR NEONATAL EDUCATION AND RESEARCH

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The title of this lecture is derived from a book of Jerome Rifkin "The European Dream", which informs about different attitudes, strength and weaknesses of both, the European and American society.

INPUT and OUTPUT comparison

We all know that European countries spend too little on Research and Development (abbr. R&D) in comparison to USA or Japan. Governmental spending in Europe is similar to the US and higher compared to Japan. However, European R&D lacks Business supported research and development. Within Europe there are the large differences in R&D spendings. Finland being the "Musterschüler" spends about 3 times higher percentage of Gross National Product and on a per capita basis on R&D compared to Italy. A similar total amount of researchers work on both side of the Atlantic, despite the EU population being three

times the one of the USA. We Europeans are about at the same level of scientific literacy as the USA, Korea and Japan, but the number of university (tertiary) graduates in Europe are fewer than in USA, Korea and Japan. The index of innovative potential and creativity more or less parallels and reflects the expenditure on R&D

Whereas the number of publications in the E.U. is steadily increasing, the rate is declining in the U.S. The average annual growth has risen, on average, by 3 percent from 1995 to 1999 in the E.U., while it has essentially stabilised in the U.S. Citations for these papers (a proxy for measuring their impact) also lessened in the U.S. In 1996, the last year for which these data are available, citations were higher in the E.U. for all research fields. Analysis of the top 1% of publications in terms of citations, however, reveal a discouraging evidence for Europe. US researchers publish approximately one-third of world scientific papers; but they receive half of world citations, and account for no less than two-thirds of the world's most highly cited papers and scientists (see <http://www.isinet.com>). We are not good in what the Eu Research Commission call "Frontier Research" and the history of "Hypothermia Trials" is a typical example.

Wasting intellectual resources

I see four causes for wasting intellectual resources: 1. wrong idea about science and research work, 2. wrong allocation of time and tasks to colleagues, 3. no renewal of resources and fostering elite 4. Not halting losses.

1. Misunderstanding the nature of science and research work. Scientists in Europe are often judged as "not performing any work", but spending time in the laboratory, sitting in the library, going to congresses, etc. The attitude towards research work has to change, since it is hard work, which requires time, support, tolerance and recognition to be successful.

2. Wrong allocation of time and tasks to colleagues

While we meticulously separate paper from glass garbage, green glass from white glass etc. European doctors are used independent of whatever talent or interest or inclination for clinical, educational, administrative and research work. We even choose the wrong sequence and false timing of skill acquisition. The concept, that a doctor should first finish clinical training and only then start with research is completely wrong. We first have to develop the curiosity, the urge to know, to be critical and precise. Our own experiment and experience in the Department of Neonatology, Medical University Innsbruck, Austria, shows that avoiding these mistakes, results in success. We introduced a time budget. Time allocation for research is negotiated according to planned projects and previous output. Allocating 50% time to two young motivated researcher and 30% to two others, within 3 years yielded more than 4 million € grant money, three to five top publications per year, ten presentations at the

highly competitive American Pediatric Society Congress and twenty-two presentations at the national Neonatology Congress.

3. Renewal of resources and raising elite. We Europeans are reluctant and do not foster elite. Most of European colleagues learn all about research by trial and error, often until old age, the painful auto-didactic way. Each individual is making the same mistake over and over – for example: no proper formulation of hypothesis, no proper statistical planning, lots of work goes into futile attempts to publish it.

ad 4. Prevention of Loss of resources. We do not pay any attention to the loss of talented colleagues to the USA, Canada or even Singapore. We feel so comfortable with our intellectual resources, but we need to create attention to that loss, and a fund or mechanism to keep these talents in the country or in Europe

LACK OF COOPERATIONS and proposals to improve

Galileo Galileo was and is a prototype of a European researcher: a brilliant individualist, ignoring and refusing to co-operate with his competitors, attempting to be popular and getting mixed up in politics. Hypothermia investigation is a typical recent example: Westin proposes hypothermia for asphyxiated newborns in 1960s, published a series of cases.

Swedish colleagues design and investigate Cerebral Function Monitor. Hellström-Westas, Linda deVries and Toets, Kuwaiti Postdoc Naqeeb in London demonstrate the predictive power of aEEG for neurological outcome. Marianne Thoresen does pilot trials on hypothermia. Thus all the basis for a hypothermia trial is work done in Europe, by Europeans. Simbruner G proposed a cooperation with David Edwards, UK, based on Gluckman's protocol. Edwards refused. The discussions lasted a year until the final "Protocol neo.nEURO.network" was submitted to DFG in 2000. The DFG review lasted one year, although maximum

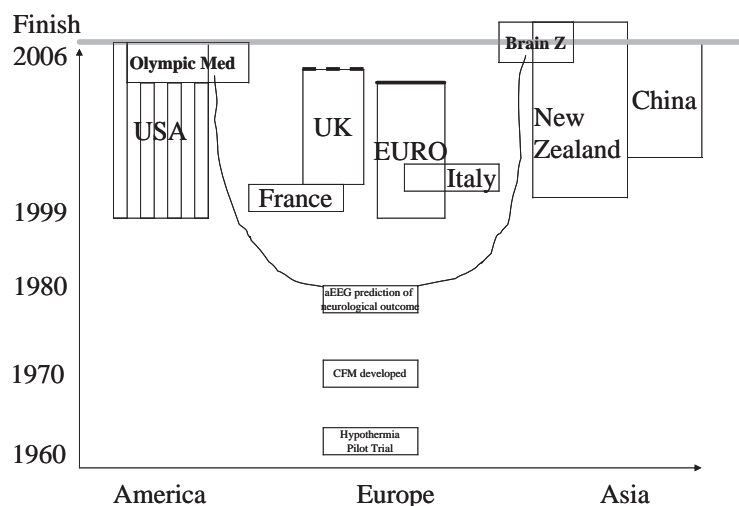
review time is 6 months. The neonatal DFG reviewers were Speer and Bartmann, opponents of Simbruner G. In the UK Azzopardi and Edwards start their own study with factually identical protocol and identical device.

At the beginning of 2006 when first reports/publications of Gluckmann, Shankaran and Chinese group have appeared, neo.NEURO.network lacks 25 patients to complete their trial enrolment. The UK group has recruited half of patients needed. Combining data is refused by UK group; French and Italian group individually published observational results on hypothermia in groups of 50 patients each. The neo.nEURO.network stops recruitment of patients April 2006; British decision to stop pending. Though the idea and most pilot trials were done in Europe, ambition, distrust, non-cooperation of Europeans resulted in trials results being published first by NewZealand (Gluckman PD et al.), then by USA (Shankaran S. et al.) and Chinese groups, and European group will most likely publish two years later (see figure).

We need more cross Europe co-operations and sharing of ideas and techniques.

Pierre Gressens, INSERM Paris shared with us the mouse model. We from Innsbruck went to Paris, learnt it how to do it and since then produce good science and passed this method on to colleagues in Budapest and who ever wants to have it. George Simbruner now has a new device of Electrical Impedance Tomography (EMS-Ortner), measuring the electrical impedance i.e the content of water in relation to air in four lung segments, and offers it to anyone interested. We needed a modern EUROPEAN roof organisation which functions more like an enterprise than a political party; which offers what single national societies cannot achieve, we European neonatologists and perinatologists need an European address, where colleagues from the world can turn to.

The "Union of European Neonatal & Perinatal



Publication of papers on hypothermia world wide

Societies" (abbr. UENPS), was founded on June 30 and July 1, in Vienna, and closely co-operates with IPOKRATES, the worldwide postgraduate society (in analogy to "Daimler-Benz"). UENPS now unites at present 17 European Neonatal & Perinatal Societies under one organisational roof and will provide the network and structure necessary to make the European dream for neonatal education and research to come true in the not so distant future. The goals and organisational structure is available on internet (see internet <http://www.ipokrates.info/UENPS>).

III SESSION: What next?

Chairman: R. Paludetto

Moderators: G. Temporin, S. Vendemmia

SURFACTANT TODAY AND TOMORROW

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Surfactant, present in the lung of all mammalian species, is a complex mixture of lipids and proteins. Surfactant obtained from alveolar wash is composed of approximately 80% phospholipids, 10% proteins and 10% neutral lipids. The main phospholipid components are phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and phosphatidylethanolamine; of these phosphatidylcholine is the principal tensioactive component. There are four main surfactant-associated proteins, termed surfactant protein (SP)-A, SP-B, SP-C and SP-D, as well as a wide variety of other, mostly serum-derived proteins.

The hydrophobic surfactant-associated proteins SP-B and SP-C are essential for lung function and pulmonary homeostasis after birth. These proteins enhance the spreading, adsorption and stability of surfactant lipids required for the reduction of surface tension in the alveolus. They also participate in the regulation of intracellular and extracellular processes critical for the maintenance of respiratory structure and function (1). The hydrophilic surfactant-associated proteins - pulmonary collectins SP-A and SP-D - play an important role in innate host defense by binding and clearing invading microbes from the lung. SP-A and SP-D also influence surfactant homeostasis, contributing to the physical structures of lipids in the alveoli and to the regulation of surfactant function and metabolism. In addition to binding and opsonizing infectious pathogens, pulmonary collectins SP-A and SP-D bind to the surfaces of host defence cells, promoting or inhibiting immune cell activity through multiple cellular pathways (2).

Although genetic factors are assumed to have a role in the etiology of respiratory distress syndrome (RDS), specific genes underlying this susceptibility are incompletely known. The most promising candidates are the genes coding for the lung-specific protein components of the surfactant (3).

In congenital absence of SP-A in mice, lung mechanics or surfactant homeostasis is normal. However, there is an increased susceptibility to infections. To date, no human infants who lack SP-A have been identified, and SP-A polymorphisms are not currently useful for estimation of individual risk of having an affected infant.

Surfactant protein B plays an essential role in the structure of tubular myelin. Mutations resulting in an absence of SP-B have been identified. They cause a recessively inherited, progressive respiratory disease. More than 27 loss of function mutations have been identified in the SP-B gene that result in lethal neonatal respiratory failure. The consistent phenotype is exhibited by infants with a homozygous genotype. The clinical presentation in infants homozygous for a SP-B mutation is full term infants who develop RDS within the first 12-24 hours of life. Surfactant replacement therapy fails to reverse this outcome, and without lung transplantation, death occurs within the first 1-6 month of life. SP-B mutations may also result in milder phenotypes. These mutations, consisting in reduced synthesis of SP-B, appear to be family-specific and result in RDS, but sometimes with more gradually progressive or chronic respiratory failure.

Surfactant protein C plays a role in the stabilization of surfactant and may also have a role in the intracellular processing of the surfactant complex. SP-B is important in the intracellular processing and production of SP-C. Although SP-C deficient mice are viable and survive to adulthood without obvious pulmonary abnormalities, their lung have reduced viscoelasticity. Human respiratory disease in the neonatal period caused by the loss of function mutations in the SP-C gene has not been identified. However, an autosomal dominant inherited mutation at the SP-C gene causes chronic interstitial lung disease.

Surfactant protein D is a member of the collectin family like SP-A, therefore it opsonizes pathogens and enhances their phagocytosis by alveolar macrophages and neutrophils. Unlike SP-A, it does not contribute to lowering surface tension. SP-D deficient mice have no respiratory abnormalities at birth, but it causes development of emphysema and predisposition to specific infections. No human infant or child with RDS and mutation in the SP-D gene has been identified.

Although not primarily a surfactant protein, ATP-binding cassette (ABC) proteins - in particular ABCA3 - are attracting a lot of attention in scientific literature (4). ATP-binding cassette proteins are transmembrane proteins involved in membrane trafficking, the ABCA subgroup is predominantly involved in lipid transport across membranes. ABCA3 deficiency was first reported in infants who presented in a similar fashion to SP-B deficiency but in whom further investigation did not support this diagnosis. Fourteen ABC genes have been associated with distinct genetic disease in humans.

The ABCA3 is an important component in maturation

of lamellar bodies and surfactant production. Electron microscopy of lung tissue from infants with ABCA3 transport mutations shows characteristic abnormalities in lamellar body structure. Lamellar bodies in ABCA3-related lung disease are small and contain densely stained inclusions. Tubular myelin is absent and the airspaces are filled with macrophages, as well as lipid and proteinaceous debris.

Although the prevalence of ABCA3 transport mutation is not entirely clear, this disorder seems to be more common than hereditary SP-B and SP-C deficiency. More than 75 different mutations have been identified in patients with ABCA3 deficiency. The ABCA3 gene has been mapped to the short arm of chromosome 16. More recently ABCA3 gene mutations have been shown to be associated with interstitial lung disease beyond the neonatal period (5).

The first successful trial of exogenous surfactant replacement therapy for RDS was reported in 1980. Since then there have been numerous randomised trials demonstrating the efficacy of surfactant treatment in reducing pulmonary air leaks, increasing survival and assessing various other aspects of therapy (6). These studies show that multiple doses may be needed if surfactant is used to treat established RDS but early or prophylactic treatment is superior for infants with gestational ages less than 30 weeks. Natural surfactants containing proteins are more effective than synthetic products that are protein free, the latter now being infrequently used.

Natural surfactants vary and should not be considered to be equivalent in their effects.

Chronic lung disease remains a problem for premature babies but it is hoped that early treatment with surfactant combined with extubation to continuous positive airway pressure will reduce this complication of prematurity.

Intensive research has provided increased understanding of molecular mechanisms of various surfactant components. These efforts have led to development of peptides which may mimic the functions of the hydrophobic proteins SP-B and SP-C. Lucinactant is a new synthetic surfactant, which contains a novel peptide, sinapultide, a surfactant-associated protein B mimic. Randomised clinical trials suggest that this compound is a safe and effective treatment for RDS in preterm infants, although not superior to existing natural surfactants (7). However, as it is more resistant to inactivation, is being actively investigated for other indications, including meconium aspiration syndrome, treatment of bronchopulmonary dysplasia in neonates, acute RDS and asthma. A novel aerosol formulation administered with nasal continuous positive airway pressure is also under development for treatment of respiratory insufficiency in neonates (8). Its non-animal origin make it an attractive alternative to animal-derived surfactants by eliminating the risks of infection and immunogenicity related to the latter. Finally, the large scale production of the newer synthetic surfac-

tants, which could be produced less expensively than natural surfactants, may make this therapy more affordable in the deprived parts of the world.

Future studies will focus on widening the indications for surfactant treatment, development of non invasive means of administration and assessing the role of newer synthetic surfactants.

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RSV INFECTION: EARLY AND LONG TERM EFFECTS

F. Macagno

SOC Patologia Neonatale – A.O.U. di Udine

RSV is ubiquitous: approximately half of infants are infected with RSV during the first year of life and almost 100% have been infected by age two.(1)

RSV is now recognized as the leading cause of serious lower respiratory tract disease in infants and children.(2,3,) The virus is both a major cause of hospitalization and a major risk for hospitalized infants.(4) RSV can spread very rapidly in the hospital environment.

The spectrum of RSV – related morbidity collects acute (bronchiolitis, pneumonia, intensive care needs, death) and chronic (wheezing, asthma) events.

During their first year of life, up to one quarter of infants born with bronchopulmonary dysplasia (BPD) will require rehospitalization because of viral respiratory infections (usually RSV). Preterm infants without BPD are also at significant risk for rehospitalization.(5) In children, 80–90% of bronchiolitis hospitalizations and 25% of pneumonia hospitalizations are due to

RSV.(6) In USA alone, it has been estimated that RSV infection causes more than 90,000 hospitalizations and a 2% mortality rate among hospitalized infants annually.(3)

In addition, there is increasing evidence of a link between RSV infection and reactive airways disease in childhood.(7)

In the Italian RADAR (8) study the prevalence of RSV was 42% in 1232 children younger than two years who were hospitalized for LRTI. The prevalence of RSV in infants with a GA of less than 36 weeks was 9.6%, as compared with 8.5% in the entire study population and 4% in the general population in Italy. While 48% of children born first to third in their families had RSV-positive infection, the prevalence rates increased to 60.7% in those born fourth or fifth and 83% in those born later. Passive smoking was also correlated with a diagnosis of bronchiolitis, whether RSV-positive or not. In other Italian epidemiological study "Osservatorio VRS"(9), performed between 2000 to 2004 seasons, 21% of children with symptoms of respiratory infection were found to be RSV-positive (range 15% to 31%) over the course of the study. Of these, 49% required hospitalization (range 37% to 57%). The RSV season began in October or November, peaked in February and ended in May.

The palivizumab meta-analysis developed by Simoes (10); includes all studies using palivizumab that had "common denominators" or evaluated subjects in a manner that could be compared with each other. These data show that the ICU hospitalization rates and length of stays were significantly decreased in the palivizumab-prophylaxed group of infants versus the non-prophylaxed group. (Tab. 1)

Greenough (11) has prospectively followed preterm children with BPD for over five years. Her data shows that the children who had RSV-proven bronchiolitis in infancy had significantly higher numbers of outpatient visits, hospital admissions, days in hospital, days in PICU (pediatric intensive care unit) and days in the pediatric ward. (Tab. 2)

The prospective 2-year Sampalis study (12) objectives were to measure health care utilization and morbidity subsequent to RSV hospitalization (in the first year of life) in non-BPD premature infants born between 32-35

weeks gestational age for 2 years. The RSV-hospitalized (n=2,415) versus those not hospitalized for RSV (n=20,254) were matched 1:10 for gestational age, sex, and province of birth. RSV disease can also contribute to long-term fatal outcomes. The Sampalis study revealed an 8% mortality rate in 32-35 wGA infants with previous RSV hospitalization. (Tab. 3)

Preterm infants (33-35 wGA) without BPD/CLD who are hospitalized for RSV LRI experience statistically significant and clinically relevant acute and chronic outcomes versus their non-hospitalized counterparts:

- hospitalization (2 times greater)
- ICU visits (50% more)
- use of respiratory therapy (3 times greater)
- physician consults (4 times greater)
- length of hospital length of stay (3 times greater)
- outpatient admissions (2 times greater)
- asthma incidence (5 times greater)
- deaths (5 times greater)

Bont (13) performed a study on the health-related quality of life (HrQoL) in infants who experienced RSV infection. These children were 3 years old at time of evaluation. He has extensively studied the incidence of recurrent wheeze after RSV LRI and has demonstrated that recurrent wheeze is associated with RSV LRTI, but also rQoL scores were significantly lower in infants who had previously experienced RSV infection than in control infants or premature even several years following the RSV hospitalization.

Tab.1
ICU Admissions – RSV Prophylaxis Reduces RSV Severity in Preterms with & w/o CLD

	ICU Rates (%)	ICU Days (per 100 children)
Preterms w/CLD- non-prophylaxed (n=467)	3.0	15
Preterms w/CLD- prophylaxed (n=1919)	1.0	6.9
Preterms no CLD – non-prophylaxed (n=1388)	1.7	15
Preterms no CLD- prophylaxed (n=2376)	0.5	6.9

Simoes EA F. *Resp Res*, 2002

Tab. 2
RSV in Premature Infants with CLD 5-year Follow Up

(mean no.)	RSV proven	Bronchiolitis	Other res. p.	No/Other admission	P value (Between all admission groups)
Outpatient visits	24	12	18	15	0.0006
Hospital admissions	8	5	5	2	<0.0001
Days in hosp.	53	20	23	5	<0.0001
Days in PICU	3	1	0.8	0	0.005
Days in Paed ward	44	17	19	4	<0.0001

Greenough et al. *Arch Dis Child* 2004;89:673-8

Tab. 3
Chronic Outcomes

Hospital Admission for Specified Diagnosis / Morbidity Among Infants Born 32-35wGA	% Cases (n=2,415)	% Controls (n=20,254)	ORDS RATIO	P-value
Respiratory Problem:				
URT	22.2	0.88	3.19	0.001
Pulmonary collapse	3.8	0.21	1.84	0.001
Bronchiolitis	64.1	1.27	1.23	0.001
Bronchitis	2.7	0.23	11.3	0.001
Pneumonia	6.8	1.12	11.8	0.001
Dyspnea	8.8	1.15	6.6	0.001
Asthma	5.3	1.06	6.0	0.001
Other Conditions:				
CHF / media	7.2	1.2	6.4	0.001
Mortality:				
Total	6.1	1.6	3.5	0.001
Known causes	2.8	1.3	1.6	0.005
Sudden death, unknown cause	6.1	0.3	21.9	0.001

Sampalis J. *Pediatr* 2003; 143:8150-8156

cine (PCV7). Before the employment of the eptavalent conjugated vaccine (PCV7) the total incidence of the invasive diseases from PNC, in the European pediatric population, ranged between 6.6/100,000 and 60/100,000 (1). In Italy recent studies have evidenced an incidence of invasive disease from PNC of 58.9/100,000 in < 3 years-old children and 50.4/100,000 in < 5 years-old children. In North America from the year 2000, a widespread vaccination of more susceptible populations was performed, in particular all the children of < 1 year-old and children until 5 years-old attending the community (asylum or maternal school) underwent vaccination. In new Italian Vaccine Plane of years 2005-2007, the anti-pneumococcal vaccination have been included between those recommended.

The effectiveness of the vaccination, estimated on the invasive types, ranges between 56% and 80% in several studies. After 2-3 weeks from the vaccination children develop specific antibodies. The polysaccharidic antigens are poor immunogenic in the 2-3 years-old children. In addition they do not induce subsequently an immunological memory. The conjugated eptavalent vaccine (PCV7) contains polysaccharides of the 7 stocks. Everyone is conjugated to a carrier represented by CRM-197, a modified diphtheria toxin. Furthermore this conjugation allows to activate the T cells. This determines a specific antibody production also in children of 2 months of age, an immunological memory of long duration and ability to give a fast booster effect with a new dose of vaccine. The protecting effectiveness in vaccinated children is 97.4%, and manifested protection in 85.7 % already in children who have only received 1 or 2 doses of vaccine (2). The regimen of three doses, at 3, 5 and 11-13 months of age, is commonly adopted in countries like Italy (3). The vaccine PCV7, administered at 2, 4, and 6 months with a 4th dose between 12 and 18 months, has reduced dramatically the invasive pathology. In children under the 5 years the reduction of invasive pathology was of 94% for the pathogens included in the vaccine, and of 75% for serotypes not included in the vaccine (4).

The population immunity of children older than 5 year-old has determined, from 1998-99 to 2003, a reduction of the invasive disease of 62%, for the pathogen included in the vaccine (4). It was observed an increase of disease from serotypes of PNC not included in the vaccine however. The advantages are obvious because the active balance of the cases has been in 2003 of 24.878 cases. Of these, 12.786 were children of less than 5 years, while the others 12,092 have been the cases of older than 5 years and adults (4).

Some authors, from 1997 to June 2004, have evidenced a reduction of invasive pathology from PNC, in the first 90 days of life. This is because the vaccinated subjects (children), not being carriers of PNC in the pharynx, more difficulty are able to transmit such infection to (sibling) newborn (Table 1). Moreover it was observed a reduction of the number of the PNC resi-

stant to beta-lactams and macrolids (more frequently employed antibiotics) because of their minor use of the antibiotic (5). In the attempt to widen the spectrum of action of the vaccine in the underdeveloped nations, it is under consideration a vaccine conjugated against a greater number of stocks, nine and eleven, that have demonstrated optimal preventive activity (Table 1). For these new vaccines it will be necessary further and deepened surveying in order to confirm the therapeutic action without adverse effects.

Antimeningococcus vaccine

The Meningococcus (*Neisseria meningitidis*) is a Gram negative bacterium (sero-groups A, B, C, Y, and W135) responsible of serious invasive meningococcal diseases (IMD) like meningitis and sepsis. In general population the number of healthy carriers of the bacterium is 10-25%. However the disease cases are limited. After 2-10 days of incubation, the subject involved develops a clinical evident disease. The symptomatology can evolve towards a disseminated intravascular coagulation, septic shock, insufficiency of organs, and fulminating septic purpura of Waterhouse-Frederiksen. The subjects more frequently involved, 30% of the cases, are < 5 years-old but this pathology is frequent also between the 15-19 years children. In Italy the incidence of Meningococcus meningitis (group B and C, both responsible of all the cases of IMD disease) is less than 1/100.000 cases. Moreover a progressive increase of cases has been taken place from sero-groups C (in the year 2004 and 2005 of 57 % and 56% respectively) of cases of meningococcal meningitis. From few years is available the conjugated vaccine against the meningococcus type C, which has a protecting action for a long time. With the exception of the old polysaccharide vaccines, the vaccine conjugated against the Meningococcus of type C is effective also under the 2 years of age because it stimulates the antibodies reply, and the memory of the immune system. Therefore it protects the subjects vaccinated for a long period of time (6). It is still experimental the vaccine against serotype B, but within the next few years it could be available. The mass vaccination of children, adolescents and adults until the 24 years of age, performed in Great Britain from November 1999, had the magnitude to reduce the high number of cases of meningococcal disease of group C. Frequently the disease involves the children from 0 to 5 years, and the adolescents of the college from the 15 to the 19 years. England and later other countries such as Ireland, Spain, Nederland, Canada and Australia have adopted the mass vaccination against the disease from Meningococcus type C, for the protection of the groups at risk, namely children and the adolescents. In Italy, the new PNV of 2005-2007 included the vaccination against Meningococcus C in the scheduled new vaccination of infancy. The dosages indicates 2 doses plus 1 under the age 1 year already from the 2nd month of life and at least 2 months from the other vac-

ination (generally 3rd – 5th and 11th month - like recommended in the PNV) or one single dose after 1 year of age. In some regions the vaccine is offered to Pugh risk categories, that are children and adults with particular conditions of hymmuno deficit (asplenia, anatomical or functional, deficit of the complement), and all the newborns, and to children who attend community.

During the year 2006 no case of invasive disease from Meningococcus type C was observed in Tuscany. To the same time it has been showed a reduction of the total number of the invasive diseases from Meningococcus. Moreover recently, it has been confirmed the possibility to associate the vaccine anti-meningococcus of group C with the vaccine exavalent scheduled in infancy. Vaccine MCV4 is today advised from the American Academy of Pediatrics (7). The vaccine administered in single dose for injective way seems to give a protection in 85% of the cases with duration of effectiveness of 7-8 years. The vaccine is recommended in adolescent with hymmuno-deficiency situations, for those who attend the university colleges, for the pilgrims, for travellers in countries at risk (8).

Antivaricella vaccine

The varicella is an infective, exantematic, epidemic disease, very contagious with recurrence every 3-4 years, above all in winter and spring. It is caused by a DNA varicella-zoster virus (VZV), more frequently transmitted from direct contagious.

In Italy, the varicella virus infect every year about 500,000 subjects, in prevalence during the paediatric age between 0 to 14 years of age, with a peak of incidence between 4 and 5 years of age (9).

The complications in the children are in 3-5% of all cases and in 10% of the subjects with specific pathology (immunodeficiencies, chronic pulmonary or cutaneous diseases, etc.) (10). The more serious complications are the interstitial acute pneumonia, the acute cerebellites, with an incidence of 1:4,000, characterised by persistent ataxia for several weeks although the outcome is generally benign. The encephalitis is a rare and often fatal extremely severe complication (1:40,000). Other rare complications are the meningitis, the mielitis trasversa, the syndrome of Guillain-Barrè, and other types of neuritis, and the Reye syndrome. The VZV virus diffuses in all the organs and apparatus. The incidence of the varicella in pregnancy has been estimated in 1:1,000 pregnancy in United States and 2-3 cases every 1,000 pregnancy in United Kingdom. The risk of the infection of the fetus increases with the gestational age (11). Several studies have confirmed that, subsequently the maternal varicella infection, the risk of malformations is of 2%, with a greater risk between 13 and 20 weeks of gestation in comparison to the infection within first 13 weeks (2.4 % versus 0.4%) (11, 12). The maternal infection from varicella in perinatal age, between the 5 days before and 3-5 days after the delivery, determines a neonatal varicella in

20% of the neonates. The rate of mortality of the baby for the infection from varicella in this period of the life is 30% (11). In relation to the several complications reported a great number of hospitalizations are expected. In Italy in the last hear 10 death related to varicella were reported (12).

In Sicily, a pharmaco economic evaluation of the cost for varicella infections in the year 2001 has suggested an estimated sanitary cost from 2,6 to 3,6 million of Euros. The estimation included the cost for the Acyclovir consumption. Studies carried out in Germany and USA has demonstrated that the vaccination against Varicella has a good costs/benefits balance. The vaccination introduced in the USA showed a reduction of the cases of varicella and the hospitalization that had exceeded 80% (13). In Italy, to obtain a drastic reduction of the disease, the vaccination should be made in limited times to cover almost 80 % of infantile population till the 2nd year of life and the adolescents with negative history for the varicella at least to cover 50% of the 12 years old children.

The Italian Plan Vaccine (PNV) suggests to vaccinate, for the varicella, the subjects at high risk to contract or to transmit the infection, including the susceptible women in fertile age or adolescent and those with negative anamnestic history for varicella. Some Italian regions have suggested the free vaccination even the problem will probably resolved, on a national level, which the availability tetravalent vaccine for morbillo, parotitis, rubella and varicella (MMRV). In September 2005, the Food and Drug Administration (FDA) has authorised, after a survey, the commercialization of the tetravalent attenuated vaccine for morbillo, parotitis, rubella and varicella (MMRV) (ProQuad®, Merck & Co) for children aged between 12 months and 12 years. The Advisory Committee on Immunization Practices (ACIP) suggested the administration, at least of one month of interval, of two doses of the vaccine after the 1st year of age or at the 12 years (4). Besides, in two groups of children of 12 - 23 months of age, were administered two doses of vaccine at 90 days of interval. The MMRV, administered in the first group, and the trivalent MMR plus the vaccine antivaricella Varivax, administered at the second group, were equally tolerated and determined similar good immunity reply (14). Since within short time it will be available the vaccine MPRV for without history of morbillo and varicella and never had been vaccinated for this disease. So it will be advised to administrate two doses of vaccine MPRV. Who had already received a dose of MPR can use the first dose of the varicella vaccine MPRV and the second dose of the Varivax vaccine after at least one month later.

Anti Rotavirus vaccine

The Rotavirus is a DNA virus of the Reoviridae family. Three sierological groups (A, B and C) may infect the humans. They are known like the main causes of

serious acute gastroenteritis, above all in the first infancy. Such pathogens, particularly the group A, may cause serious episodes of diarrhoea in infants and children during the winter and in areas with moderated climate. The period of incubation ranges from 1 to 3 days. Often the first symptom is vomit, followed by diarrhoea for 4-8 days. In some cases, the virus causes an isotonic dehydration with acidosis. From a meta-analysis of 124 studies in 52 different countries between 1973 and 2003, 4 sero-types (G1P, G2P, G3P and G4P) are responsible in the children of 88.5% of the cases of diarrhoea in the world and more of 90% in Europe. The G4 sero-type is more common in Europe.

In Europe every year the acute gastroenteritis from Rotavirus (R-AGE) causes 100,000 hospitalizations in paediatric age. Moreover every year 1 million of children need of specialised visits and rotavirus causes 385 deaths (15). The infection from rotavirus in the European children of less than 5 year-old is responsible, every year, of 3.6 million of episodes that determine 700,000 paediatric visits, 87,000 hospitalization, and 231 deaths (16). Therefore the disease remarkably affect on the sanitary costs in Europe. One recent survey has estimated the impact of the costs for patients of less than 4 years. In consideration that the children under the 36 months are near 1.6-1.8 million and that have 1.5-1.7 million episodes of enteritis every year the expensive is about 150-180 million of Euro. The 75% of the costs of such disease are due to the loss of the working time of the parents. Moreover the therapy, administered above all to the children of less than 36 month-old, is consistent for 47% in pre and/or probiotic, 26% in re-hydration solutions, 11% in antipyretics, and 11% in anti-emetic drug.

In order to prevent a severe disease, the vaccine anti-Rotavirus has been developed. Two types of oral vaccine have been commercialized, one in the USA and one in Europe, one monovalent and the other pentavalent, with an optimal effectiveness and safety profile. The effectiveness of these two vaccines is quantified in a reduction at least of 85% of the cases of serious disease from rotavirus. Furthermore there is a reduction of beyond 40% of the hospitalization for diarrhoea in South America, USA and Europe and, and a reduction of mortality rate in the developing countries. It has been obtained a recombinant virus human-bovine (G1, G2, G3, G4, P1) (PRV) not pathogenic for the human but in degree of induction a high immunity reply. This vaccine employed in a multicentric double blind randomized and controlled international study with placebo on 70,301 children of Italy, Finland, Germany, Sweden, Belgium, Mexico, Guatemala, Costa Rica, Jamaica, Taiwan and United States. Only healthy children to which the dose of vaccine or placebo have was administered at an age ranges between 42 and 84 days. The second and the third dose have been administered at intervals of 4-10 weeks (Pediatric Academic Societies, Washington, 2005).

Nobody of vaccinated children has showed serious side effects during the follow-up. Very rarely, the days immediately before the administration of the dose of vaccine/placebo has been showed side effects such as irritability, gaseous cholic, vomit, fever and alteration of the stools (17, 18, 19, 20). At term of the follow-up lasted 2 years the vaccinated children have showed a reduction rate of the hospitalization of 95.8% and the visits at the first aid of 93.4%. The investigation with the human-bovine vaccine (PRV) has showed a reduction in the demand for hospitalization and the sanitary requirement for infection determined gives from Rotavirus. Besides, it appears obvious that the vaccine finds maximal indication in the newborns beginning from the 6 weeks of life with the advantage that it can be administered contemporary with the normal schools vaccination. Many authors believe that the vaccine PRV, easy administrable and without side effects, will have in brief time a wide diffusion for the several uneasiness and costs that may avoid.

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IV SESSION: Round table on malformations

Chairman: M. Silvetti

Moderators: I. Barberi, M. Puddu

INTEGRATED MANAGEMENT OF CHILDREN WITH MALFORMATIONS: THE EXAMPLE OF DOWN'S SYNDROME

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Down's syndrome remains, despite the spread of cytogenetic prenatal diagnosis for pregnancies at risk, the most frequent single cause of mental retardation.

Pediatricians, and especially neonatologists, play an important role in the assistance to the newborn with DS, from a medical point of view, as well as a social and human ones. They have to inform parents about it and are involved in all the follow-up programs.

Epidemiology and pregnancy screening

The prevalence of Down's syndrome at birth has recently decreased in different socio-economical nations (from one in 700 to about one in 1000) (1). In Italy the evaluation of the rates of prevalence at birth has shown a progressive and meaningful decrement in time: from 14.4x10.000 born in the period 1978-1984, it has gone down to the 8.7 in the period 1993-1997 (2).

For a long time we have known that the risk of recurrence of Down syndrome increases in proportion to the increasing maternal age: from one in 1.650 in 20 year-old women, to one in 250 in those of 35 years old, one in 50 in women →45 years old). In the past few years in Italy there has been a progressive rise in the average age of conception: on the basis of ISTAT data we can observe an increase in the percentage of pregnant women over 35 years of age. If we submitted all these women to prenatal diagnosis, we could prevent the birth of only around 30% of patients with Down syndrome (3); therefore, maternal age alone is not a good criteria to select the population at risk. We need of more sensitive and specific screening programs.

In the 1st. trimester of pregnancy the most effective screening test is the combined use of maternal serum screening with Plasma protein A (PAPP-A) and human chorionic gonadotropin (β-hCG) with fetal ultrasound testing to identify the thickening of the nuchal fold, that may have an 80-86% detection rate with 4-5% rate of false-positives 4. In the second trimester of pregnancy

several prospective studies have confirmed the efficacy of the triple screening test, that measures, in relationship to the maternal age, serum alphafetoprotein, human chorionic gonadotropin and unconjugate estriol with 78% detection rate with 7% rate of false positives (4).

When the cytogenetic prenatal diagnosis confirms the presence of a Down fetus, a genetic counselling must be provided giving all the informations about Down syndrome, its natural history, possibilities of intervention and follow-up, accompanying the parents in the decision making process (non-directive approach).

Diagnosis and neonatal follow-up

Although the phenotype is variable, a clinical diagnosis is possible at birth in most of cases in relation to the characteristic physical (5).

Difficulties can arise in very small babies, as in the case with premature or small for age newborns, or if there are severe clinical problems that turn the attention away from the phenotypic characteristics of the infant.

In 91.8% of cases a standard trisomy 21 originating from a non meiotic chromosome disjunction is revealed, in 4.8% a trisomy by unbalanced robertsonian translocation (of which the most frequent is the 14/21) can be found, and in 3.4% there is a mosaic trisomy with the presence of two cellular lines (cells with normal karyotype and trisomy 21) (6). Only in 0.2% of the cases is there partial trisomy because of the duplication of the critical region of the chromosome 21 (21q22.2q22.3) (7).

The communication of diagnosis is, perhaps, the most delicate moment of the whole follow-up period of the child with Down syndrome (8) and it must be done in two stages: when the diagnosis is suspected and when it is confirmed.

The diagnostic suspicion must also be communicated to both parents together to avoid putting the burden of communicating it to the other on one of the parents when their relationship may already be difficult owing to the fact that the child that was born is different from the one they expected. The suspected diagnosis should be communicated referring to the child by his name to underline that each child is a single patient and must be treated as such because it has its own rate of physical and mental development.

The communication must be unhurried and undertaken in a quiet environment: the neonatologist should spend enough time with parents to establish a mutual relationship so that personal feelings can be freely discussed and questions asked and dealt with sensitively, using clear and comprehensible language. Once the diagnosis has been confirmed, parents must be met again and try to give them the impression that he knows all about the syndrome and the problems they will have to face. He will have to reassure them, trying to give them quietness, discussing with them. He will also underline the child's potentialities and resources,

involving the parents in the decisions regarding the child.

The clinical examination will include a detailed evaluation of growth (growth charts of Down syndrome) (9-11) and neurological development (hypotonia, difficulty with feeding).

Congenital heart defects are the most common and severe malformation: about 40-50% of newborns are affected. The most frequent structural defects are: atrioventricular septal defect (45%); ventricular septal defect (35%); secundum atrial septal defect (8%); persistent patent ductus arteriosus (7%); tetralogy of Fallot (4%) and other lesions (1%) (1).

All newborn babies with Down syndrome must be submitted to an echocardiogram, even in the absence of symptoms (12 13).

Among congenital gastrointestinal tract malformations (7.3%) (14), the most frequent anomalies are: duodenal stenosis (4-7%), congenital megacolon (1%), anular pancreas and anal imperforation.

Sensory defects are frequent in Down syndrome (15). It is necessary that all the newborns with Down syndrome are submitted to investigations related to the auditory ability: otoacoustic emission at birth and/or brainstem auditory evoked response at 3 months.

Red reflexes should be checked at birth, as their absence is an important clinical sign of congenital cataract (15%). Other ocular abnormalities are frequent in Down syndrome: dacryostenosis, glaucoma, strabismus and nystagmus.

During the neonatal period there are frequent: polycythemia (18%) (that should be treated, in order to avoid cerebral damage); transient myeloproliferative disorder (characterized by an increased number of white blood cells, often with a high percentage of blast cells, usually without anemia or trombocytopenia) (16); thrombocytopenia, thrombocytosis, macrocytosis, lower or higher leukocyte count and congenital leukæmia (<1%).

Transient myeloid disorder is a self-regressing myeloproliferative disorder that is found mainly in newborns with Down syndrome during the first 4 weeks of life, affecting as many as 10% of all neonates, (17). The management of transient myeloid disorder is conservative, most often involving watchful waiting or supportive care.

Congenital hypothyroidism is much more frequent than in the general population (1%) (18). Screening for thyroid disease must be carried out at birth.

Breastfeeding has a protective effect towards different particularly frequent pathologies in Down children (immunitary disorders, infections and obesity) (19). It favours, besides, the bonding and the relationship of the newborn with its mother (20). It is necessary that only the newborns with the most severe pathologies requiring intensive care as well as surgical treatment must be maintained into a hospital for a long time.

On the child's discharge pediatrician, besides programming the clinical and instrumental follow-up, will have to provide the parents with all the necessary

information on the territorial realities. Not only will he deal with the clinical controls, but he will offer support to the couple, "accompanying them" on that long and complex follow-up.

Neonatologists must give parents of DS newborn all the informations about the existence of Parents' Associations which can represent an important instrument to face the problems related to the birth of an infant with DS. Parents' Associations provide information on territorial services, offer support to the family, refers on the mutual exchange of experiences among people that live the same problems. Families must be active part in the educational and rehabilitative follow-up project of the Down child.

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CRANIO FACIAL ANOMALIES – CRANIOSYNOSTOSIS

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At birth the skull is made up of five bones held together by seams called sutures. The sutures remain open until age one to allow for brain growth.

Craniosynostosis is a congenital anomaly caused by early closure of one or more of these sutures, resulting in limited or distorted head growth. Other names used for this condition are synostosis or cranial stenosis. In some cases, in addition to abnormal head shape, craniosynostosis can result in raised pressure inside the skull.

Craniosynostosis can occur as part of a syndrome or as an isolated defect (nonsyndromic). Craniosynostosis is called "simple" when only one suture is involved and "compound" when two or more sutures are involved. The sagittal suture is affected in 40 to 60 % of cases, the coronal suture in 20 to 30 % of cases, and the metopic suture in less than 10 % of cases; true lambdoid synostosis is rare. Syndromic craniosynostosis is less common (20 %), even though more than 100 syndromes with craniosynostosis have been identified. In cases of syndromic craniosynostosis, multiple sutures are involved.

Craniosynostosis is also seen in the context of a variety of syndromes. The most common syndromes encountered in clinical practice are Crouzon, Apert, Saethre-Chotzen, and Pfeiffer. These syndromes are

all characterized by bilateral coronal synostosis of varying severity, often combined with some degree of sagittal synostosis. The typically observed brachycephaly is due to a shortened anteroposterior diameter of the skull and corresponding enlargement of the bitemporal and biparietal diameter. Other suture involvement can result in oxycephaly, scaphocephaly, and turricephaly. Combinations of all these deformities can be seen in complex cases

Scaphocephaly corresponds to sagittal synostosis. Plagiocephaly corresponds to unilateral coronal synostosis. The term posterior plagiocephaly corresponds to lambdoid synostosis. Trigonicephaly corresponds to metopic synostosis.

The terms brachycephaly, oxycephaly, and turricephaly are used for various forms of synostosis affecting both coronal sutures in the former or in combination with the sagittal and sphenofrontal sutures in the latter forms, usually encountered in syndromic types. Brachycephaly is derived from the Greek word *brachys*, meaning short. Oxycephaly is derived from the Greek word *oxys*, meaning sharp, and is a high, conical head with sharp bossing in the region of the anterior fontanelle. Turricephaly is derived from the Latin word *turris*, meaning tall, and is a round head, like a tower.

Positional nonsyndromic plagiocephaly is a form of craniosynostosis. In 1992, the American Academy of Pediatrics recommended that infants sleep on their backs to reduce the risk of sudden infant death syndrome (SIDS). This successfully reduced the number of infants with SIDS, but also increased the number of infants suffering from positional plagiocephaly due to back sleeping. An infant with positional nonsyndromic plagiocephaly has a flattened skull at the back of the head. Positional plagiocephaly can also occur if the child was born prematurely, has torticollis, cervical spine abnormalities or problems with their eyes. In deformational plagiocephaly, the skull shape abnormality may not have been present at birth and may improve over time. One side of the back of the head is flat, but the involved ear is pushed forward and the forehead may appear fuller. This is in contrast to lambdoid synostosis that is present at birth, worsens with time and has an ear that is pulled back toward the affected suture. In some cases, the growth of the skull is restricted enough to cause increased pressure in the head and can lead to headaches, visual problems, or developmental delays. When there is excessive pressure on the brain, problems with neural development, including visual problems can occur. These may be subtle or mild, such as learning disabilities, or more severe, such as mental retardation and loss of vision. Most children never develop such severe symptoms. However, because we cannot predict which children will have these problems, treatment of the craniosynostosis is recommended to prevent the possible increased intracranial pressure. Plain radiography is the first step in the evaluation of suspected craniosynostosis and is sufficient for diagnosing single-suture craniosy-

nostosis. Anteroposterior and lateral views of the skull are usual. It is important to evaluate the entire length of each suture because only a small segment may be involved. The signs of craniosynostosis on plain radiography include bony bridging across the suture that produces beaking or heaping up of bone; sclerosis, straightening and narrowing of the suture; and loss of suture clarity. The diagnostic value of the CT scan outweighs that of plain radiography because the sutures can be identified more accurately on a CT scan. In addition, CT scanning helps in evaluating the brain for structural abnormalities and in excluding other causes of asymmetric vault growth. Three-dimensional surface reconstruction using CT scanning can help the surgeon to accurately delineate the craniofacial deformity and plan surgical reconstruction. Once the diagnosis of craniosynostosis is confirmed, the treatment is surgical correction. The best time to intervene is when the infant is between three and nine months of age. However, infants with symptoms and signs of increased intracranial pressure require urgent decompression. The treatment of craniosynostosis requires surgery in order to release the involved suture and reshape the malformed bones of the skull. The goals of surgery remove the involved suture so that brain growth can occur normally and improve the facial appearance

Etiology. Autosomal dominant inheritance has been clearly identified in syndromic forms of craniosynostosis, although a number of patients have spontaneous new mutations. Familial cases are frequent, constituting from 25-46% of the total number of cases, variable for the different syndromes. Complete penetrance has been observed in all inherited cases. No inheritance pattern has been identified for nonsyndromic forms of craniosynostosis, although a familial occurrence has been observed in 4-10% of the patients, variable for the different types of syndromes. In familial cases, variable vertical and horizontal penetrance has been observed.

The presence of mutations in the group of genes coding for fibroblast growth factor receptor (FGFR) in patients with Apert, Crouzon, and Pfeiffer syndromes is now clearly established. Almost all cases of Apert syndrome are due to 1 of the 2 described mutations of the FGFR2 gene, located on chromosome 10. Currently, 25 mutations have been identified on the FGFR2 gene and implicated in the pathogenesis of Crouzon syndrome. Mutations of both FGFR1 and FGFR2 genes have been described in Pfeiffer syndrome, each corresponding to phenotypes of different clinical severity. Mutations in TWIST1 are causative of Saethre-Chotzen syndrome. Inheritance is autosomal dominant.

Apert's syndrome (acrocephalosyndactyly) is an autosomal dominant disorder that occurs in one of every 160,000 live births. The syndrome is caused by nucleotide alterations resulting in amino-acid substitutions, leading to a mutation in the FGFR2 gene located on chromosome 10. Craniosynostosis and symmetric syndactyly of the extremities are hallmarks of this

syndrome. The clinical features include misshapen skull caused by coronal suture synostosis, wide-set eyes, midface hypoplasia, choanal stenosis, and shallow orbits. Intracranial anomalies include megaloccephaly, hypoplastic white matter, and agenesis of the corpus callosum, leading to cognitive impairment.

Pfeiffer's disease is also a close relative of Apert's syndrome, although is less severe. It is characterized by acrocephaly, broad thumbs and great toes and partial soft tissue syndactyly of the hands. It also follows an autosomal dominant mode of transmission. Most cases are familial, but sporadic cases exist.

Crouzon's disease is inherited through an autosomal-dominant pattern. Nearly 60 percent of cases are new mutations, and many are associated with paternal age older than 35 years. Crouzon's disease occurs in one of every 25,000 live births and accounts for 5 percent of cases of craniosynostosis. Nucleotide alterations causing amino-acid substitutions at the FGFR2 gene on chromosome 10 lead to the Crouzon phenotype. Clinical findings include brachycephalic craniosynostosis, significant hypertelorism, proptosis, maxillary hypoplasia, beaked nose and, possibly, cleft palate. Intracranial anomalies include hydrocephalus, Chiari 1 malformation, and hindbrain herniation. Pathology of the ear and cervical spine is common. Infants with Crouzon's disease do not have anomalies of the hands and feet as do infants with Apert's syndrome.

Saethre-Chotzen syndrome has a more variable presentation. The most consistent features of this syndrome include acrocephaly, hypertelorism, nasal septal deviation, lid ptosis, and mild syndactyly of the hands and feet. Saethre-Chotzen is possibly the most common of all the craniosynostosis syndromes. Despite its commonness, however, the syndrome is probably underdiagnosed. This is due to the great variability in expression even within affected families, as well as the relative mildness of the abnormalities.

SGA DUE TO GENETIC REASONS

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SGA (Small for Gestational Age) definition is statistical and refers to every newborn infant whose anthropometric parameters are usually < 10th percentile (fixed cut-off), but sometimes < 3rd percentile or < 2 DS.

IUGR (Intra Uterine Growth Restriction) definition is clinical (inability to reach an appropriate genetic growth): so, the auxological evaluation is longitudinal, prenatal and concerning a coming-event.

The auxological evaluation of SGA condition, instead, is transversal, pre or postnatal and concerning a result.

The incidence of SGA infants is 2.5% in that developed countries and 20% in developing ones. Mortality is from 5 to 20 fold more than observed in AGA (Appropriate for Gestational Age) infants and, in the

long run, from 5 to 10 fold more frequent neurodevelopment and sensory-outcomes are observed.

Usually we have to divide the heterogeneous SGA infant group into:

1. subjects with IUGR. This condition is defined "symmetric" when all the 3 growth parameters are involved, and "asymmetric" when there is a minor/no head sparing. These infants are more likely to incur perinatal asphyxia, but with time, have a better prognosis.

2. small-constitution SGA infants.

The SGA new-born infant is not a specific pathological entity, but is the product of different causes due to maternal uterus-placenta risk-factors (80%) or fetus metabolic-infective and genetic reasons (20%).

Very often infants with Congenital Malformations (CM) are also IUGR, as reported in several studies.

In a 1988 population based study, Khoury showed that 22.3% of CM infants were also IUGR. The percentage of IUGR was 73% among the infants affected by anencephaly and 83% among those with trisomy 18.

In 1991, Van Vught, having considered 261 IUGR infants, showed that 21.8% were affected by CM. The most frequent IUGR associated congenital malformations were chromosomal anomalies (6.1%), genetic and urinary system disorders (6.5%) and CNS malformations (4.2%).

In 1991, Lituania conducted a population retrospective study on 270 CM infants born between 1987 and 1990. Among the infants, he found 18% IUGR cases. Among these, 36% were associated with a chromosomal anomaly.

Finally, Mc Cormick (1992) showed that IUGR infants have twice the risk of CM than AGA; the risk increases 3 fold if the weight is very poor (< 2 DS).

We cannot know exactly how much genetic reasons increase the percentage of SGA-infants, however malformation syndromes have from 15 to 20% intrauterine growth restrictions. Therefore, 600 syndromes, at least, can be IUGR associated.

1982-2002 IMER Register data show that 10,233 of 511,425 newborns are affected by CM, that is to say a 2% birth rate.

The CM infant can be divided into three groups : SGA, AGA and LGA (Large for Gestational Age), 2,159 infants (24.5%) are SGA, 6,116 (69.4%) AGA and 538 (6.1%) LGA.

Infant-distribution analysis based on the evidence of one or more malformations and BW <10th centile shows that:

a. 6,948 infants (78.8%) are affected by an isolated CM, but 1,865 (21.2%) have multiple malformations or a syndrome;

b. 21.3% (1,482) of the infants affected by isolated CM are SGA, while among the others the incidence increases to 36.5% (p<0,001).

The most frequently observed malformations in the SGA infants were:

1. Chromosomal abnormalities (35.1%)
2. Hypospadias (29,7%)

3. Congenital Heart Defects (27,3%)

4. Cleft lip and palate (22.5%).

1) The chromosomal anomaly most frequently associated with SGA birth is trisomy 18 with a relative risk (rr) = 72. Trisomy 13 and 21 are correlated also with a SGA condition, but in a less significant way : rr = 8.3 and 4.9 respectively.

2) About hypospadias, IMER data (1982-2002) show that 881 out of 5,256 CM male infants (1,260, i.e 24%, were SGA) were affected by hypospadias, that is to say 16.8% of the whole population. 262 (29.7%), out of these 881, were SGA.

Most infants (78%) show a mild hypospadias, a medium can be seen in 14.9%; finally, a severe hypospadias is observed in 6.4%. The incidence of hypospadias in SGA infant is increased from 27.4% for the mild form, 34.4% for the medium and 53.7% for the severe one. This suggests a significant association between hypospadias and SGA.

3) 27.3% of infants affected by CHD are SGA. In this group of newborns the defect most frequently observed is Tetralogy of Fallot (33%). We also saw a high SGA percentage in CoAo, HLHS, TGV and DIV cases.

4) Cleft lip and palate are often SGA associated anomalies. From IMER data we can see that 22.5% of these infants are SGA.

The Neonatologist, when he faces a SGA infant, has to collect first all the case history data necessary to understand the phenomenon (family, parental, pregnancy and birth histories), and, after a deep and careful physical examination, he has to go on with all the instrumental and laboratory investigations that are essential to reach a diagnosis.

Other associated CM have to be investigated because of the great risk of occurrence. Their presence can suggest specific conditions, variously due to heredity. Anyway, it is necessary to remember that the great amount of syndromes associated with SGA condition make a diagnosis more difficult. It is not always possible to make an antenatal diagnosis. In most cases, clinical factors alone cannot lead to a sure diagnosis both because of the extreme variability of clinical expression and because of the changeability of the phenotypical outline during the gestation. Many times, there is no specific analysis for a sure diagnosis, except organizing the clinical data.

Morphologically it is useful to divide the SGA associated conditions into:

a) Syndromes with a symmetric restriction in all the anthropometrical parameters

b) Syndromes with an asymmetrical restriction. In this case we can divide this group into: syndromes in which the parameter most affected is length (foetus-neonatal osteochondrodysplasias) and those in which the most affected parameter is head-circumference (microcephaly).

Etiologically, all these syndromes may have different ways of transmission: chromosomal, by Mendel-type heredity, autosomic, recessive or dominant, X-linked,

monogenic, environmental, etc.

Focusing on SGA genetical conditions, we can underline that almost all the aneuploidy and chromosomal unbalancing conditions may show a poor birth weight and a complex phenotypical outline characterized by: dysmorphic cranio-facial, major malformations and neurologic and behavioural abnormalities, very often present at birth.

Paradigmatical examples are: trisomy 18, trisomy 13, some partial monosomies like Wolf-Hirschhorn's, Cri du Chat's etc.

Asymmetrical syndromes include fetal-neonatal osteochondrodysplasias (Hypochondroplasia, Ellis-Van Creveld, Robinow, etc), and among microcephaly associated ones we find the syndromes: Cockaine's, Miller-Diecker's, Seckel's and Seckel's-like.

SGA associated symmetrical syndromes include both forms with an identified gene (i.e. Bloom, Smith-Lemly-Opitz, Williams, Cornelia de Lange, etc) and forms without an identified gene (i.e. Dubowitz, Johanson-Blizzard, etc.).

Conclusion

The malformation syndromes, associated with SGA, are many, quite heterogeneous and non-specific: when separately considered, they are rare and complex to diagnose.

SGA infants are frequently correlated with genetical pathologies.

The theories and mechanisms that are most likely to justify this correlation are different, but they can be thus synthesized: an intrauterine growth restriction is a direct consequence of a genetic pathology whether the pathology is due to monogenetic defects or to a more complex defect such as aneuploidy or chromosomal pathologies.

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NEWBORN LARGE FOR GESTATIONAL AGE AND OVERGROWTH SYNDROMES

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Introduction

A newborn is classically defined large for gestational age when has a birth weight more than or equal to the 90th percentile or two standard deviations above the mean, for a given gestational age (1). However Goldenberg et al (2) have indicated that birth weights have increased with the time. This type of threshold for abnormality is termed isolated normality.

The use of this definition includes a significant proportion of the normal population.

Another popular mean to categorize the large infant is by using the term macrosomia: macrosomia has several thresholds for its definition, but the most widely used definition is when birth weight exceeds 4,000 g. The percentage of infants who are LGA and born with genetically-driven factors can exceed 70% (3); the remaining 30% of infants are those can be considered infants who are abnormally LGA and will require special clinical attention.

Maternal risk factors for macrosomia included maternal obesity, hypertension, gestational diabetes, smoking, increasing age, parity, post-term pregnancy, previous macrosomic infant and even high socio-economic status.

Over 4000 g mortality increases with birth weight. LGA infants have an high incidence of birth injuries. The incidence of hypoglycemia and of congenital anomalies is also higher than in term infants of normal weight. The more frequent clinical problems in LGA newborns are a mild hypotonia, poor feeding and a significant decrease in neonatal weight in the first days of life.

Maternal diseases (diabetes for example) or fetal diseases (overgrowth syndromes) can involve, other than a large birth weight, also important clinical problems in neonatal period and subsequently.

The infants of diabetic mother (IDMs) share certain distinctive morphologic characteristics, including long size, macrosomia and high morbidity risk. The IDMs tend to be large and plump owing to increased body fat and enlarged viscera, with puffy, plethoric facies. The IDMs tend to be "jumpy", tremulous, and hyperexcitable during the 3 first days of life, although hypotonia, lethargy and poor sucking also may occur. They may have any of the diverse manifestations of hypoglycemia. There is a greater incidence of hyaline membrane disease in IDMs than in infants of normal mothers born at comparable gestational age. Cardiomegaly is common (30%) and heart failure occurs in 5 to 10% of IDMs. There is also an increased incidence of hyperbilirubinemia, renal vein thrombosis and congenital anomalies (cardiac and skeletal are most common) (4).

Overgrowth syndromes

The main overgrowth syndromes are: Beckwith-Wiedemann syndrome, Sotos syndrome, Weaver syndrome, **Simpson-Golabi-Behmel syndrome** (5). In their typical and complete presentation, newborns with overgrowth syndromes in addition to an excessive weight and length frequently have neonatal hypoglycemia, specific findings and various associated typical anomalies.

Beckwith-Wiedemann syndrome (BWS, OMIM # 130650) is the most common overgrowth syndrome, with an incidence of approximately 1 in 13,000 births. The key clinical findings are omphalocele, macroglossia and macrosomia. However, given the extreme

variability of the BWS phenotype, a panel of diagnostic criteria has been proposed, recommending at least two major and one minor criteria be identified to confirm the diagnosis. The major criteria include positive family history, macrosomia, anterior linear ear lobe creases/posterior helical ear pits, macroglossia, abdominal wall defects, visceromegaly, embryonal tumors in childhood, hemihyperplasia, renal abnormalities, adrenocortical cytomegaly, cleft palate (rare); the minor criteria are polyhydramnios, prematurity, neonatal hypoglycemia, probably related to hyperplastic changes in the pancreas, facial nevus flammeus, capillary dysplasias, characteristic face, cardiac anomalies, diastasis recti, advanced bone age, monozygotic twinning.

BWS is associated with an increased incidence of prematurity, perhaps as high as 50%.

Frequently reported prenatal findings include fetal overgrowth, polyhydramnios, enlarged placenta, a distended abdomen or omphalocele, long umbilical cord. Some recent studies reported an unexpectedly high incidence of BWS in neonates conceived with assisted reproductive technology.

In BWS patients average birth length is 52,5 cm and average birth weight is 4 Kg; BWS children show a growth velocity above the 90th centile up to 4-6 years of age, with a decreasing rate from mild-childhood through puberty. Bone age may be markedly advanced and particularly so during the first 4 years of life. Hemihyperplasia occurs in about 25% of cases, but it may not be apparent at birth. The face may be "coarse" with prominent metopic suture, large anterior fontanel, prominent occiput, midfacial hypoplasia and infraorbital creases.

Mentally development is generally normal in BWS although it may be delayed if there is a chromosomal duplication involving 11p15, and/or complications of prematurity, and/or uncontrolled or undetected hypoglycemia. Cardiac malformations have an incidence ranging from 9 to 34%, cardiomegaly being the diagnosis in about half of these cases.

Children with BWS have an elevated risk of developing malignancies (7.5-12%): the most common occurrences are Wilms tumor, hepatoblastoma, neuroblastoma and rhabdomyosarcoma.

BWS is a complex multigenic disorder caused by modifications of growth regulatory genes on chromosome 11p15. Paternally expressed/maternally silenced genes implicated in the etiology of BWS include IGF2 and KvLQT1. Also include in this class of genes are paternally silenced/maternally expressed H19, whose untranslated mRNA may function as a tumor suppressor CDKN1C, which negatively regulates cell proliferation and KvLQT1 which encodes for a part of a potassium channel protein complex.

Most BWS cases are sporadic, due to either de novo chromosome rearrangement, or uniparental disomy (UPD), or putative imprinting errors.

Sotos syndrome (SoS, OMIM #117550) is characterized by increased birth length and weight, excessive

growth during the first 4 years of life, advanced bone age and distinctive facial traits. Overgrowth is commonly evident in the newborn, with birthweight averaging 4,200 g in males and 4,000 g in females. Mean birth length is 55.6 cm in males 57.3 cm in females. SoS patients have frequently hand and foot length above the 97th centile, early dental eruption and tendency to early puberty. In 84% of SoS patients bone aged is advanced. Final height is often within normal range. Occipitofrontal head circumference tends to exceed the 95th centile by the age of one year. In the neonatal period poor feeding and hypotonia are common.

The typical facial gestalt included marked frontal bossing, high frontal hairline, frontoparietal balding, downslanting palpebral fissures, narrow bitemporal diameter without true hypertelorism, flue cheeks, high palate, facial flushing.

Seizures are found in 50% of patients, but in approximately half of these they are of febrile origin. Delay in expressive language has been observed during infancy and a mean DQ/IQ in SoS patients is 78. Performance deficit in SoS may be accompanied by autistic behaviour and characteristic MRI pattern: prominence of trigonus, prominence of the occipital horns and ventriculomegaly. The incidence of heart defects is estimated at approximately 8%, gastrointestinal and urogenital anomalies have also been reported.

Children with SoS have an elevated risk of developing malignancies vs general population

Most cases of SoS reported in literature were sporadic and thought to result from new mutations of postulated dominant genes. In 2002 Kurotaki et al. found rearrangements of the NSD1 gene in SoS patients harboring a chromosomal translocation, encompassing 5q35. Haploinsufficiency of NSD1 is the major cause of SoS, but in other cases it's possible to found a 5q35 microdeletion.

Weaver syndrome (WS, OMIM #277590) is a condition characterized by persistent overgrowth of prenatal onset, accelerated bone maturation, distinctive craniofacial appearance, developmental delay, a hoarse, low-pitched cry, congenital hypotonia, widened distal long bones and camptodactily. Mean birth weight is 4785 in males and 3,883 in females, length 56 and 53, and OFC 366 and 35.2, respectively. Skeletal growth is more accelerated than skeletal maturation, resulting in excessive height in adults.

Craniofacial traits include macrocephaly, broad forehead, flattened occiput, sparse hair, hypertelorism, long prominent philtrum, relative micrognathia and redundant nuchal skin folds; the ears are large, low-set, and may be of abnormal structure. Other common findings are camptodactily, prominent finger pads, thin and deeply set fingernails, broad thumbs, clinodactily of toes, foot deformities and limited extension of the elbows and knees. The infant cry is typically low-pitched and hoarse.

Children may have breathing and swallowing difficulties, but frequently end up with a voracious appetite.

Few cases of tumors associated to WS have been reported.

In only one study was identified a NSD1 mutation in 42% of WS patients: the authors suggested that probably WS and SoS are allelic conditions. However, this hypothesis was not confirmed by other authors and other international studies.

Simpson-Golabi-Behmel syndrome (SGBS, OMIM # 312870) is an X-linked semi-dominant condition, characterized by pre and postnatal overgrowth, facial anomalies and various congenital anomalies. The clinical spectrum is broad, ranging from mildly affected female carriers to multiple malformations and even neonatal death in males.

Overgrowth, typically of prenatal onset, with birth length, weight and head circumference all above the 97th percentile in affected males, continues postnatally with final height usually above the 97th percentile. SGBS patients have macrocephaly, "coarse" face with hypertelorism, downslanting eyes and epicanthic folds, hypotonia, supernumerary nipples, rib/vertebral anomalies, advanced bone age, hepato-splenomegaly, neonatal hypoglycemia.

Hands and feet are usually broad with polydactyly, cutaneous syndactyly, fingernail hypoplasia, especially of the index finger.

The incidence of heart defects is estimated at approximately 35%, while ECG abnormalities at 12%. Like other overgrowth syndromes, SGBS is characterized by an increase risk of embryonal-childhood tumours.

The syndrome is caused by mutations in the GPC3 gene, mapped in Xq26. GPC3 is likely involved in regulating cell proliferation and apoptosis, and modulating cellular responses to growth factors.

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MAGISTRAL LECTURE

Chairman: S. Chiappe

NEONATAL THROMBOCYTOPENIAS

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Thrombocytopenias are very common in the neonatal period, especially in preterm or sick neonates. Infact, approximately 1/3 of the neonates admitted to intensive care nurseries are affected by thrombocytopenia. A useful approach in the differential diagnosis of thrombocytopenia in the newborn is to divide the patients into three distinct categories: thrombocytopenia in acutely ill infants, thrombocytopenia in infant who have also congenital malformations and thrombocytopenia in well appearing infants. Thrombocytopenias in acutely ill neonates is a severe complication being associated with high mortality and morbidity. Common neonatal condition associated with thrombocytopenia are hypoxemia, bacterial sepsis, necrotizing enterocolitis, polycythemia and thrombosis. Among the neonates not acutely ill with thrombocytopenia, are included those with congenital infection (CMV, toxoplasmosis, rubella), Kasbach-Merritt syndrome and thrombocytopenia-absent radius syndrome and other inherited thrombocytopenic syndromes. In a well appearing infant the most common causes of thrombocytopenia, in decreasing order of frequency, are neonatal alloimmune thrombocytopenia (NAIT), maternal ITP, maternal LUPUS and the inherited forms. NAIT, which accounts for 10-20% of cases of neonatal thrombocytopenia, results from the development of maternal IgG antibodies directed against fetal antigens (most commonly HPA1A) inherited from the father. These antibodies cross the placenta and destroy the fetal platelets. Mothers of these infants are well and do not have thrombocytopenia. First born infants can be affected but the severity of the disease tend to increase in subsequent affected siblings. Establishing a diagnosis is critical to prevent or trait neonatal haemorrhage which occur in 10-25% of these infants. Diagnosis is confirmed by demonstrating platelet antigens incompatibility in the parents and the presence of maternal antibodies against the father's platelets in the neonate/fetus. In future siblings, the risk of intracranial haemorrhage is approx 25%. Adequate counselling of the parents is warranted. The options for antenatal treatment of the fetus are object of discussion and include: administration of IgG to the mother eventually associated with prednisone and/or in utero transfusion of antigen-negative platelets to the fetus. In newborns affected,

therapy is based on the infusion of concentrated, washed and irradiated maternal platelets or donor platelets negative for the involved father antigen associated with intravenous immunoglobulin. The thrombocytopenia resolves in 3 weeks.

The inherited forms show a marked genetic and clinical heterogeneity. The differential diagnosis is based on the analysis of the size (macro and micro-thrombocytopenia) and morphology of the platelets, the association with alterations of other blood cell lineages and the presence of associated anomalies. The most common are the amegakaryocytic thrombocytopenia, the thrombocytopenia with absent radius, MYH9-related diseases and Bernard-Soulier syndrome.

Chairman: A. Corrias†
Moderators: D. Rosatelli, G. Serra

NEW ASPECTS OF HEME METABOLISM

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Free radical reactions are involved in several pathologic conditions i.e. atherosclerosis, ischemia-reperfusion injuries, aging disorders, neonatal pathological states, retinopathy of prematurity, bronchopulmonary dysplasia, intraventricular hemorrhages, periventricular leukomalacia, renal vein thrombosis and necrotizing enterocolitis (1-10). Oxygen is frequently used in neonatal disorders. Oxygen itself is a free radical, and having an unpaired electron can generate uncontrolled free radical reactions. The consequence of the unlimited chain reactions is the destruction of lipoproteins, glycoproteins, nucleic acids, and the consumption of antioxidants leading to cell-, tissue injury. Oxygen derived free radicals also play beneficial role in host defense against infective agents. Neutrophil granulocytes, macrophages generate superoxide anion, hydrogen peroxide, hypochlorite, nitric oxide in significant amount at special places close to the invading organisms or targeted organs in order to provide protection for the human body. Administration of oxygen during treatment needs strict control, since large studies proved the role of hyperoxia in retrolental fibroplasia and bronchopulmonary dysplasia. Healthy organisms keep oxygen concentrations in the blood between narrow edges, so in case of pathologic conditions the same strict limits has to be followed. Oxygen, as a drug owns those characteristics as other drugs, the right dose and the right time needs to be determined in all circumstances, in this way significant amount of side effects of oxygen treatment can be prevented.

Accepting the well known dogma, i.e. transition metals

as catalytic agents are necessary for oxygen toxicity, the study of the role of these metals is compulsory in free radical disorders. Using tissue homogenate, iron catalyzes lipid peroxidation generated by oxygen free radicals. Chelation of iron by desferrioxamine, a water soluble iron chelator, blocks the free radical chain reaction totally. In case of living cells iron depletion makes them resistant to free radicals. The hypothesis derives from these observations, i.e. iron accumulation of cells and tissues is significant risk factor for oxygen toxicity. The hypothesis is strengthened by the fact that accumulation of ferritin, a large molecular weight cellular protein having an iron storing function, may lead to hepatic cirrhosis, cancer and heart failure. To develop model systems for studies with iron rich cells and tissues is extremely difficult since the iron uptake by cells and organs are strictly regulated. Those iron complexes which work in tissue homogenates for generating free radical reactions do not work in living cells. The first study which was able to load living cells with iron used lipid soluble iron chelator, 8-hydroxyquinolin, and target cells, vascular endothelial cells became extreme sensitive to free radicals coming from the extracellular space or derived from intracellularly (11). It suggests that hydrophobic iron complexes may bypass the well controlled and regulated iron uptake routes, and iron can accumulate in the intracellular hydrophobic cellular milieu. After this significant observation research started to discover the natural, hydrophobic, endogenous iron complex or complexes having the same characteristics as 8-hydroxyquinolin-iron complex owned.

Small molecular weight iron complexes and proteins participating in iron transport and storage were not able increase the iron content of living cells and could not sensitize them towards reactive oxygen species. The only complex which was able to mimic the effect of 8-hydroxyquinolin-iron complex was heme (12). The synthesis of heme is under strict control, and the cellular heme content is mainly located in heme proteins. The intracellular heme transport is mediated by specialized proteins. The main intracellular heme proteins are the hemoglobin, myoglobin, cytochromes and other heme containing enzymes, for example nitric oxide synthase. In terms of quantity the most significant heme protein is the hemoglobin. Intracellularly a repair system keeps hemoglobin in reduced state where the final enzyme of the system is the methemoglobin reductase. During the aging process the efficacy of the repair system decreases, and the heme, escaped from the globin, intercalates into the lipid bilayer of the red blood cell causing cell membrane lipid peroxidation and cell elimination by the spleen. In that circumstance when hemoglobin escapes from red blood cells resulting in intravascular hemolysis, haptoglobin serves as main transport plasma protein for free hemoglobin in order to eliminate it from the circulation. The human plasma contains a specific heme binding protein too, hemopexin, serving as a heme transporter from the plasma to the liver. Since it has a significant

amount of heme binding capacity, 25 mikromol/l, underlies the danger of free heme and its significant catalytic activity in free radical reactions. The human organism handles the free heme carefully, since albumin also contains heme binding sites. Since the discovery of the significance of free heme in cellular injury, more and more observations have been accumulating which prove the pathologic role of heme in many disorders.

Vascular endothelium is responsible for the smooth blood circulation, trafficking of nutrients through the vascular wall, the anticoagulant state of the vasculature, vasculogenesis and several inflammatory processes. Any injury of the vascular endothelium disturbs this complex function leading to vascular disorders, among them the retinopathy of prematurity, bronchopulmonary dysplasia, periventricular leukomalacia and so on. This is the reason why research has been done on vascular endothelial cells and free heme is tremendous. Our research group was the first who brought the heme idea into the scientific field (12).

Heme as a natural hydrophobic iron complex sensitizes vascular endothelium towards free radicals generated by activated neutrophils, or any other free radical sources. Free heme also behaves as a catalytic agent in the lipid peroxidation of lipoproteins, in low density lipoproteins firstly demonstrated by our group (13). During cell injury and lipoprotein destruction heme falls apart, and free iron accumulates in lipid domains of cells or particles. The iron found in tissues and organs are the footprints of heme catalyzed cellular toxicity in many pathological states. The kinetics of heme destruction is parallel with the kinetics of the lipid peroxidation chain reaction and can be used for monitoring of the lipoprotein damage or cell injury. The methodology was published by our group (14).

The question is the source of free hem in different pathological states. Since in normal conditions heme is located in heme proteins, studies are focusing on their role in cell and tissue injury. Although in several models heme proteins themselves can generate and initiate free radical reactions, the effective catalytic agent is the free heme. The most abundant heme protein is the hemoglobin, normally present in reduced forms in red blood cells carrying oxygen. The oxidized form of hemoglobin is methemoglobin containing ferric iron and it is present in red blood cells in low concentration. The cell uses a significant amount of energy to keep the methemoglobin content at low levels. If the hemoglobin escapes from the intracellular space there is no such system which reduces methemoglobin back to hemoglobin. The formation of methemoglobin is especially fast at low pH, during inflammation, in the extravascular compartments i.e. subendothelial space, in the interstitium, different cavities of the human body or in the urine. Among the heme proteins methemoglobin possesses a unique capability releasing its heme very easily (15). The released free heme incorporates into surrounding cells, tissues and lipoproteins. In this way one can understand why hemoglobin- and heme

binding proteins in the plasma provide protective properties against the heme catalyzed free radical reactions and cell-, tissue injuries. Intriguingly, other heme proteins and their oxidized forms can not release their heme, or special circumstances are necessary for heme release. Based on the results of these studies the methemoglobin has to be considered to be the main heme source in pathologic states. Since intravascular or extravascular hemolysis frequently occurs in many diseases and inflammation also joins the process in the pathogenesis, the scenario is given for heme toxicity.

If the heme idea is a significant part of the pathogenic process in different disorders, a natural protective mechanism must persist against it. Our group was the first who proved living cells are capable to protect themselves against heme stress. Short, acute heme stress makes vascular endothelial cells extremely sensitive towards free radicals but a more prolonged heme stress makes them resistant. The chronic heme stress induces gene expression and new protein synthesis. This adaptive mechanism represents a new form of stress adaptation processes which was discovered by our group (16). We observed that not only heme but other free radical sources and stress reactions can induce the same genes and proteins. The two main players of this endogenous, inducible stress adaptation are the heme oxygenase enzyme and ferritin. By using many models researchers proved that heme oxygenase-ferritin system is a valuable protective stratagem not only for cells and organs but for the whole organism as well.

The heme oxygenase enzyme is responsible for the enzymatic degradation of heme (17,18). In the presence of NADPH and oxygen heme oxygenase opens the porphyrin ring of heme molecule producing biliverdin, carbon monoxide and elementary iron. Today three isoforms of heme oxygenase is known. Heme oxygenase type I is the inducible form of the family representing one of the most important stress proteins of mammalian cells. The heme oxygenase II is a constitutive expressed protein, can not be induced by free radical stress. The third form of the enzyme behaves similarly to heme oxygenase II. The promoter region of the heme oxygenase I gene has many regulatory elements sensible to heme, free radicals, metals, viral proteins and so on. After heme or free radical stress the gene induction occurs within a few hours, in our models the peak induction can be observed at the 4 hour time point measured by mRNA expression (16). The maximum cellular heme oxygenase I protein content and enzyme activity peaks at the 8 hour time point. The quantity of the induction is tremendous, the level of mRNA increase by 100-200 folds, the enzyme activity by 50 folds. The high activity of the heme oxygenase enzyme results in several beneficial consequences. The first advantage is the removal of the dangerous free heme. At the same time biliverdin is formed which is converted to bilirubin by biliverdin reductase. Bilirubin serves as a significant, endogenous

lipophylic antioxidant. The other endproduct, carbon monoxide plays an important messenger function by inhibiting platelet aggregation, adhesion, promoting vascular relaxation, influencing vasculogenesis, having antiinflammatory properties (19,20). More and more data are accumulating which proves that heme oxygenase plays a central role in apoptosis, cell necrosis, cell proliferation, vasculogenesis and inflammation. This new form of stress adaptation underlies the importance of heme oxygenase enzyme and its substrate, heme. In summary, acutely heme is a toxic molecule but chronically it induces protective mechanisms.

Ferritin is the second player of this newly described protective mechanism (16). Ferritin is a multimeric protein containing heavy and light chains. Ferritin had a bad reputation. Since high ferritin content of hepatic cells is associated with cellular necrosis, cirrhosis, hepatic carcinoma and in the case of primary or secondary hemochromatosis with heart failure, it was a reasonable explanation that ferritin is causative agent. The negative role was emphasized by experiments where iron generated lipid peroxidation was augmented by ferritin. Our model was the first in the literature providing solid evidence against the dogma. Using side directed ferritin mutant we showed that the ferroxidase activity of the heavy chain of ferritin is critical for its protective function in heme toxicity (16). The reason of that is the following. One of the endproduct of the heme oxygenase enzyme is the catalytically active free iron. The heavy chain of ferritin oxidizes the reduced form of iron to ferric iron, and the ferritin complex stores it in the core. One ferritin complex contains 24 subunits and stores a tremendous amount ferric iron inhibiting the participation of the iron in free radical chain reactions. Ferritin induction occurs in our model at the time point of 8 hours, peaks at 24 hours and lasts for days. The quantity of the induction is more than 10 folds. Induction of ferritin is regulated in a post-transcriptional way. The higher intracellular iron content removes the iron responsible protein from the iron responsible element of ferritin mRNA allowing translation. Today ferritin is considered to be a protective and storing protein for iron, and not the cause of tissue injury (21,22). High ferritin concentrations in plasma and tissues represent a protective stratagem against free radical stress.

Our group was the first who introduced the heme toxicity and the new form of stress adaptation, the heme oxygenase-ferritin system, in the literature and developed many models for its study (16).

In cell culture models, the first was the vascular endothelium. This model is a basic work in this field. It provides a totally new view of the pathogenesis of vascular disorders, and a detailed description of methodological circumstances for future research. Those circumstances are being used nowadays by many research groups all over the world. Using a low density lipoprotein model we proved that heme is a very active catalytic agent for lipid peroxidation and during the process

heme degrades (13,23). This degradation is the second form of heme catabolism besides heme oxygenase enzyme. Although its participation in the total heme degradation can not be high, but because of its pathological location it must be significant, especially in the subendothelial space. The heme mediated low density lipoprotein modification influences the endothelial cell function. In short term cells suffer toxicity, become procoagulant, cell detachments occur. In long term, if endothelial cells survive, modified low density lipoproteins induce heme oxygenase and ferritin in endothelial cells (24,25).

In cancer cell model we found the same system working (26). Acute heme toxicity sensitizes breast cancer cells to free radicals, chronic heme effect changes them resistant by the induction of heme oxygenase – ferritin system. We also made basic observation on the relationship between heme oxygenase – ferritin system and the nitric oxide system (27). Nitric oxide itself influences the iron cluster and the aconitase activity of the iron responsive protein influencing the ferritin mRNA translation.

In a rat kidney failure model our group proved that induction of heme oxygenase in kidneys provided protection not only for the kidneys but for the animals who survived the lethal dose of glycerin given intramuscularly causing rhabdomyolysis, kidney failure and death (28). The next organ was the lungs (29). We could prove that hemoglobin given intravascularly in methemoglobin form induced heme oxygenase and ferritin in endothelial cells, alveolar epithelial cells and macrophages. So lungs have the capability to protect themselves against different types of stress where the described system has beneficial role. The role of heme, heme oxygenase and ferritin was tested in heart transplant (30). Induction of heme oxygenase protects the transplanted heart against the acute rejection where the carbon monoxide plays critical role. Recently studies turn their attentions towards biliverdin and bilirubin, products of heme metabolism.

Human neonates right after birth experience a rapid, huge change in iron metabolism (31). Plasma iron content drops in few hours after birth parallel with the change of heme catalyzed free radical reactions. Not only iron levels drop but a significant ferritin induction occurs right after delivery. Is it part of the adaptation of newborns to the outside world, or just the consequence of many changes, we do not know yet, as we do not know the existence of this phenomenon in cases of premature newborns. The size of these changes is tremendous that can not be seen later on of life.

The heme catabolism and the heme oxygenase - ferritin system, as an inducible protective adaptation, are critical for life (18,24,25,32). Mutant animals missing the ferroxidase activity of the heavy chain ferritin are not viable. Mutants in heme oxygenase I enzyme can not tolerate stress, their survival is also limited (33). Our research group participated in the description of a complex syndrome presented by the first known heme oxygenase I deficient child (34,35). The child suffered

severe vascular injury leading to intravascular hemolysis, glomerular injury and atherosclerotic vessel wall modification. Haptoglobin was consumed, methemoglobin was present in the plasma, low density lipoprotein particles were oxidized containing large amount of iron, and in contrary to the hemolysis there was no bilirubin formation. The case represents a complex pathology where vascular changes are dominating the heme oxygenase I deficiency.

Scientists believe that the removal of free heme and the induction of heme oxygenase – ferritin system is a beneficial reaction of the human body in the vast majority of pathological states, and active research is going on to discover the regulation of the whole mechanism in details in order to find specific inducers (36). Heme itself is an effective inducer but its use is limited because of toxicity. In the heme-arginate molecule, heme is bound to arginin, and the complex is effective drug in prophyria without significant side effect. Our group studied heme-arginate in a vascular endothelial cell model (37). The good news about the complex is that arginin decreased the toxic side effects of heme but the induction of heme oxygenase – ferritin system remained. There are many other inducers also, and new questions have been raised how different target organs can be reached by them. Since organisms need time for developing the protective reactions in hyperacute situations, administration of the endproducts of the heme oxygenase – ferritin system is a way of solution. These aims are under investigations by many research groups.

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NEONATAL ANEMIA

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Introduction

The red blood cells produced during the fetal life are different from those produced in the postnatal life. Therefore, in the newborn period hematopoiesis is characterized, as several organs and functions, by a transition with relevant modifications that influence the hematologic values at birth and the postnatal changes. Macrocytosis (MCV 100 – 110 fl), increased mean hemoglobin (15 to 18 g/dl), reticulocytosis (3 to 7 %) and presence of nucleated red blood cells are the typical neonatal red blood cell indices. Moreover, at birth between 55 to 65 % of total hemoglobin synthesis consists of HbF (1). The normal hemoglobin values are influenced by several factors including the gestational age, type of labor, clamping time of the umbilical vessels, site and time of sampling. In the first days after birth the rate of hemoglobin synthesis and red cell production consistently decreases. This sudden and mar-

ked decrease in erythrocyte production is initiated by the rapid increase in the blood oxygenation. Studies using ⁵¹Cr survival indicate that the actual life span of newborn red blood cells is shorter than that of adult red blood cells (60 – 80 days vs 120 days) (2). Modifications in membrane function and an increased susceptibility to mechanical damage have been suggested as responsible for the decreased life span of the fetal erythrocytes (3).

Causes of neonatal anemia

Neonatal anemia is defined by a hemoglobin concentration lower than 2 standard deviations below normal values. Diagnosis can be complicated by chronologic variations in normal hemoglobin levels and by factors that may affect the laboratory values, including delay in cord clamping, timing and method of obtaining blood samples. Causes of neonatal anemia can be subdivided in three major groups: 1) blood loss; 2) increased erythrocyte destruction; 3) decreased erythrocyte production.

1) **Blood loss.** Blood loss can occur at any time during the prenatal, perinatal and postnatal periods and accounts for 5 – 10 % of all cases of severe neonatal anemia (4). Blood loss may be the result of occult hemorrhage before birth (fetomaternal hemorrhage and twin-to-twin transfusion), obstetric accidents, malformations of the placenta and cord, internal hemorrhages (into the adrenal glands, kidneys, spleen, retroperitoneal area, rupture of the liver, cranial hemorrhages) or excessive blood sampling. The clinical manifestations depend on the volume of the blood loss and the time to develop (i.e. acute or chronic). Chronic hemorrhage is characterized by intense pallor, normal or elevated venous pressure, microcytosis and hypochromia. If the losses are acute and higher than 20% of the total blood volume, newborns may have signs and symptoms of hypovolemic shock and red blood cells are normochromic and macrocytic. Frequent and excessive blood drawing for laboratory tests is a major cause of anemia in severely ill neonates, particularly if they are low birth weight infants. Several approaches to reduce the blood sampling for laboratory tests should be used, including use of micromethods, reduction of the blood drawn in excess of that needed for the analysis, use of transcutaneous monitoring techniques (5).

2) **Increased erythrocyte destruction.** Several intrinsic, extrinsic, congenital and acquired abnormalities of the red blood cells may further decrease the normally reduced erythrocyte life span resulting in hemolytic anemia. Hemolytic anemia in newborns is associated with increased unconjugated bilirubin levels, usually above 10 mg/dl. It should be pointed out that in newborns, the hepatic metabolism of bilirubin is less efficient than in adults, in part because of deficiency of the cytoplasmic acceptor protein ligandin and in part because of decreased activity of the diphosphoglucuronyl transferase (6, 7). The most common causes of

hemolytic anemia in the newborn include: a) immune-mediated red cell destruction; b) hereditary disorders of red cell membrane; c) erythrocyte enzyme deficiencies; d) hemoglobin defects; e) infections; f) other causes.

a) **Immune-mediated red cell destruction.** If fetal erythrocytes with different surface antigens enter the maternal circulation, they stimulate the production of IgG antibodies. These antibodies cross the placenta, enter the fetal circulation, coat fetal red cells resulting in hemolysis of variable severity, that can be symptomatic in utero or at birth. Incompatibility of Rh (in the past) and ABO system are relatively common, although minor blood group incompatibilities should be considered. Several factors, including the prevalence of the different blood antigens, the type and titer of the produced antibody and the medical intervention affect the occurrence and the severity of the hemolysis. The administration of antiRh (D) antibody in Rh negative mothers at 28 weeks gestation and up to 72 hours after delivery, has dramatically reduced the incidence of Rh sensitization to about 0.1 % (8, 9). Rh incompatibility usually occurs in the second pregnancy with an Rh-positive fetus, after an exposure occurring usually during the first pregnancy as a consequence of fetomaternal hemorrhage or obstetric procedures.

ABO incompatibility is more common and less severe than Rh incompatibility (10). It has been reported in about 15 % of the pregnancies, but results in symptomatic hemolysis in approximately 3 % (11). Because of possible previous exposure to A and B antigens present in some foods and bacteria, hemolysis from ABO incompatibility can occur during the first pregnancy.

b) **Hereditary disorders of red cell membrane.** Hereditary disorders of the red cell membrane (spherocytosis, elliptocytosis, stomatocytosis and xerocytosis) may manifest in the neonate as hemolytic anemia or hyperbilirubinemia. The severity of the hyperbilirubinemia can be influenced by the coinheritance of genetic defects in bilirubin metabolism (12). Hereditary spherocytosis, the most common defect, manifests in the newborn in about 50 % of the affected patients, with 75 % of them requiring transfusions (13). Anemia can be worsened by the blunted reticulocyte response in the first months of life. Diagnosis of hereditary spherocytosis can be difficult at birth because the osmotic fragility test is not always reliable at this age and because spherocytes on the peripheral smear can also be present in the ABO incompatibility (14). Elliptocytosis and the other less common membrane defects present rarely in the neonatal period with anemia and hyperbilirubinemia.

c) **Erythrocyte enzyme deficiencies.** Hereditary deficiencies of the red cell enzymes result in alterations of the metabolic pathways, in significant damage to the erythrocytes and hence hemolysis and / or hyperbilirubinemia. In Mediterranean countries defects of the glucose-6-phosphate dehydrogenase (G6PD) are the most common causes of hyperbilirubinemia sometimes associated to hemolytic anemia, particularly in

presence of an oxidative stress due to infections or administration of certain medications (15). Piruvate kinase deficiency, the second most common red cell enzyme defect, may manifest in the newborn with anemia sometimes requiring transfusions (16).

d) **Hemoglobin defects.** Hemoglobin defects in the newborn (hemoglobinopathies) are represented by structural defects (hemoglobin variants) and by synthetic defects (thalassemias). Overall hemoglobinopathies are not a common cause of anemia in the newborn. Clinical manifestation associated with structural variants in the newborn depend from several factors, including the globin chain type, the amount of the affected chain, the severity of the structural defect. Therefore since the hemoglobin A (alpha2-beta2) represent less than 30 % of the normal hemoglobin at birth, beta chain mutations generally do not produce clinical symptoms at this age. Alpha chain mutations rarely result in clinically symptomatic forms. One example is the hemoglobin Hasharon (alpha 14 Asp → His), a relatively common alpha variant, which is mildly unstable. In the newborn the tetramere formed by the alpha Hasharon and gamma globins is highly unstable and one case of hemolytic anemia associated with Hb Hasharon has been reported (17). The gamma chain variants generally do not produce severe hematologic alterations in the newborn and are usually detected during neonatal screening programs. The only exception is the HbF-Poole (alpha2-Ggamma cd130 T→G Trp→Gly) an unstable variant that result in hemolytic anemia in the first weeks of life. (18). Some HbF variants present alterations in the oxygen affinity (i.e. HbF M Osaka, HbF LaGrange, HbF Onada). One characteristic of the gamma chain variants is that they disappear in the first few months of life, as the gamma chain synthesis decreases up to less than 1.5 % and so the associated disorder spontaneously resolves.

In the newborn, defects in hemoglobin synthesis rarely manifest as anemia. The only exceptions are the clinically significant forms of alpha-thalassemia (hemoglobin H disease and HbBart's hydrops fetalis syndrome) and the deletion gamma-delta-beta thalassemia. The deletion or inactivation of 3 out of 4, or of all 4 alpha globin are the molecular defects associated respectively HbH disease and HbBart's hydrops fetalis syndrome. A significant neonatal anemia with severe reduction of MCV and MCH and a marked increase (about 25 %) in HbBart's (tetramere of gamma chains) are present in newborn infants with HbH disease. HbBart's hydrops fetalis syndrome is the most severe form of alpha thalassemia and is characterized by a very marked anemia, hepatosplenomegaly, ascites and in some cases developmental anomalies (microcephaly, hydrocephaly, urogenital abnormalities). The hemoglobin composition is characterized by absence of HbA and presence of HbBart's with traces of Hb Portland. Affected infants die in utero or soon after birth. Attempts of treatment with red cells transfusions in utero and a continuous post delivery transfusion

regiment have been performed (19, 20). Relevant maternal complications, such as eclampsia, have been reported in pregnancies with HbBart's hydropic fetuses (21). This severe form of alpha thalassemia, which is very rare in the Mediterranean countries, can be prevented with prenatal testing and genetic counselling.

Deletion of gamma-delta- and beta gene is a very rare thalassemic defect that has been described in the heterozygous state, in a full term infant with microcytic hypochromic hemolytic anemia, and nucleated red cells on the peripheral blood smear (22). As the infant matured the anemia improved and a beta-thalassemia-like hematological phenotype with normal HbA2 was present.

e) **Infections.** Neonatal bacterial sepsis may result in hemolysis, disseminated intravascular coagulation and hemorrhage. Endotoxins produced by bacteria cause increased red cell destruction often associated with a microangiopathic process (23). Congenital viral infections due to cytomegalovirus, toxoplasmosis, rubella and herpes simplex may also be associated with hemolytic anemia. Fetal and neonatal infection with parvovirus B19 can result in severe anemia, hydrops and fetal demise (24). B19 parvovirus replicates in erythroid progenitors stopping their proliferation and maturation. The resulting anemia is hypoplastic, but also hemolysis has been reported (25). Congenital HIV infection is usually asymptomatic, but infants born to mothers on zidovudine treatment may have hypoplastic anemia as a side effect of the drug (26). f) **Other causes.** Rare conditions resulting in a hemolytic process in the neonatal period include disseminated intravascular coagulation, macro- and microangiopathic anemias (associated with cavernous hemangioma, large vessel thrombi, renal artery stenosis and severe coarctation of the aorta), galactosemia, prolonged or recurrent metabolic or respiratory acidosis and pyknocytosis.

3) **Decreased erythrocyte production.** The disorders of erythrocyte production are infrequent causes of neonatal anemia. Diamond-Blackfan anemia is a rare condition characterized by a failure of erythropoiesis. Twenty five percent of the affected infants may be anemic at birth (27) with hemoglobin values up to 9.4 g/dl and reticulocytopenia. Affected newborns may have a low birth weight and anomalies, such as microcephaly, cleft palate, anomalies of the eye, thumb deformity (28). Pearson syndrome, is another reported cause of reduced red cell production in the newborn (29). Exocrine pancreatic dysfunction, sideroblastic anemia and vacuolization of bone marrow precursors are the main characteristics of Pearson syndrome. Cases of congenital dyserythropietic anemia resulting in severe fetal and / or neonatal anemia have been reported (30, 31). Congenital infections, mainly due to rubella, cytomegalovirus and parvovirus, have been associated with hemolysis (see above) and impaired red cell production (32, 33).

Diagnosis of neonatal anemia

In view of the numerous causes responsible of neonatal anemia and some newborn peculiarity (i.e. low blood volume, difficulty in obtaining blood samples, coexistent pathologies) a careful and systematic approach is required. A detailed history (family, maternal, prenatal, perinatal and postnatal) should be obtained. Physical examination should include vital signs, skin, liver and spleen evaluation. The identification of signs and symptoms of associated conditions that can be responsible of the neonatal anemia (i.e. infections, hemorrhage) and the presence of congenital abnormalities may be useful in defining the etiology of anemia and in choosing the most appropriate treatment. Laboratory studies should follow a stepwise approach to avoid unnecessary testing. Initial laboratory evaluation should include, as in the adult, simple tests such as a complete blood count, reticulocyte count and examination of a peripheral blood smear. A useful approach is based on the reticulocyte count. In presence of increased reticulocytes and / or hyperbilirubinemia the diagnostic focus will be the hemolytic anemias and further evaluation will be planned on the basis of the diagnostic possibilities among this group of anemias. The diagnosis of bone marrow suppression or dysfunction is suspected if the reticulocyte count is low. In both cases the diagnostic work-up will take into account the history of the patient and the associated clinical features.

Treatment

The treatment of neonatal anemia depends on its severity and on the underlying cause. The need of erythrocyte transfusion should be established on the basis of the severity of anemia and on the presence of other clinical findings including evidence of respiratory distress and / or hypovolemia. Given the potential risks of transfusion, this therapeutic intervention should be carefully planned. To minimize donor exposure dedicated units should be divided in several aliquots and administered to the same newborn. Other therapies, such as exchange transfusion, iron supplementation, erythropoietin, steroid therapy, are etiologic-specific and will be used when the exact diagnosis has been clearly established.

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TRANSFUSION GUIDELINES IN THE NEWBORN

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The transfusion requirements of the neonate are recognized as unique and in particular most preterm infants with birth weight of ≤ 1000 g will require multi-

ple red blood cell (RBC) transfusions. Moreover preterm infants are especially vulnerable to the potential infective and toxic effects of transfusion. They have immature immune and metabolic processes, and are still undergoing rapid neurodevelopment.

Cytomegalovirus (CMV) infection may be transfusion transmitted, and is a particular risk for causing severe disease in very low birth weight (VLBW) neonates. The immaturity of the fetal and neonatal immune system leads to concerns regarding the risk of transfusion-associated graft vs. host disease (TA-GVHD).

Taking into account these considerations, the Neonatal Hematology Working Group of the Italian Society of Neonatology and the Italian Society of Transfusion Medicine and Immune-hematology have released a document on the transfusion guidelines for neonates.

Because of a lack of evidence from controlled studies in a number of areas, many transfusion recommendations in the neonatal period are largely based on consensus opinion.

In this document, indications for transfusion, product selection, compatibility testing and administration of blood products, have been considered.

The definitions of the levels of evidence and the grading of recommendations used in this guideline originate and derived from the US Agency for Health Care Policy and Research.

General recommendations

Donors: Components for transfusion in utero or to neonates must be prepared from blood donated by usual donors.

Prophylaxis of CMV infection (evidence level VI/grade C): It is essential to use blood components CMV-safe (blood components from CMV seronegative donors or blood components leucodepleted to $< 5 \times 10^6$ /unit) for:

1. Intrauterine transfusion.
2. Recipients of allogeneic, organ, bone marrow, or stem cell transplants or likely candidates for such transplants.
3. VLBW neonates or with gestational age < 30 weeks.
4. Neonates with congenital or acquired immunodeficiency.
5. Women during pregnancy.

Prophylaxis of TA-GVHD (evidence level III/grade B): It is essential to irradiate all red cell and platelet components [with the exception of fresh frozen plasma (FFP)] for:

1. Intrauterine transfusion.
2. RBC or PLT transfusion after intrauterine transfusion.
3. When the donation is from a first- or second-degree relative or a human leukocyte antigen (HLA)-selected donor.
4. Neonates with congenital or acquired immunodeficiency.
5. Recipients of allogeneic, organ, bone marrow, or

stem cell transplants or likely candidates for such transplants.

Pretransfusion testing for neonates

Wherever possible, samples from both mother and infant should be obtained for initial ABO and RhD group determination.

Investigations on the maternal sample:

- ABO and RhD group.
- Screen for the presence of atypical red cell antibodies.

Investigations on the infant sample:

- ABO and RhD group.
- Direct anti-globulin test (DAT) performed on the neonate's red cells. In the absence of maternal serum, screen infant's serum for atypical antibodies by an indirect anti-globulin test.

Infants rarely produce atypical red cell antibodies other than following repeated large volume transfusion. Small volume transfusions can be given repeatedly over the first 4 months of life without further serological testing, provided that there are no atypical maternal red cell antibodies in the maternal/infant serum, and the infant's DAT is negative when first tested (evidence level IIb/grade B).

Exchange transfusion

Exchange transfusion (ET) may be used to manage severe anemia at birth, particularly in the presence of heart failure, and to treat severe hyperbilirubinemia, usually caused by HDN.

- **Severe anemia at birth** (Hb <8g/dL): to treat this condition, if associated with heart failure it is indicated the partial ET technique, using packed red cells with a hematocrit of around 80% (the exchange volume is 20-80 mL/kg to obtain a postexchange Hb of <12 g/dL).
- **HDN with hyperbilirubinemia:** to treat this condition is indicated the double volume ET technique (160-200 mL/kg), using reconstituted whole blood. The aim is to remove both the antibody-coated red cells and the excess bilirubin.

ET is a specialist procedure associated with a poten-

tial for serious adverse events. As such, it should be undertaken only by staff who are experienced in the procedure.

Small volume transfusions

As transfusions of small volume it is meant a transfusion of blood components in doses of 15±5 mL/kg.

1 RED BLOOD CELLS

RBC transfusions in VLBW neonates

Restrictive guidelines have been developed which have decreased donor exposure and transfusion number, but several factors continue to contribute to the need to transfuse. These include iatrogenic anemia, oxidative hemolysis often from sepsis, and rapid growth with concomitant protein and iron deficiency. Clinicians who transfuse according to agreed local guidelines give fewer transfusions and it is recommended that local transfusion protocols be established in all neonatal units (evidence level Ib/grade A).

Dedicating aliquots of stored RBCs from a single donation to allow sequential transfusions from the same donor for VLBW neonates who are likely to be repeatedly transfused is considered good practice.

Suggested transfusion thresholds for VLBW neonates are showed in table-1.

RBC transfusions for post-hemorrhagic, haemolytic or hypo-regenerative neonatal anemia

- **Prenatal and early onset severe neonatal anemia.** Severe anemia (Hb <8g/dL) at birth may be associated with two conditions: hypovolemic shock or heart failure (for this last condition see ET section). Acute blood loss may occur in relationship to vasa previa, abruptio placenta, cord accidents, fetomaternal transfusion or other conditions. The resulting hypovolemic shock may necessitate emergency RBC transfusion. To maintain perfusion pressure, the circulating blood volume can be re-expanded by crystalloid solutions or colloids. However, a significant fall in hematocrit usually requires RBC transfusions.
- **Early onset mild neonatal anemia.** Mild anemia in

Table 1

Indications for RBC transfusions in VLBW infants

□ Infants with artificial ventilation or FiO ₂ > 0.4	with Ht value < 40%
□ Spontaneously breathing infants	
• during the first 2 weeks of life	with Ht value < 35%
• during the third to fourth week	with Ht value < 30%
• more than four weeks	with Ht value < 25%
□ At any Ht value, when life-threatening anemia or hypovolemia are suspected or if surgery is planned	
* with reticulocyte count < 100.000/μL	

FiO₂ indicates inspired content oxygen; Ht, hematocrit. From Maier RF et al. J Pediatr 1998;132:866-870

neonates with associated heart or lung congenital disease may require RBC transfusion to maintain Ht > 35-40%.

• **Late onset neonatal anemia.** A RBC transfusion, to restore or maintain adequate tissue oxygen delivery, might be necessary in symptomatic anemic neonates. Clinical signs of anaemia include respiratory irregularity, tachycardia, poor weight gain, lethargy, poor suck and increased blood lactate levels. All of these signs are susceptible to be influenced from other factors and they could not be consistent with inadequate tissue oxygenation resulting from anemia. Moreover, in order to assess the transfusion requirement, it is important to evaluate the rate of bone marrow erythrocyte production: values of reticulocytes > 100.000/ μ L suggest a good production of erythrocytes.

2 FRESH FROZEN PLASMA

The transfusion of FFP is indicated when there is bleeding, or when an invasive procedure is planned in a patient with documented coagulation factor deficiency, or a significantly prolonged prothrombin time (PT) and/or partial thromboplastin time (PTT). FFP should only be used if a specific factor concentrate is not available or in the case of liver failure where multiple factors are simultaneously decreased.

Neonates with a significant coagulopathy, prolonged PT and/or PTT more than two times the age-related normal values, and significant risk of bleeding [e.g. preterm infants less than 28 weeks of gestational age and/or intubated, previous periventricular hemorrhage (PVH) in the last 48/72 hours] or who are about to undergo an invasive procedure should receive FFP (evidence level VI/grade C).

FFP is used in the treatment of DIC, confirmed or suspected, when therapy of the underlying illness has begun and there is acute bleeding, along with a prolonged prothrombin time and/or partial thromboplastin time (evidence level VI/grade C).

The clotting times of normal newborn blood may be longer than those of adults, and those of premature infants may be even longer.

FFP is not indicated for volume expansion. Routine administration to preterm infants to try to prevent PVH has been shown to confer no benefit and should therefore be avoided (evidence level Ib/grade A). Fresh frozen plasma should not be used to treat polycythaemia unless there is a co-existent coagulopathy. FFP has not been proven to have clinical benefit when given to septic patients in an attempt to improve immune function.

3 PLATELETS

Thrombocytopenia is common in sick preterm infants and is associated with an increased risk of severe periventricular bleeding. However, the administration of platelets to manage moderate thrombocytopenia (platelets 50-100 x 10⁹/L) did not appear to reduce the severity of bleeding. In the absence of randomized,

controlled trials in this patient group, recommendations for platelet transfusion must be made on the basis of clinical experience.

Term infants are unlikely to bleed if the platelet count is maintained above 20-30 x 10⁹/L. In preterm infants a higher threshold is generally recommended (50 x 10⁹/L), particularly during the first few days when the risk of PVH is highest or if there is a co-existent coagulopathy (evidence level VI/grade C). Platelet transfusions in neonates with platelet counts more than 50 x 10⁹/L should be reserved for patients with active major bleeding.

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TWIN-TO-TWIN TRANSFUSION SYNDROME

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Twin-to-twin transfusion syndrome (TTTS) is associated with a high risk of perinatal morbidity and mortality (1). Severe mid-trimester TTTS complicates about 15 % of monochorionic twin pregnancies (2). If left untreated, the mortality is 80-100% (2). The pathophysiological prerequisite for the onset of TTTS is unequal blood flow via arteriovenous placental anastomoses from the so-called donor to the recipient twin (2). This can result in hypovolemia, hypotension and oligo-anuria in the donor with the up-regulation of renin angiotensin angiotensinogen system (RAAS) and hypervolemia, hypertension, poliuria and, finally, heart failure in the

recipient and a consequent down-regulation of activity of RAAS (2). The diagnosis and treatment of TTTS has progressed to a staging system to allow directed therapy with the addition of laser to traditional serial amniocentesis (3). Leading sonographic signs of TTTS include severe oligo- or anhydramnios and a small or absent bladder filling in the donor in contrast to polyhydramnios with increased bladder filling in the recipient (2). Although ex vivo and in vivo studies fail to identify a unique anastomosis signature, TTTS placentae are typically associated with an imbalance in uni-directional arteriovenous anastomoses with absent bi-directional anastomoses (4). Doppler detection of an artery-artery anastomosis reduces the chance of TTTS, whereas, in those that develop the disease, it improves stage-independent survival (4). Patients might present with clinical symptoms due to massive polyhydramnios (2). Treatment strategies for TTTS have remained controversial, but two main approaches have been commonly used: serial, aggressive amnioreduction and fetoscopic laser photocoagulation of the chorionic plate vascular anastomoses at the intertwin membrane (4). The main results can be obtained if the laser approach is performed at the early pregnancy stage, before the 26th week of pregnancy (5). An early diagnosis, as proposed by Sebire et al (6), leads to anticipate the surgical approach before that the uni-directional arteriovenous anastomoses could cause irreversible damages of twins. Using the laser approach, survival rates of between 18 and 83% have been described. However, 5-58% neurological morbidity has been demonstrated in the surviving infants treated by serial amnioreduction alone (3). Very interesting is the study of Senat et al (7). These authors performed a randomised trial to compare the efficacy and safety of serial amnioreduction with those of selective fetoscopic laser coagulation of the communicating vessels on the chorionic plate. Pregnant women with severe TTTS before 26 weeks of gestation were included in the study. The authors assessed perinatal survival of at least one twin at six months of age, and survival without neurological complications at six months of age on the basis of the number of pregnancies or the number of fetuses or infants, as appropriate. As compared with the amnioreduction group, the laser group had a higher likelihood of the survival of at least one twin to 28 days of age (76% versus 56%, relative risk of the death of both fetuses, 0.63; $p=0.009$) and 6 months of age ($p=0.002$). Infants in the laser group had also a lower incidence of cystic periventricular leukomalacia (6% versus 14%, $p=0.02$) and were more likely to be free of neurologic complications at six months of age (52% versus 31%, $p=0.003$). Endoscopic laser coagulation of anastomoses is a more effective first-line treatment than serial amnioreduction for severe TTTS diagnosed before 26 weeks of gestation (7). Although these encouraging data, further studies of placental and vascular pathophysiology may not only refine current treatment modalities, but

may also suggest further avenues for downstream management such as genetic predisposition testing or pharmacological intervention (4).

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ERITHROPOIETIN: THE DEFINITIVE WORD

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Introduction

Anemia of prematurity (AOP) is a normocytic, normochromic, hyporegenerative anemia that is characterized by a low serum erythropoietin (EPO) level in an infant with an extremely reduced hemoglobin concentration. Physiological factors influencing erythropoiesis and EPO biology are critical in the pathogenesis of the AOP. Growth is extremely rapid during the first weeks of life, and red blood cells (RBC) production by neonatal marrow must increase proportionately. The circulating life span of neonatal RBCs in the bloodstream is believed to be shorter than that of adult RBCs. A key clinical factor is the need for repeated blood sampling to monitor critically ill neonates. Another key reason is the relatively diminished EPO level in the plasma of preterm infants in response to anemia. In preterm

infants, adaptive mechanisms to the extra-uterine environment are incomplete. Fetal erythropoiesis is endogenously regulated by fetal EPO production, which initially occurs in the liver, with synthesis gradually shifting to the kidney after birth. By the end of gestation, the liver remains a major source of EPO. Although erythroid progenitors of premature infants are quite responsive to produced or administered EPO (1), and despite diminished available oxygen to tissues, the ability to increase serum EPO concentrations remains inadequate. The mechanism regulating the expression of EPO is controlled by an oxygen-sensing mechanism in the liver and in the kidney; it is thought that the liver has a decreased sensitivity to hypoxia compared with the kidney and requires more prolonged hypoxia to achieve an EPO response. The shift from the liver to the kidney EPO production begins between the 32nd and the 36th week of gestational age and is completed in the first weeks of life and it is not influenced by birth. Consequently, in the extremely premature infants liver remains the major site of EPO production and new RBC production appears inadequate despite the marked anemia caused by rapid growth and RBC loss due to phlebotomy, clinical bleeding or hemolysis.

Rationale for EPO treatment

Plasma EPO levels in neonates are lower than those of older children and adults and are a key motive that nadir hematocrit values of preterm infants are lower than those of term infants.

Provided that the response of erythroid progenitors to the EPO is present in preterm infants and low plasma levels of EPO is a relevant cause of anemia, it is reasonable to think that the use of recombinant human erythropoietin (rHuEPO) to prevent or treat the anemia of prematurity would be advantageous.

Unfortunately, rHuEPO has not been broadly employed in clinical neonatology practice because its efficacy is controversial. rHuEPO and iron, in sufficient doses, effectively stimulate erythropoiesis as proved by increased blood reticulocyte and RBC counts. Conversely, when the primary objective of rHuEPO therapy is to eliminate RBC transfusions, rHuEPO often fails.

Systematic reviews of rHuEPO administration in anemia of prematurity have been published (2, 3). The primary goal of rHuEPO therapy is to reduce transfusions. Most transfusions are given during the first three to four weeks of life. Because the efficacy of rHuEPO therapy in stimulating erythropoiesis is more consistent in more mature neonates (2), sick and unstable ELBW infants, who require RBC transfusions soon after birth because of a small blood volume and relatively large phlebotomies that are required during hospital stay, have inconsistent response to EPO (3).

Vamvakas and collaborators reported discrepant results, and concluded that until this dissimilarity is better understood, it is too early to recommend rHuEPO as standard treatment for the anemia of pre-

maturity (2). In another review, Garcia and colleagues concluded that administering rHuEPO to VLBW neonates can result in a modest reduction in late erythrocyte transfusions and that this effect is dependent on the dose of rHuEPO used (4). A modest reduction in transfusion has been reported by Kotto-Kome and co-workers when rHuEPO administration is begun in the first week of life. The reduction is less significant for early transfusion than for late transfusion (3). The same systematic review noted a wide range, from 90 to 1400 IU/kg/week of rHuEPO doses used, and of iron between 1 mg/kg/day to 10 mg/kg/day (3).

Adverse effects and non-hematopoietic functions of EPO

Controversial is also the potential for adverse effects. Hypertension, bone pain, rash and rarely seizures have been reported as adverse effects in adults treated with rHuEPO. Only transient neutropenia, presumably due to a mechanism of stem-cell competition, and thrombocytosis, attributable to the structural and functional similarity between the EPO and the thrombopoietin, have been reported in neonates. Recent studies have revealed an increased risk of retinopathy of prematurity (ROP) in premature infants treated with rHuEPO compared to non-treated infants. EPO has been reported to play a role in the angiogenesis, and since this mechanism is known to be responsible for the ROP, a potential role in the ocular vasculogenesis of the ROP has been supposed. Numerous clinical and experimental observations have pointed out that ischemia or hypoxia can induce the vasculogenesis mediated by an excessive production of VEGF (vascular endothelial growth factor) and EPO.

EPO has been found to have important non-hematopoietic functions in the brain and other organs during development (5). EPO would act by binding to a specific receptor on the surface of cells in the nervous system, stimulating maturation or cellular apoptosis. Administration of EPO could potentially have a neuroprotective effect in preterm infants, especially in perinatal asphyxia (5).

Additionally, aplastic anemia mediated by anti-rHuEPO antibodies has been reported in adults but never in neonates given rHuEPO, and, consequently, the use of darbopoietin, a longer-acting erythropoietin analogue, used to treat anemia commonly associated with chronic renal failure in adults, has not been considered in neonates.

Meta-analysis of clinical trials studying rhuEpo in the anemia of prematurity

Studies of meta-analysis of the controlled clinical investigations examining the effectiveness and safety of rHuEPO in the AOP, published between 1966 to 2005, have been recently accomplished to explore the extent and the causes for the conflicting results of published clinical trials. Early (before 8 days after birth) and late (between 8 - 28 days after birth) initiation of

rHuEPO treatment, and the comparison between early vs late initiation of rHuEPO treatment have been independently considered. All the examined studies reported at least one of the following outcomes: use of one or more red blood cell transfusions; total volume (ml/kg) of blood transfused per infant; number of transfusions per infant; number of donors to whom the infant was exposed; mortality during initial hospital stay (all causes); and common outcomes associated with preterm birth (6, 7, 8).

For the meta-analysis of the early initiation of rHuEPO treatment, 23 studies enrolling 2074 preterm infants in 18 countries were included.

All studies except one applied transfusion guidelines. The quality of the trials varied. Most trials were of small sample size. Only one study clearly stated that infants were excluded if they had received red blood cell transfusion prior to study entry (9). The data showed a significant heterogeneity and similar results were obtained combining differently high (>500 U/Kg/ws) or low (<500 U/Kg/w) doses of EPO with high (>5 mgK/w) and low (<5 mgK/ws) dose of iron. The conclusion was that early initiation of rHuEPO administration reduces the use of one or more RBC transfusions, the volume of red blood cells transfused, and the number of donors and transfusions the infants are exposed, but all these advantages had a limited clinical importance because of a significant increase in the rate of ROP (stage >3). Thus, owing to the limited benefits and the increased risk of ROP, as supported by animal data and observational studies in neonates, early administration of rHuEPO was not recommended (8).

The same authors have reviewed randomised or quasi-randomized controlled trials of late initiation of rHuEPO treatment vs placebo or no intervention in preterm and/or low birth weight neonates. 28 studies enrolling 1302 premature infants in 21 countries were included. As in the review previously described, the quality of the trials varied and the sample size was mostly small. Only one study clearly stated that infants were excluded if they had received red blood cell transfusion prior to study entry. Meta-analysis have shown a significant efficacy of late administration of rHuEPO in reducing the use of one or more red blood cell transfusions, the number of red blood cell transfusions per infant and the total volume of red blood cell transfused per infant. Late rHuEPO therapy did not significantly reduce or increase any of many important neonatal adverse outcomes including mortality, ROP, sepsis, intraventricular haemorrhage, periventricular leukomalacia, necrotizing enterocolitis, bronchopulmonary dysplasia, SIDS, neutropenia, hypertension, or length of hospital stay (6).

The study of meta-analysis considering early initiation vs late initiation of rHuEPO treatment identified only two high quality randomized double-blind controlled studies enrolling 262 infants. The use of early rHuEPO did not significantly reduce the primary outcome of "use of one or more red blood cell transfusions", or "number of transfusions per infant" compared to late

rHuEPO administration. The finding of a statistically significant increased risk of ROP (any grade) and a similar trend for ROP stage>3 with early rHuEPO treatment was of great concern (7).

Evidence-based medicine beyond meta-analysis

The previously described studies of meta-analysis assessed the existing controlled clinical trials and concluded warning about the increased risk of ROP associated with early initiation of rHuEPO, but were unable to clearly propose the best use of rHuEPO in clinical practices. Thus, neonatologists wishing to treat or prevent AOP with rHuEPO are in a dilemma. The relatively large or stable preterm infants, who commonly respond to treatment with rHuEPO and iron at the marrow level, receive a small number of RBC transfusions with today's conservative transfusion practices and, accordingly, have little need for rHuEPO when the objective is to avoid RBC transfusions. Conversely, critically ill and unstable extremely small preterm infants, who absolutely need RBC transfusions soon after birth, have not consistent response to treatment with rHuEPO and iron when the outcome measure is to reduce need for RBC transfusions, thus questioning rHuEPO efficacy at the clinical level.

Consequently, there is no persuasive need to use rHuEPO as routine practice to treat anemic premature infants because its role in altering RBC transfusion practices is unclear.

Conclusions

Although courageous efforts including many prospective randomized trials, critical analysis commentaries by experts, and formal meta-analysis have been made to provide definite guidelines for the use of rHuEPO in the treatment of the AOP, no recommendations are unanimously accepted by most neonatologists.

However, provided that current treatment of AOP should minimize all causes that reduce erythrocytic mass, including the diminution of phlebotomies for laboratory studies by improvement of ultramicro-methods, use of noninvasive procedures, and acknowledging the importance to perform phlebotomies only when the procedure is indispensable, rHuEPO has to be considered a tool potentially useful in the management of preterm infants.

Undoubtedly, rHuEPO has efficacy in stimulating erythropoiesis as evinced by increased reticulocytes and stable hematocrit values, but, when the desired objective is the elimination or marked reduction in need for RBC transfusions, efficacy is questionable with success demonstrated in only certain subsets of infants (10). The greatest hope of success in reducing need for RBC transfusions seems possible in extremely low birth weight infants given rHuEPO at a dose of 250 to 400 U/kg 3 times per week subcutaneously plus iron, beginning within the first few days of life and continuing for at least 6 to 8 weeks, particularly if phlebotomy losses can be minimized. For LBW and VLBW,

RBC transfusions can be reduced by following rigorous and conservative transfusion criteria, not considering whether or not rHuEPO is given. Actually, various neonatologists prefer to give RBC transfusions, per single donor programs, only when needed, instead of dealing with the unclearness of rHuEPO and iron therapy, persuaded, as they are, that the risk of transmitting donor infectious diseases can be markedly reduced, limiting donor exposures by using stored units of RBCs reserved for specific infants.

Therefore, each neonatologist or institution must critically analyze the available information and then decide what is best to give appropriate cares to the infants in their units.

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IS IRON A MARKER?

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Introduction

Iron, element 26 in the periodic table, is the fourth most abundant element in the Earth's crust. It is also the most abundant transition metal in the body, and an essential factor for growth and well-being of almost all living organisms.

This transition metal, like copper, chromium, molybdenum, cobalt, manganese, nickel and vanadium, contains unpaired electrons and therefore fulfil the criteria of being a free radical. Because of its ability to switch back and forth between ferrous and ferric oxidation states, by varying the ligands to which it is co-ordinated, iron is both a strong biological oxidant and reductant. It catalyses the reaction between the superoxide anion and hydrogen peroxide (Fenton reaction), leading to the formation of the toxic hydroxyl radical.

In moderate quantities and leashed to protein, iron is an essential element in all cell aerobic metabolism and growth, but it is toxic when unleashed.

Non protein bound iron (NPBI): a double-edged sword
The essentiality of iron is well established in view of its involvement in a large number of metabolic processes including DNA, RNA and protein synthesis, as cofactor for numerous enzymes, and myelin synthesis. It is involved in oxygen transport (haemoglobin), storage (myoglobin), utilisation (cytochrome), activation and detoxification, in nitrogen fixation, in antibacterial defence and in many reactions of photosynthesis.

Iron deficiency during early development of the brain has been related to behavioural alterations including deficits in learning and memory mediated by the hippocampus. Severe iron deficiency may lead to similar deficits in cell energy metabolism and organ performance in the brain, resulting in a reduced ability to respond to restriction of oxygen and perfusion. Studies on iron uptake by the brain have shown that iron transport and transferrin-binding sites are a maximum during the period of brain growth (second week of postnatal life) and maximum uptake by the brain occurs in 15-day-old rats. In the human brain, growth spurt begins early in life and continues throughout the first year of life. This is a critical period during which the brain undergoes several fundamental developmental phases such as maturation of axonal and dendritic outgrowth, establishment of neuronal connections, synaptogenesis, multiplication of glia cells with accompanying myelination and cell, axonal and dendritic death. This is also the period when newborn acquires many new motor and sensory faculties, including advances in spontaneous motor behaviour. The concentration of iron in the amniotic fluid progressively increases from weeks 15 to 19 of gestation, reflecting enhanced release of non protein bound iron (NPBI) as a trophic factor at an early development stage, when

growth is especially active.

Iron is a double-edged sword. In moderate quantities and leashed to protein, it is an essential element in all cell aerobic metabolism and growth, but it is toxic when unleashed. Because of its ability to switch back and forth between ferrous and ferric oxidation states iron is both a strong biological oxidant and reductant. Normally iron is sequestered in transport proteins such as transferrin and lactoferrin and stored in proteins such as ferritin and haemosiderin that maintain iron non-toxic, unable to engage in Fenton reaction. During situations of iron-overload and low plasma pH, as occurs during ischaemia, transferrin releases its iron and chelatable forms of iron (iron ions or redox active complexes of iron) escape sequestration in biological systems. Iron catalyses the reaction between the superoxide anion and hydrogen peroxide, leading to the formation of the toxic hydroxyl radical which causes extensive cell damage

NPBI as a marker

Erythrocytes were the first cells of newborns to reveal the susceptibility of the neonate to oxidative stress (OS). OS leads to oxidation of haemoglobin and damage to the erythrocyte membrane. Iron release in a reactive form play a key role in membrane protein damage via the Fenton reaction and hydroxyl radical production. The role of iron and the Fenton reaction in hydroxyl radical formation and red cell damage was demonstrated in experiments in which cells were incubated in a medium containing a number of oxidizing agents and under aerobic and anaerobic conditions. Erythrocytes depleted of glutathione demonstrated that membrane protein abnormalities observed after incubation with oxidizing agents also occurred after anaerobic incubation. It is interesting that membrane protein damage was related to the appearance of the senescence antigen. Information on how oxidative injury of the red cell is triggered may provide a partial answer to some questions about the causes of the anemia of prematurity and also about red cell involvement in neonatal hypoxia. Indeed, some peculiar characteristics of the neonatal red cell predispose it to oxidative damage.

The risk of oxidative damage to red cells and other cells depends on the balance of production and elimination of ROS. There seems to be not only a general predisposition to oxidative hemolysis, but also relationships between oxidative injury, gestational age and clinical condition. Intraerythrocyte NPBI has been found to be particularly elevated in cord blood of hypoxic preterm newborns. Release of NPBI was associated with increased lipoperoxide products in plasma. When experiments were carried out by incubating newborn red cells under hypoxic conditions, we found a much greater release of iron than in an equal period of normoxia. Hypoxia also induced faster formation of senescent cell antigen than did normoxia in erythrocytes of newborns and to a lesser extent, those of adults.

In newborns the release of NPBI in erythrocytes is correlated with plasma NPBI: the released iron has a tendency to diffuse from erythrocyte into the surrounding medium, suggesting the appearance of plasma NPBI. Iron chelators able to enter cells (ferrozine, quercetin, fluor-benzoyl-pyridoxal hydrazone) prevent both membrane protein oxidation and senescent cell antigen formation, one of the major pathways for erythrocyte removal.

After asphyxia in newborn infants there is an increase in intraerythrocyte and plasma NPBI, significantly correlated with neurodevelopmental outcome. Leakage of plasma NPBI into the brain through a damaged barrier may occur and is particularly damaging, as it is taken up directly by cells in a manner that is independent of transferrin. OS may also result from iron delocalization induced by the superoxide anion, acidosis and anoxia. Enhanced proteolytic activity occurring in injured tissue also releases iron from storage proteins. The toxicity of iron is inversely proportional to the availability of ferritin to sequester and detoxify ferrous ion, and directly proportional to the quantity of hydrogen peroxide to produce hydroxyl radicals by the Fenton reaction. Albumin appear to be the main plasma protein modified by OS in patients with high levels of NPBI. Since albumin is a major extracellular antioxidant, its susceptibility to oxidation can therefore be expected to decrease plasma antioxidant defences and increase the likelihood of tissue damage due to OS in the newborn.

After hypoxia, the expression of transferrin receptors on brain macrophages increases. This is a protective mechanism to facilitate the active uptake of excess iron that may be released by iron-rich oligodendrocytes, or may accumulate due to the disruption of its normal transport after hypoxic insult.

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LABORATORY MANAGEMENT OF IRON BALANCE

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Iron has the capacity to accept and donate electrons readily, interconverting between ferric (Fe^{2+}) and ferrous (Fe^{3+}) forms. This capability makes it a useful component of cytochromes, oxygen-binding molecules (i.e., hemoglobin and myoglobin), and many enzymes. However, iron can also damage tissues by catalyzing the conversion of hydrogen peroxide to free-radical ions that attack cellular membranes, proteins, and DNA. Proteins sequester iron to reduce this threat. Iron ions circulate bound to plasma transferrin and accumulate within cells in the form of ferritin. Iron protoporphyrin (heme) and iron-sulfur clusters serve as enzyme cofactors. Under normal circumstances, only trace amounts of iron exist outside these physiologic sinks, although stored iron can be mobilized for reuse. Iron balance is tenuous; both iron deficiency and iron overload are deleterious. Disorders of iron homeostasis are among the most common diseases of humans.

Diseases of iron deficiency

Normal erythropoiesis is influenced by several factors, especially erythropoietin (EPO), which stimulates maturation of red blood cell (RBC) precursors. Anemia, defined as hematocrit (Hct) or hemoglobin (Hb) concentration >2 SD (standard deviation) below mean for age, may be due to three general causes: blood loss, increased RBC destruction or reduced RBC production. Anemia has been defined as Hct $<45\%$ in term babies. Premaure infants with anemia do produce erythropoietin, but have lower levels than older persons with this condition. Inadequate erythropoietin production, and not unresponsive red-cell progenitors, appears to be a major cause of neonatal anemia. It is unclear why plasma erythropoietin levels are low in newborn infants, but one reason is that during the first weeks of life the liver is the chief site of erythropoietin production, rather than the kidney. This

dependence on hepatic erythropoietin production is important because the liver is less sensitive than the kidney to tissue hypoxia hence, the diminished erythropoietin response to anemia in newborns. Moreover, erythropoietin has an increased rate of clearance from plasma and an increased volume of distribution in infants, both of which lower the plasma erythropoietin level. The inability to compensate for the low level of erythropoietin by increasing production is another factor.

It is well documented that, in addition to iron-deficiency anemia, several other anemic states may lead to increased absorption of dietary iron. These conditions include the thalassemia syndromes, congenital dyserythropoietic anemias, and sideroblastic anemias. Strikingly, many other forms of anemia that are characterized by similar rates of erythropoiesis do not stimulate intestinal iron absorption. These disorders include hereditary spherocytosis, autoimmune hemolytic anemia, and sickle cell anemia. Thus, hyperproliferative anemias can be divided into two classes: those that stimulate iron absorption and those that do not. The two types can be differentiated in a simple way. The types that stimulate iron absorption have in common the fact that erythroid cells are destroyed near the site of their development within the bone marrow (a situation known as ineffective erythropoiesis). The types that do not stimulate iron absorption involve the destruction of cells in the periphery. Quantitative studies of iron balance have been limited by the lack of a method for determining the total amount of stored iron in the body. The plasma ferritin concentration and the amount of urinary iron excreted after the administration of an iron-chelating agent are only qualitative indexes of iron loading and are influenced by infection, inflammation, liver disease, ascorbate deficiency, and other factors. Phlebotomy with careful measurement of the amount of iron in the blood removed is the most accurate means of measuring total body iron stores but it cannot be used in transfusion-dependent patients. Measurement of the iron concentration in a liver-biopsy specimen is the reference method for assessing body iron stores, but the relation between the hepatic iron concentration and the total amount of stored iron in the body has been a missing link in our understanding of transfusion-related iron overload.

Infants and toddlers need relatively more iron than adults to support their rapid growth. Normal, full-term infants have a generous iron endowment at birth, totaling about 75 mg per kilogram. Premature infants, infants of mothers with diabetes mellitus, and infants who are small for gestational age have substantially smaller iron stores than normal, full-term infants. Stores are rapidly depleted, however, even in normal children, and there is little margin in iron balance. For that reason, iron-fortified infant formulas have been widely used since the early 1970s. There are no known contraindications to feeding with iron-fortified

formulas and no apparent side effects. Recent changes in clinical practice may place premature infants at greater risk for tissue iron depletion. Neonatologists are avoiding packed erythrocyte transfusions, a rich iron source, due to the implementation of lower hemoglobin/hematocrit triggers for transfusions and lower phlebotomy losses accompanying improved respiratory stability. Although it is unclear which premature infants are candidates for recombinant erythropoietin (rhEpo), the use of rhEpo adds additional risk for tissue iron depletion. Very recently, it has been present a pilot study examining the utility of measuring serial zinc protoporphyrin/heme (ZnPP/H) ratios in evaluating impaired erythrocyte iron delivery and guiding iron therapy in premature infants. ZnPP/H ratio is an underused, sensitive measure of iron deficient erythropoiesis in older patients. Zinc protoporphyrin level reflects incomplete iron incorporation into protoporphyrin, as zinc substitutes for iron when supply is limited. ZnPP/H ratio is more sensitive than hemoglobin or plasma ferritin in diagnosing iron-deficient erythropoiesis. ZnPP/H ratio could be practical for screening and/or monitoring therapy in premature infants. It is potentially sensitive, can be performed on small sample volumes, is stable on storage for 10 days, and is inexpensive.

Diseases of iron overload

Iron overload usually presents in one of two characteristic patterns. In cases in which erythropoiesis is normal but the plasma iron content exceeds the iron-binding capacity of transferrin (e.g., in cases of hereditary hemochromatosis), iron is deposited in parenchymal cells of the liver, the heart, and a subgroup of endocrine tissues. In contrast, when iron overload results from the increased catabolism of erythrocytes (e.g., in cases of transfusional iron overload), iron accumulates in reticuloendothelial macrophages first and only later spills over into parenchymal cells. Parenchymal iron loading is particularly dangerous, because it leads to tissue damage and fibrosis. The reticuloendothelial system is generally a safe sink for iron; reticuloendothelial macrophages keep it sequestered, even after rather large doses (e.g., after the administration of parenteral iron dextran). If left untreated, however, both forms of iron overload progress to parenchymal deposition and organ damage.

Liver biopsy is the gold standard for quantifying iron. The hepatic iron concentration typically exceeds 80 μmol per gram of liver, dry weight, resulting in a hepatic iron index of more than 1.9 mmol per kilogram per year (the hepatic iron index is the ratio of the hepatic iron concentration to the age of the patient in years). Iron overload may also be assessed by quantitative phlebotomy to the point of iron depletion. The diagnosis can be confirmed by direct mutation analysis of the *HFE* gene. Homozygosity for the C282Y mutation plus biochemical evidence of iron overload makes the diagnosis of hemochromatosis indisputable.

Neonatal hemochromatosis is a fulminant disease characterized by massive hepatic iron loading and liver failure in the perinatal period. Like other iron-overload disorders, neonatal hemochromatosis is characterized by the accumulation of iron in the myocardium and pancreatic acinar cells. The pathophysiology of this disorder is poorly understood, and it is not yet known whether iron loading is the primary problem or secondary to some other insult to developing hepatocytes. Rare familial cases have been reported, in some of which there was consanguinity, but the inheritance pattern has not been clearly defined. Unaffected siblings and parents do not have evidence of iron loading, and there is no genetic linkage to the HLA complex. Liver transplantation is the primary treatment, but it is often unsuccessful.

In rare instances, iron overload develops in a pattern resembling that of hereditary hemochromatosis but at a greatly accelerated rate. Several Italian families with multiple affected members have been particularly well characterized. Their disorder has been termed juvenile hemochromatosis. Perhaps because of the young age of these patients, or perhaps because of the rate of iron loading, they are more likely to present with cardiomyopathy and endocrinopathy than with severe liver disease. Patients with this disorder typically die of heart failure before their 30th birthdays. The genetic basis of juvenile hemochromatosis is unknown. The *HFE* gene has been ruled out as a possible locus, and juvenile hemochromatosis maps to human chromosome 1q. It is reasonable to speculate that the product of the juvenile hemochromatosis gene participates in the same regulatory pathway as the *HFE* gene.

The availability of a genetic test for hemochromatosis has fueled controversy about the benefits of screening for the disease. The test is simple, and the disease is highly prevalent and treatable. However, important disadvantages must also be considered. There is concern, particularly in the United States, that persons known to be homozygous for the C282Y mutation would face discrimination from health and life insurers. Furthermore, the test is not always predictive; some persons who are homozygous for C282Y never have adverse effects resulting from iron overload, and some patients with genetic iron overload do not have mutations in the *HFE* gene. A subgroup of patients with hereditary hemochromatosis, indistinguishable from those described above, do not have mutations in *HFE*, and their disease does not appear to be linked to the HLA complex.

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I.V. IMMUNOGLOBULIN AND EVIDENCE-BASED MEDICINE

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Introduction

The neonatal age is characterized by a delicate process of adaptation from intra- to extra-uterine life. The immune system is particularly subject to problems of adaptation. Indeed, the immune system is incompletely developed at birth. In addition, the fetus, who develops in a highly protective germ-free environment, lacks antigenic experience. The high incidence of infectious disease in the perinatal period is a direct consequence of the precarious host-parasite balance. In preterm neonates the immunodeficiency is more severe and prolonged, and is associated with higher incidence of infection and sepsis, and increased risk of morbidity, mortality or neurological sequelae¹. This immune deficient state is worsened in sepsis.

Both the innate (natural, non specific) and the adaptive (acquired, specific) components of host defense mechanisms are compromised in the neonate, however, the antibody deficiency is, probably, the most important contributory factors to the neonatal increased susceptibility to infection.

Several factors are responsible for the marked antibody deficiency in the neonate. The immaturity of T and B lymphocytes and of antigen presenting cells are responsible for the marked deficiency of antibody production in the neonate. Indeed, switch from IgM to other immunoglobulin isotypes is delayed, only low levels of IgM are produced during the first month of life in response to antigenic challenge, and no response to lipopolysaccharide antigen may be observed. In addition,

levels of IgG are low in preterm infants because transplacental passage from the mother mostly occurs during the last trimester of gestation. Moreover, physiologically the rate of catabolism of maternally acquired antibodies exceeds neonatal de novo production. Therefore, preterm neonates may lack the protection ensured by maternal derived pathogen-specific IgG².

The combined neonatal deficiency of immunoglobulin, complement and neutrophil activity results in increased susceptibility to systemic infections from encapsulated pathogens, such as Group B Streptococci, Staphylococci and Klebsiella spp., that require opsonization for efficient phagocytosis and killing.

Intravenous immunoglobulin (ivIgG) prophylaxis and therapy

About 25 years ago, new preparations of intravenous immunoglobulin became available; these can be administered intravenously in high doses, contain intact antibody molecules, and maintain normal biologic activities of the Fc fragment, such as complement activation, binding to cell surface receptors, catabolization, and opsonic activity, although with significant variability among available preparations. Furthermore, the IgG subclass distribution is similar to that found in normal serum and includes antibodies to most of the pathogens found in neonatal infections.

Intravenous immunoglobulin may produce a favorable effect in neonatal sepsis through several possible mechanisms of action³:

- Offering broad spectrum of antibodies.
- Neutralisation and clearance of Endotoxins, exotoxins and superantigens.
- Help killing the organism by increasing opsonisation.
- Increase macrophage surveillance.
- Increase number and activity of PMN's.
- Increase in opsonisation and phagocytosis.
- Inhibition of release of pro-inflammatory cytokines and stimulation of the release of their antagonists.
- Improve chemotaxis.
- Complement mediated killing of pathogens.
- Scavenging of activated complement factor C3b and C4b.
- Regain Immunological balance.
- Reduction in bacterial cell adherence.
- Modulation of pro and anti-inflammatory cytokines.

Several studies have been carried out to evaluate the efficacy of ivIgG for the prophylaxis of infection in high risk infants, with variable results, because of the different methods applied for patients selection, different methods of care, study designs, and ivIgG preparations and dosages used (in most trials 0.5 g/kg weekly, while in other studies ivIgG dosage has been individually regulated to maintain IgG serum level > 400 or > 700 mg/dl).

These studies have pointed out a common finding: no significant immediate or late side effects have been recorded after treatment, which suggests that ivIgG

are well tolerated by preterm infants, when used at total doses not exceeding 2-2.5 g/kg⁴.

Ohlsson and Lacy selected for meta-analyses 19 studies on the use of ivIgG for prophylaxis in the neonate. These included approximately 5,000 preterm and/or LBW infants. When all studies were combined there was a statistically significant reduction ($p = 0.02$) in sepsis, RR [0.85 (95% CI 0.74, 0.98)] and RD [-0.03 (95% CI 0.00, -0.05)], NNT 33. A statistically significant reduction was found for any serious infection, one or more episodes, when all studies were combined [RR 0.82 (95% CI 0.74, 0.92); RD -0.04 (95% CI -0.02, -0.06.); NNT 25 (95% CI, 16.7, 50)]. There were no statistically significant differences for mortality from all causes, mortality from infection, incidence of NEC, BPD and IVH or length of hospital stay⁵.

These data show that the addition of ivIgG to standard therapies is of minimal but demonstrable benefit in preventing sepsis in very low birth weight infants. However, the routine administration of intravenous immunoglobulin to all preterm infants cannot be recommended due to the high number of infants that would be treated unnecessarily.

The overall improvements in neonatal intensive care have favored a gradual spontaneous decrease of the infection rate in neonatal units; therefore, the prophylaxis of infection with ivIgG is no more required in most units where, thanks to the optimal intensive care, the incidence of infection is very low. On the other hand, in those units where admission of infants at very high risk for infection is prevalent, the prophylaxis with ivIgG can be considered. The cost/benefit ratio of this therapy can be improved by a strict selection of infants with the lowest birth weight and the highest risk of infection.

As for the use of ivIgG for therapy of sepsis, fewer data are available because of the difficulty to evaluate an adequate number of septicemic infants with homogeneous characteristics for a reliable statistical analysis. However, Jenson and Pollock in a review of 110 cases of neonatal sepsis in three studies of ivIgG treatment showed a significant increase in the survival of infants receiving ivIgG ($p = 0.007$, OR = 0.173, CI = 0.031 - 0.735)⁶.

In a more recent meta-analysis of 262 neonates with proved infection, treatment with IVIG did result in a statistically significant reduction in mortality (RR 0.55; 95% CI; 0.31, 0.98).

IvIgG dosage most commonly used in these studies were 0.5-0.8 g/kg/day to a maximum of 2-2.5 g/kg total dosage.

Although meta-analyses suggested that treatment with ivIgG was of benefit in preventing death in neonates with sepsis (the survival rate could be improved two to six fold when ivIgG was added to standard therapies in septicemic infants), the authors concluded that there is insufficient evidence to support the routine administration of IVIG preparations to prevent mortality in infants with suspected or subsequently proved neonatal infec-

tion⁷.

On the contrary, based on the same data others believe that ivIgG in the management of neonatal sepsis is worthy of serious consideration³. Researchers should be encouraged to undertake well-designed trials to confirm or refute the effectiveness of ivIgG to reduce adverse outcomes in neonates with suspected infection.

Future trials should also evaluate the association of ivIgG with G-CSF for treating neonatal neutropenias and improving the depressed neutrophil function of the neonate with sepsis⁸.

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XXIII CONGRESS "CHILD HEALTH PLAN" - THE NEWBORN

The International Congresses "Child Health Plan", started in autumn 1982 after a meeting with Prof. Ettore Rossi, at that time Chief of the Pediatric Clinic of the University of Berne, Prof. Giorgio Maggioni, Chief of the Puericultura Institute of the University of Rome, Prof. Gianni Bona, Chief of the Pediatric Clinic of the University of Novara and myself.

During these years the goal of the meetings was to promote constructive comparisons among medical science, technology and medical humanities, focusing on the child's dignity, since the soul of Medicine, a noble Art, is in the medical/patient relationship.

In the past years the contribution of international authoritative researchers, coming from 28 different nations was determinant in the success of the meetings.

An old aphorism says that "only what becomes tradition has a value". In this view, the 23rd Congress "Child Health Plan" is included in the prestigious Workshop of Cagliari with the help of renowned colleagues.



Prof. Giuseppe Caramia

Former Head, Pediatric and Neonatal Division, Salesi Hospital, Ancona
President 23rd Congress "Child Health Plan"

I SESSION: Apporti multi-disciplinari in neonatologia

Chairman: G. Sabatino

Moderators: G. Pusceddu, G. Carboni

INTERPRETATIVE MODELS OF THE PHYSICIAN/PATIENT RELATIONSHIP: THE COMMUNICATION OF CRISIS

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Introduction

In a context of multidisciplinary relationships (Medicine, Psychology, Education) a research group has been created by the Faculty of Medicine and the Faculty of Education Science.

This research group started an experimental program planned to define theory, methodology and applicative requirements in the relationship between physicians and patients, especially in the crisis communication area.

The project is the result of a cooperation between the two Faculties, which involves exchange of teachers and experience.

Research goals

Research goals were as follows:

- to introduce and to evaluate active learning methodologies (role-play) in medical education, including dramatization of the different roles (doctors, patients and parents);

- to apply these methodologies in a constant way during the educational process;

- to insert this relevant communication experience in the curriculum of the single student;

- to learn the models of communication, especially in the management of history collection and diagnosis transmission in critical situations;

- to develop a maturation process in medical humanities.

Methodology

Eight clinical cases were selected (6 adult cases; 2 pediatric cases) as models of crisis communications. In particular the two pediatric cases concerned a 5 years old child with leukaemia and an infant with meningitis. Written histories and clinical findings were distributed to 32 students (16 men, 16 women) of the 6th year of Medicine, who assumed the role of medical doctors.

Four actors (2 men, 2 women) received the role of patients (or parents in the pediatric cases)

Experimental protocol

- 1st phase: distribution of written histories and clinical findings and individual reading in separate rooms,

either for the doctor, either for the patient or parent of the same clinical case.

- 2nd phase: meeting between the patient (or parent) in a real medical surgery with video-recording of the interview. For each clinical case 4 different role-play situations were registered, with 4 different students and different doctors.

- 3rd phase: at the end of the registration a questionnaire was administered to both patient (or parent) and doctor for a cross-evaluation: doctor versus patient (or parent) and patient (or parent) versus doctor.

- 4th phase: cross video analysis and compilation of a specific form with the indication of strong and critical points in communication.

- 5th phase: small group video analysis and evaluation of filled forms.

- 6th phase: small group meeting with a tutor.

- 7th phase: plenary session with all the 32 students, the project coordinators and the psychologists.

Comparative evaluation of all the collected material (video, forms) and statistical analysis.

Qualitative analysis with the program Atlas T was performed (topic evaluation with the network view method) stressing the different behaviours of doctors, patients and parents.

Quantitative analysis has considered different parameters such as the time duration of the interview, the frequency of the different categories of relationship, the content of meanings of words and phrases, types of pauses, conflicts, body position and movements.

Future directions

This project belongs to the area of psychology applied to medical profession, where a multidisciplinary approach can allow an adequate management of the doctor/patient relationship.

This approach can prevent or minimize situations of cognitive and emotional disturbances suggesting how to use in a correct technical way (work protocol) the individual attitudes for human relationship of physicians in daily life.

QUALITY OF CARE IN PAEDIATRICS AND NEONATOLOGY

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Within the Italian Society of Paediatrics, a Committee for the Continuity and Quality of Care has been formed, together with an Accreditation and Continuous Improvement of Quality of Care Study Group (GSAQ). FIMP, the Italian Federation of Paediatricians, has a Central Office for Quality of Care and the Italian Society of Neonatology has formed a Neonatal Quality of Care Study Group, or QCN. The membership of these institutional bodies has often been the same but his the concern has always been to co-opt colleagues and broaden their scope and interest for quality of care

in different medical settings. These pathfinders have had at their side, in their various capacities, the large number of paediatricians and neonatologists whose daily practice, research and publications are witness and warrant of the quality of Italian paediatric care (1). The search for quality in the healthcare system goes apace with a precise definition and a method of quality assessment, the latter being an indispensable premise for setting out on an improvement pathway. Thus, in 1998, GSAQ neonatologists and paediatricians issued an Accreditation Manual for the subspeciality of hospital-based paediatrics in which they proposed to assess quality of care in paediatric and neonatology wards. This manual-based method is in the tradition of Australian, Canadian and US accreditation blue books and stems from a suggestion of the Institute for Social Research. It follows the pattern of voluntary accreditation manuals used in healthcare settings as diverse as emergency departments, or psychiatric care, begun under the Italian Society for Quality in Public Health Care. The Paediatric Manual was used on a trial basis by some paediatrics and neonatology hospital departments, mainly in Lombardy, both as a self-assessment instrument and as an external validation tool, using medical and nursing staff from other hospitals as quality assessors. An updated 2002 edition integrated the experience handed down from the trial. Accreditation visits have helped define critical areas of paediatric care and suggested improvement pathways that were, in fact, found to have been taken into consideration, during follow-through visits in several paediatric wards (2).

Over the following few years, all major stakeholders in paediatrics healthcare have been interlinked, reaching out from neonatologists and NICUs to family physicians with an interest in paediatrics. Each group, subsequently, put out its own quality assessment manual.

Both the GSAQ and the QCN (the equivalent neonatologists' group) branched out, culturally, from quality assessment in healthcare to training, risk management, excellence network culture, appropriateness of prescribing, guidelines and clinical pathways.

These concepts have been linked into a natural pathway, geared towards the goal of quality of care, and are the foundations underpinning clinical policy management, or rather clinical governance. This is the central tenet of Britain's new National Health Service, born in 1998 with the intent to guarantee a continuous improvement in general standards of clinical care, to reduce variation in outcomes, to ensure universal access to services and, whenever possible, to base clinical decisions on up-to-date, documented evidence (3).

In the Italian healthcare context, the culture of quality of care (and how it translates into clinical practice) has failed to spread evenly. Implementation remains patchy and there is clearly a need for continuous communication and drive. Besides training, operators in the field who dedicate part of their professional time to

continuous improvement are running up against two major challenges in years to come: the identification and use of outcome measures, on the one hand, and the need to involve even more health care users and their family into care pathways, on the other. The central role of the care user is frequently discussed, but it appears to have immobilised the user in a sort of focal point around which the various practitioners interact, rather than to have operated a true Copernican revolution. Seeing the disease through the patient's eyes is not an easy art, but it should be now obvious that, without conscious patient participation, not even the most efficacious treatment can yield sufficient clinical result (4).

In the international sphere, too, paediatricians remain attentive to developments in quality of care. The American Academy of Pediatrics has become actively involved in quality of care pathways and, among other initiatives, has published updates on the possible reduction of risk in the prescription or administration of treatments (5).

There is an abundant production of recent paediatric guidelines which claim to be evidence-based. That their quality actually appears to be high has been demonstrated by the use of the AGREE (Appraisal of Guidelines, Research and Evaluation) assessment instrument applied by a group of Dutch paediatricians, whereas the same cannot be said to be true for other specialties outside paediatrics (6).

An exponential expansion of the culture of quality of care among Italian health care-givers comes up against a possible fall in quality standards themselves in the paediatric ward, owing to the lack of resources and the constraints of savings at all costs.

The idea of quality itself does not agree with the reality of neonatologists and paediatricians' professional lives:

- Cutting down on in-hospital stays (for the sake of children patients' rights and hospitalisation appropriateness) is difficult, when there are delays in ancillary services and, sometimes, a lack of cooperation from the outpatient services.
- Finding time for updates, teaching and study is becoming increasingly arduous.
- Clinics are often points of care of excellence in their own right, but their management is complex and they can be hugely labour-intensive when they are to fit with pared-down, on-duty staff rosters. The spectre of 'waiting lists' is also constantly raised by hospital administrators and Health Authorities and pointed out as "shameful", even when demand obviously outstrips resources.
- The unstoppable and escalating onslaught on 'Emergency Rooms', with all cases seen and felt as "emergencies", beggars description: a medical facility has to remain open 24 hours a day, seven days a week, to cater for users of services entitled to stroll in an Emergency Rooms after dinner or a shopping-trip in the city centre, for reassurance regarding the least complaint. The paediatrician in the first line of this

assault lives in constant angst of an unrecognized, but actual emergency, as sometimes indeed happens. Triage, which necessitates qualified training and hard-to-get ad hoc personnel, remains an available option for very few Italian centres.

These are some of the negative aspects we have to contend with, together with spending cuts implemented according to criteria best left to the Health Authorities themselves.

Neonatologists and paediatricians are well aware of the meaning of quality of care and of the various implications it entails in its multiplex applications. What they do not yet know, however, is how to put it into practice and how to defend it in the current context. At all events, one thing is clear, it is neither compromise nor hiding behind fine words that will accomplish such a mission. It may be that the new dimension introduced by quality of care to look up to in future years will be only that of transparency: the available resources are sufficient only for this goal and for no other and as paediatricians are not ultra-gifted people from Planet Krypton. Quality will mean acknowledging these limitations as such and playing our hand to the best of our abilities. The real test of the future will be how all stakeholders surmount these hurdles within the health care system.

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UNLICENSED AND OFF-LABEL DRUG USE IN THE NEWBORN

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Introduction

Until now, little has changed as regards to the labelling of drugs prescribed to newborns. This derives from the fact that few therapeutic indications are unique for this patient population, the number of drugs required is relatively small and difficulties in developing formulations appropriate are of concern to drug companies. In addition, practical difficulties in carrying out clinical trials and the lack of adequate research funding could explain why medicines have not been adequately studied in newborns (1).

Recently, in the USA paediatric research grew as a result of financial incentives for the pharmaceutical industry and 87 products underwent labelling changes for children use from 1998 to 2004 (2). In Europe, in the last years there have been tangible steps to increase and expand therapeutic research in children with the recent institution of a European Paediatric Clinical Trials Register (3) and with the decision of the European Commission (June 2006) to introduce incentives for conducting research into the paediatric use of medicines (4).

The lack of availability of suitable licensed medicines for neonatal care means that who administer drugs to newborns frequently prescribes outside the terms indicated in the product license (off-label use as regards dose, age group, route of administration, different indication) or in an unlicensed manner (formulations modified, extemporaneous preparations, imported medicines, chemicals used as drugs) (5). This does not imply that a drug is contraindicated or disapproved, but insufficient data are available and the risks or benefits of using a medicine in a particular situation have not been examined. Little is known about a possible corre-

lation between this kind of prescribing and adverse effects development, but certainly the need to use adult formulations exposes neonates to an increased potential risk for medication errors (6). Recently, a document has been released by EMEA (7), focusing on evidence of harm from off-label or unlicensed medicines in children.

With this presentation, an updated overview of the worldwide situation of off-label and unlicensed drug use in the neonatal population will be given, since the prevalence to prescribe medicines outside their product license is evident everywhere. Furthermore, the contribution to ADR occurrence of drugs used in newborns in an off-label or unlicensed manner will be discussed.

Methods

The incidence and nature of unlicensed and off-label drug use in newborns have been investigated in different therapeutic and geographical areas through a Medline and Embase search performed between 1990 and 2006, involving the terms "off-label", "unlicensed" and "drug use" combined with the terms "newborn", "adverse reactions". An additional search examined the aspect of ADRs risk in case of an off-label drug use in newborns.

Results

Incidence and nature of unlicensed and off-label drug use in Neonatal Intensive Care Units

As shown in Table 1, from the analysis of the literature 7 studies were identified, 5 involving exclusively neonatal intensive care units (NICUs) (8, 9, 10, 11, 12) and 2 where data on NICUs were extrapolated since authors considered also other wards (13, 14). Among the neonatal population, the number of subjects receiving at least one off-label or unlicensed drug prescription generally is high, ranging between 80% and 93%. Moreover, antibiotics are the drugs more commonly prescribed in an off-label manner.

From a prospective study conducted by Conroy et al (8) over 13 weeks in the NICU of Derbyshire Children's

Table 1

Summary of studies reporting off-label (OL) and/or unlicensed (UL) drug prescriptions in NICUs

Country	Number of prescriptions	OL prescriptions	UL prescriptions	Patients receiving an OL/UL prescription
UK ⁸	455	55%	10%	90%
France ⁹	257	63%	10%	n.i.
Israel ¹⁰	525	63%	16%	93%
Australia ¹¹	1442	47%	11%	80%
Spain ¹²	236	50%	13%	n.i.
Netherlands ¹³	621	14%	62%	90%
UK ¹⁴	323		55%	n.i.

n.i. =not indicated

Hospital (UK), 455 prescription episodes were administered to 70 babies (49 preterms, 21 terms) and only 161 (35.4%) regarded licensed drugs: 249 prescriptions (54.7%) were referred to drugs used in an off-label way and the remaining 49 (9.9%) to unlicensed drugs. 90% babies received at least one unlicensed or off-label prescription, mostly regarding drugs such as gentamicin, benzylpenicillin, folic acid, dalivit, albumin, vitamin K, caffeine, flucloxacillin, morphine.

Avenel et al (9) studied the incidence of prescriptions without marketing product license in a NICU (Creteil, France) from January to February 1998. Forty babies (G.A. 25-40 weeks, 88% preterm newborns with a birth weight <1000 g) were included and received 55 different medicines (corresponding to 257 prescriptions), mostly antibiotics, analgesics and drugs for respiratory diseases. 26 (10%) of the prescribed medicines had no product license and 161 (63%) were off-label. A higher rate of unlicensed use was found in preterm newborns compared to the other neonates.

Among 525 prescriptions given to 105 neonates admitted to the NICU at the Assaf Harofeh Hospital (Zerifin, Israel) during a 4-month period (10), 331 (63%) were off-label and 87 (16%) unlicensed. 98/105 newborns (93%) received at least one off-label prescription. The major reason for prescribing an off-label medication was the age of the patient (morphine, cimetidine and cisapride) or a dosage higher than that recommended (antibiotics and theophylline). The reason for giving unlicensed drugs was a not approved indication (theophylline for apnea in premature infants) or a change in the formulation of vitamin E, furosemide, spironolactone and sodium thyroxine.

During a 10-week period, a total of 1442 prescriptions (related to 69 different drugs) was administered to 97 newborns (median G.A. 31 weeks, median B.W. 1560 g) admitted to the NICU of the Royal Women's Hospital of Melbourne (11). 681 prescriptions (47%) were off-label, 152 (11%) unlicensed. 78 infants (80%) received either an unlicensed or an off-label prescription or both, but the percentage rose to 93% in ELBW. The most frequently off-label drugs used were: morphine (safety and effects in neonates not established), methylxanthines (not indicated for prevention or treatment of apnea of prematurity), phosphate (not indicated for prevention or treatment of osteopenia of prematurity), paracetamol (administration to infants under 1 month not recommended), dopamine and dobutamine (not indicated for treatment of hypotension in neonates). Spironolactone and sodium chloride were considered unlicensed because respectively modification of the licensed drug and "homemade" product.

A total of 236 prescriptions (involving 48 different drugs) was administered to 48 newborns, 29 (60%) preterm and 19 (40%) term infants, admitted to the NICU of Vall d'Hebron Hospital (Barcelona, Spain) during a 3-month period (12). 117 prescriptions (50%) were off-label, 32 (13%) unlicensed. A not approved indication was the most common category of an off-label use and regarded theophylline (given iv for the

prophylaxis or treatment of neonatal apnea), carnitine (administered to improve lipid metabolism in newborns submitted to parenteral nutrition) and the association ampicillin plus gentamicin (given to prematures for infection prophylaxis). An inappropriate dose or dose interval was another reason for a use outside the product license in 27 cases (11%) that mostly regarded cefotaxime, given to a dosage of 300 mg/kg/day instead of the recommended dosage of 150-200 mg/kg/day. In 19 cases (8%) acetylsalicylic acid, dipyridamol, propacetamol and ipratropium bromide were administered despite no indication of use in the neonatal age. Among the unlicensed drug prescriptions, 19 (8%) regarded dose adaptations of diuretics and 13 (5.5%) were related to caffeine not available in Spain as oral solution.

During a 5-week period, 'T Jong et al (13) conducted a prospective study in a Dutch academic Children's Hospital, taking into account 4 different units comprised the NICU. 66 newborns received a total of 621 prescriptions, among which 84 (14%) were administered in an off-label manner (different dose and frequency compared to that recommended in the product license) and 384 (62%) were considered unlicensed, due to formulations manufactured or modified by the hospital pharmacy. The most frequently drugs used unlicensed and/or off-label were caffeine, tobramycin, dexamethasone, antiasthmatics, vitamins D3 and E.

Turner et al (14) studied prospectively for 13 weeks all drugs administered in five wards at Alder Hey Children's Hospital, comprised the NICU. A total of 323 prescriptions were administered to 100 newborns: 178 (55%) were given in an unlicensed or off-label manner.

Risks associated with an unlicensed and off-label drug use in newborns

An off-label or unlicensed use of a medicine does not necessarily reflect an inaccurate administration: most of drugs are given based either on longstanding experience or evidence obtained from the literature. However, the use of drugs that lack paediatric labelling may contribute to an increased risk of ADRs in newborns, due to their different pharmacokinetic and pharmacodynamic characteristics or errors in adjusting doses or formulations (6). Impicciatore et al (15) identified, among factors predisposing to ADRs occurrence in the paediatric population, also an off-label use of drugs. Nevertheless, this problem could result underestimated, being the effects mostly subtle. As reported by McLay et al (16) that assessed attitudes to off-label prescribing among 257 Scottish paediatric hospital consultants and specialists, no neonatologist admitted to have observed any ADR and only 24% to have observed treatment failure due to an off-label medicine use, probably due to difficulties in identifying an ADR in a sick neonate.

From the analysis of the literature, while the general problem of ADRs in paediatric patients has been summarized in a systematic review (15), only a few number of authors examined the contribution to ADRs

occurrence by drugs used in an off-label or unlicensed manner and no paper considered in detail this aspect in the newborn. However, some information could be extrapolated by two works by Turner et al (14) and Clarkson (17). The first author carried out a prospective surveillance on five different paediatric wards, comprised a NICU, at Alder Hey Children's Hospital for 13 weeks. Among the 157 ADRs detected in the study period, 6 ADRs occurred in newborns admitted to the NICU, where 55% of prescriptions were unlicensed or off-label. A significant relationship was observed between the percentage of unlicensed and off-label drugs and a higher risk of ADRs. Clarkson et al (17) reported 165 medically significant suspected ADRs in children, of which 45 (27%) involved unlicensed or off-label medicines. Among 10 fatalities associated with a suspected ADR, 6 involved either an off-label use or unlicensed medicines. The use of atracurium in pre-term neonates accounted for the majority of the off-label medicine reports and was associated to 3 cases of cardiac respiratory arrest in the first week of life.

Conclusions

In this paper, the unlicensed and off-label drug use in newborns has been limited to NICUs, even if this situation is not confined to intensive care settings. This derives from the fact that data available in the literature are mainly referred to this reality, while in other settings (paediatric wards, community) it is difficult to extrapolate data referred to newborns as the paediatric population is generally discussed altogether. In every case, as previously reported (18), NICUs have much higher proportions of a use of medicines outside the product license: the younger the patient and the more critical and rare their illness, the more likely they will need a treatment involving unlicensed/off-label drugs.

In relation to drug safety, the risk of prescribing off-label and unlicensed drugs in newborns is not clear, but several studies suggested that there may be a greater risk in relation to such use. The problem of underreporting ADRs, common in the paediatric field (14) regards also drugs used in an off-label manner, resulting even amplified. In fact, whereas licensed drugs are monitored by epidemiological surveys or surveillance systems, there is currently no similar process for collecting information on ADRs due to unlicensed and off-label drug use and spontaneous reporting is the only available chance to signal a side-effect.

Clinical trials, with targeted pharmacovigilance studies, are the gold standard to establish the risk/benefit ratio of drugs used in children. The detection rate of ADRs would be implemented either by an extensive surveillance system (17) or by local computerized monitoring systems, resulting in the dual effect to increase the percentage of correctly identified ADRs as well as to stimulate physicians to be aware of the occurrence of ADRs.

Fortunately, in the last period some encouraging steps

have been made to improve knowledge for new products. In fact, the European Paediatric Clinical Trials Register is freely available to both health professionals and the public. Moreover, the new European proposal for a regulation on drugs for children intends to introduce incentives to pharmaceutical companies to conduct research into the paediatric use of medicines. However, a major problem remains with many existing drugs commonly used in children. Future research should be directed towards the identification of individual drugs that cause serious ADRs in the paediatric population and lack product information. Moreover, information on optimal drug use in newborns should be made readily available to those who care for these patients and collaboration between Europe and the other regions of the world should be encouraged: only a strong contribution by all those dealing with drug use in newborns as well as a harmonization of interventions will ensure that this patient population do not remain therapeutic orphans.

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PROBIOTICS AND IMMUNITY

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The role of the gastrointestinal tract is to digest and absorb nutrients and to act as a barrier against antigen from food and microorganisms. Protection against harmful agents is ensured by many factors such as saliva, gastric acid, peristalsis, mucus, intestinal proteolysis, epithelial cell membranes with intercellular junction complexes, and intestinal flora. The establishment of normal indigenous bacterial population in the gut prevents the overgrowth of potential pathogens and it is associated with immunophysiologic regulation. This is the reason that has led to the introduction of a novel therapeutic interventions based on the consumption of probiotics that are "microbial cell preparation or component of microbial cells that have a beneficial effect on the health and well being" (Marteau, 2002).

Probiotic agents exert their multiple mechanisms through interplay with the immune system of the host. Some of this effects are local like as systemic.

In the gut, probiotics can occupy binding sites on the gut mucosa, preventing pathogenic bacteria from adhering to the mucosa which may lead to bacterial

translocation to systemic organs and tissues. Lactobacilli produce proteinaceous compounds, namely bacteriocines, that act as local antibiotics against more pathogenic organisms and decrease production of pro-inflammatory cytokines such as IFN-gamma, TNF- α and IL-12. The toll-like receptor 9 signaling is essential in mediating the anti-inflammatory effect of probiotics (Rachmilewitz, 2004). Lactobacilli also produce acetic and lactic acid with the inhibition of pathogens growth.

Probiotics stimulate immunoglobulin A (IgA) antibody production in the mucosal surface competing with bacterial pathogens for nutrients and modifying toxin produced by pathogens or toxin receptors found in the gut wall.

Prerequisites for the use of probiotics are to be effective and safe. The principal characteristics of an effective probiotic are the resistance to digestion by gastric acid and bile and by enteric or pancreatic enzymes, and then the ability to prevent adherence, establishment and replication of pathogens in the gastrointestinal tract (Saavedra, 1995).

The treatment with probiotics is safe even if in some rare case they are potentially pathogenic in immunosuppressed patients during mechanical ventilation or with venous and urinary catheter (Muniz, 2005).

The principal probiotic bacteria are members of the Lactobacilli family such as *Lactobacillus rhamnosus* GG and *Lactobacillus reuteri*, bifidobacteria and *Saccharomyces boulardii*.

Many bacteria can be qualified as probiotics, but different bacteria have different actions in different disease states and for this reason some disease are better treated with a specific probiotic.

Preterm infants in Neonatal Intensive Care Unit (NICU) develop abnormal bowel colonisation compared with healthy infants. Adult faecal flora may have more than 400 different bacterial strains compared with less than 20 in preterm infants. The use of antibiotics and infection control procedures, such as hand washing and sterile feeds, reduce exposure of preterm infants to normal commensal microflora and this lack of microbial diversity predisposes them to become a reservoir for fungal and antibiotic resistant bacteria which can cause infections. The predominant facultative bacterial species in the fecal flora of preterm infants are staphylococci, enterobacteriaceae and enterococci. Clostridia are the most common anaerobes, in contrast with healthy breast fed term infants in whom bifidobacteria predominate.

It is possible to encourage bowel colonisation with a normal flora through the administration of probiotics bacteria thanks to a gut colonisation who provide benefit to the host.

We have studied the effect of two different probiotics, *Lactobacillus reuteri* and GG, in preventing the infections in 184 newborns, all outborn, with gestational age of 34.9 ± 2.6 weeks and birth-weight of 2262 ± 130 g, admitted to our NICU in the last 2 years. This infants was divided into three groups: group I (67 babies), who assumed *L.reuteri*; group II (55 babies)

who assumed LGG and group III (62 babies) who did not assume probiotics.

The Candid infections rate were: 1,4% in the group I; 3,6% in the group II and 8% in the group III.

The percentage of bacterial infections were 1.4% in the group I; 3.6% in the group II and 6% in the group III.

In the newborns with Candid infection, an antifungal therapy associated to probiotics allowed a faster elimination of the infection (19±9.3 days) versus the control group (40.7±16,2 days). The use of the probiotics produced a decrease of hospitalisation period (P<0.05) with an improvement in clinical outcome.

In conclusion our study showed the use of probiotics should be really effective (P<0.05) in prevention of nosocomial bacterial and fungal infections.

UPDATES IN PEDIATRIC ANESTHESIA

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Anesthesiological problems

The most important concept in neonatal anaesthesia is that physiology and pharmacology in this period of life are very different from adults.

The knowledge of neonatal physiology and pharmacology, the technical skills and the clinical experience in paediatrics is absolutely necessary for the anaesthesiologist who intends to care for newborns and children.

In the neonatal period fetal circulation is replaced by the adult circulation. This process takes place immediately after birth and becomes steady during the first month (1-2): nevertheless during the first weeks it can be unstable and a sudden increase in pressure in the pulmonary artery can cause the restoration of fetal circulation (flip-flop circulation) and the shunt of blood through the ductus arteriosus and foramen ovale, which may be not yet anatomically closed. This condition can happen as a consequence of hypoxia, hypercapnia, acidosis, infection, hypothermia and it is frequent in premature infants.

Cardio-circulatory apparatus in newborn babies shows a deficit of contractile muscle cells; the ventricle is less compliant and unable to deal with a sudden fluid overload and an increase of cardiac afterload³. This condition of immaturity can result in congestive cardiac failure.

Cardiac output in these conditions depends exclusively on the heart rate. The bradycardia induced by hypoxia and anaesthetics can make worse cardio-circulatory failure.

Neonates have a reduced supply of ionized calcium therefore the myocardium is more vulnerable to negative inotropic effects of inhaled anaesthetics and to the property of fresh frozen plasma and blood transfusion

to lower the serum calcium.

For these reasons hypocalcemia in neonates must be aggressively corrected either during the pre-operative or the intraoperative period.

The neonatal airway differs from the adult airway in five ways (4 -5):

- the tongue is relatively larger;
- the larynx is located higher in the neck;
- the glottis is shaped differently and angled over the laryngeal inlet;
- the vocal cords are angled;
- the narrowest portion is in the subglottic portion at the level of the cricoid cartilage.

Consequently, ventilation can be difficult and is simplified by positioning of a small roll under neonate's shoulders and neck. Laryngoscopy can be simplified by using straight blades. Cuffless small diameter tubes are used. Special attention must be paid to the bag ventilation to avoid high pressures in the airways and gastric distention, reflux and risk of pneumonia ab ingestis.

Neonatal renal function is immature until the 2nd month. At birth a decrease of glomerular and tubular filtration is observed, therefore drugs eliminated by the kidney and their metabolites accumulate in plasma (6). Also hepatic function is incomplete at birth. Some enzymes of the cytochrome P450 catalyzing phase I reactions are immature whereas others are 50% efficient. Phase II reactions (increasing drugs solubility in water) are usually reduced in newborn babies until the 2nd year. This fact has important clinical implications because the kinetics of anaesthetics, especially benzodiazepines, curares such as vecuronium, and morphine can become unpredictable (7-8)

Digestive apparatus is complete at birth; deglutation and breathing reflex coordination is imperfect until the fifth month and this determines gastroesophageal reflux in neonates and premature infants. The eventual presence of digestive diseases manifests within 48 hours and must be rapidly corrected (9).

A frequent problem in newborn babies is the vulnerability to hypothermia due to the high between surface to body weight ratio and their poor capability to produce heat. Anaesthesia blunts thermoregulation mechanism, due to low ambient temperature; the exposure of internal organs and the use of room temperature fluids, gases and disinfectant increase heat loss. The use of thermic beds, warmed gauze, the heating of operating-theatre and the humidification of gases are recommended during surgery; fluids and blood derivatives must be warmed (10).

In neonates, especially if premature, the response to drugs is modified by many factors: body fluid composition, protein bond, body temperature, cardiac output distribution, blood brain barrier maturity, liver and kidney function.

The distribution of body fluid compartments depends on age, at birth water percentage is very high, consequently the volume distribution of water-soluble drugs, initial dose of most anaesthetics (such as succinylcho-

line), is increased while liposoluble drugs (thiopental) dosage must be decreased as their distribution depends on the amount of body fats. The high volume of distribution as well as reduced kidney and liver function, cause an increase in anaesthetics' half-life and consequently of general anaesthesia duration. For this reason is recommended, if possible, the use of short term drugs with kinetics of elimination not depending on hepatic metabolism or renal excretion.

Inhaled anaesthetics (sevoflurane) are widely used in induction in anaesthesia.

Special attention must be paid to the prevention of inhaled anaesthetic overdose, due to the kind of inhaled (sevoflurane at 2.5 MAC) (11).

Commonly used intravenous anaesthetics can be utilized in neonates by adjusting dosages to their variable clinical conditions. Opioids are widely used in paediatric patients. While the greatest experience has been with Fentanyl, Remifentanyl is now the most commonly used. Its short half-life, its metabolism by aspecific plasmatic esterases with no active metabolites, make its use reliable, predictable, adjustable with no adverse effects in postoperative period (12).

Muscle relaxants are not always necessary in paediatric anaesthesia. If necessary succinylcholine can be used at a higher dose in newborns (2 mg/kg/bw). However in premature infants can cause cardiac arrest due to release of cellular potassium. The choice of the muscle relaxant should be done considering the level of neuromuscular blockade aimed and the duration of surgery.

Atracurium, cisatracurium, mivacurium and rocuronium are the most used drugs; vecuronium and pancuronium must be used with caution as they have long half-life and prevalent hepatic metabolism (13).

Another problem in newborn babies is the preoperative fast. At present a fast of 4 hours after infant feeding and of 2 hours after liquids is suggested. Parenteral nutrition must not be interrupted neither before nor after surgery. Water needs depend on age, type of disease and surgery, intraoperative loss and fast. The water needs in newborns are between 70 ml/kg on day 1 and 120 ml/kg on day 7, they are higher in premature infants. Losses related to the type and the duration of surgery has to be added; fluids most commonly used are crystalloids.

Premature infants required continuous glucose infusion in order to prevent hypoglycemia; blood sugar has to be regularly checked during anaesthesia and hyperglycemia has to be corrected with insulin. Criteria for administering blood and its products are the same as for surgery and has to be adequately justified. Blood volume is 120ml/kg in premature infants, 90 ml/kg in newborn babies and 80ml/kg in children between 3 and 12 months. Minor blood loss can be replaced with Ringer Lactate (3ml of Ringer for each ml of blood loss). If more blood loss is expected cross matched blood must be available.

Premature infants younger than 60 weeks from the beginning of pregnancy, especially if anaemic, present

an high risk of postoperative apnoea, therefore they must be checked adequately after surgery.

There are no doubts that newborns, even if premature, are able to perceive pain so anaesthesia has to be adequate; postoperative pain treatment should be planned in any case, using drugs or regional anaesthesia (14).

From a cultural and technical point of view neonatal anaesthesia is a challenge for anaesthesiologist. Skills required are the result of a long period of training and a constant clinical practice. The best performances are obtained in specialized structures where anaesthesiologist, surgeons and paediatricians cooperate for the care of the child.

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NEONATAL RESUSCITATION

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Introduction

Neonatal resuscitation is one of the most widely practiced medical procedures. Perinatal asphyxia and extreme prematurity are the 2 complications of pregnancy that most frequently require resuscitative measures by skilled personnel.

Currently, approximately 4 million out of the 130 million worldwide annual births suffer from perinatal asphyxia, with subsequent neonatal death in approximately 25% of cases and sequelae in 25% of cases (1). Successful neonatal resuscitation should prevent a large percentage of these deaths, as well as improve the outcomes of surviving asphyxiated infants.

Preterm infants make up the largest proportion of newborns requiring some degree of resuscitation, particularly those born at <32 weeks of gestational age (GA) and <1,500 g of birth weight (BW). During the past 2 decades, the advances in obstetric and neonatal interventions have gradually improved the survival of extremely preterm (EPT) infants and lowered the limit of viability. However, long-term morbidity rates continue to be high in this category of newborns. A large study by Horbar et al (2) evaluated trends in mortality and morbidity for 118,448 very low birth weight (VLBW) infants from 362 neonatal intensive care units (NICUs) enrolled in the Vermont Oxford Network Database between 1991 and 1999. The rates of mortality, pneumothorax, intraventricular hemorrhage (IVH), and severe IVH declined from 1991 to 1995, whereas from 1995 to 1999, pneumothorax rate increased and there were no significant changes in the rates of mortality, IVH, and severe IVH. In particular, mortality rate declined from 18.1% in 1991 to 14.5% in 1995, and then increased to 14.8% in 1999.

Recently, early death, morbidity, and need of treatment among EPT infants were prospectively assessed by Markestad et al. (3) Of 636 infants with GA of 22 to 27 weeks or BW of 500 to 999 g born in Norway between 1999 and 2000, 174 infants (27%) were stillborn or died in the delivery room, 86 (14%) died in the NICU, and 376 (59%) were discharged to home. The risk of being registered as stillborn or not being resuscitated increased with decreasing GA below 25 weeks. Overall survival rates were 0% for <23 weeks, 16% for 23 weeks, 44% for 24 weeks, 66% for 25 weeks, 72% for 26 weeks, and 82% for 27 weeks' GA.

The rate of survivors without severe neurosensory or pulmonary morbidity increased from 44% for 23 weeks' to 86% for 27 weeks' GA.

Reported survival and morbidity rates for EPT infants vary considerably because of selection bias, demographic characteristics, organization of care, outcome measures, and attitudes toward resuscitation at extreme prematurity.

The question of providing life support or not for EPT infants is an important and ongoing debate.

Evidence-Based Medicine and Neonatal Resuscitation: development of the Neonatal Resuscitation Program (NRP) Guidelines

In the past, multiple organizations around the world have made recommendations for neonatal resuscitation based solely on the opinions of experienced clinicians or on suboptimal studies. Evidence-based medicine (EBM) is an approach to health care practice in which clinicians are aware of the evidence and the strength of the evidence that supports their clinical practices. Sackett et al (4) described EBM as "the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients". Ideally, all medical practice should be evidence-based. The development of the most recent NRP guidelines was an attempt to implement EBM regarding neonatal resuscitation.

The new NRP Guidelines

The new NRP Guidelines were codeveloped by the American Heart Association and the American Academy of Pediatrics, and published on November 28, 2005 (5). These guidelines apply primarily to newborns undergoing perinatal transition (newly born infants), but are also applicable to newborns requiring resuscitation during the first weeks after birth. There are major changes in the new NRP guidelines concerning the administration of oxygen during neonatal resuscitation, intrapartum suctioning of meconium-stained babies, use of devices for assisting ventilation, use of epinephrine and other drugs, temperature control, noninitiation of resuscitation and discontinuation of resuscitation efforts.

Administration of oxygen. Currently, it is discussed whether depressed newborns should be resuscitated with room air or 100% O₂. There are concerns about the adverse effects of pure oxygen, essentially the potential tissue damage from O₂ free radicals. Conversely, there are also concerns about tissue damage from hypoxia during and after asphyxia. A recent meta-analysis by Saugstad et al (6) demonstrated that neonatal mortality is significantly reduced when depressed newly born infants are resuscitated with room air instead of 100% O₂ (8.0 vs. 13.0%, respectively). Furthermore, recovery was faster in infants resuscitated with 21% O₂ as compared with infants given 100% O₂. However, current evidence is

insufficient to resolve all questions concerning the use of supplemental O₂ during neonatal resuscitation. For babies born at term, the new NRP guidelines recommend use of 100% supplemental O₂ when there is cyanosis or when positive-pressure ventilation (PPV) is required. Resuscitation may also be started with less than 100% O₂, but supplemental O₂ up to 100% should be given if there is no appreciable improvement within 90 seconds after birth. If supplemental O₂ is unavailable, PPV should be administered with ambient air. On the other hand, resuscitative measures used for very preterm infants (<32 weeks' GA) should prevent excessive tissue oxygenation. Thus, the use of oxygen blender and pulse oxymeter is recommended in very preterm infants, because these devices allow starting the PPV with oxygen concentration between room air and 100% O₂, and subsequently adjusting O₂ concentration to achieve an oxyhemoglobin concentration from 90% to 95%. Nonetheless, when oxygen blender and pulse oxymeter are not available in the delivery room, and there is insufficient time to transfer the mother to another facility, O₂ management described for a term newborn is appropriate.

Clearing the airway of meconium. Aspiration of meconium before delivery, during birth, or during resuscitation may be responsible for aspiration pneumonia. Past NRP guidelines (7) recommended that oropharyngeal and nasopharyngeal suctioning be performed after delivery of the head but before delivery of the shoulders (intrapartum suctioning) in order to try to decrease meconium aspiration. But a recent large multicenter randomized trial showed no effectiveness of this practice for the prevention of meconium aspiration syndrome (8). Therefore, current recommendations no longer advise routine intrapartum airway suctioning for meconium-stained infants.

Devices for assisting ventilation. Self-inflating and flow-inflating bag-and-mask equipment and techniques remain the cornerstone of achieving effective ventilation in most newborns requiring resuscitation. However, flow-controlled pressure limited mechanical devices, especially T-piece resuscitators, are recognized as an acceptable method of applying PPV during resuscitation of the newborn, especially the preterm infant.

Laryngeal mask airway. The use of this device has been shown to be an effective alternative for assisting ventilation in case of failure of bag-and-mask ventilation or endotracheal intubation, especially for term or near-term newborn infants.

Medications. They are rarely indicated in neonatal resuscitation, and include epinephrine (1:10,000), volume expansion, sodium bicarbonate, naloxone, and vasopressors. With regard to epinephrine, intravenous administration of 0.01 to 0.03 mg/kg per dose of this drug should be preferred. However, while access is being obtained, endotracheal administration of a higher dose (up to 0.1 mg/kg) may be considered, but the safety and efficacy of this practice is not proven.

Temperature control. It is an important issue in neo-

natal resuscitation. The use of polyethylene bags during resuscitation may be helpful in maintaining body temperature in VLBW infants. Therapeutic systemic or selective cerebral hypothermia after resuscitation of newborns with asphyxia may reduce the extent of brain injury, but there are insufficient data to recommend routine use of this practice. Conversely, avoidance of hyperthermia in resuscitated newborns is particularly important in order to mitigate hypoxic-ischemic brain injury.

Withholding resuscitation. Resuscitation is not indicated in conditions (GA <23 weeks, BW <400 g, anencephaly, and chromosomal abnormalities incompatible with life) associated with almost certain early death and unacceptably high morbidity among the rare survivors. On the other hand, in conditions with uncertain prognosis in which survival is borderline, the morbidity rate is relatively high with a potentially high burden to the child, parental desires about initiation of resuscitation should be supported.

Discontinuing resuscitative efforts. In infants without signs of life after 10 minutes of continuous and adequate resuscitation, discontinuation of resuscitative efforts may be justified because of the high mortality or severe neurodevelopmental disability associated with this condition.

Conclusions

Remarkable progress has been made in neonatal resuscitation over the past 3 decades. However, many of the principles of neonatal cardiopulmonary resuscitation are based on accepted practice rather than research data, and therefore it is important that more research continue to assess the effectiveness of the resuscitative interventions. In particular, further studies focusing on the modalities of oxygen treatment, the roles of intratracheal epinephrine administration, sodium bicarbonate, CPAP in the delivery room, and post-resuscitation therapeutic hypothermia might contribute to improve the outcomes of newborns requiring delivery room resuscitation.

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TOXOPLASMOSIS

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Introduction

Toxoplasma gondii infection acquired during pregnancy can cause congenital toxoplasmosis. The problem of diagnosis of toxoplasmosis in a pregnant woman is complex because most pregnant women who acquire toxoplasmosis are asymptomatic. Prevention is the first best therapy against congenital toxoplasmosis. Pre-pregnancy and antenatal screening offers the opportunity to give dietary and other advice to reduce the risk of infection to seronegative women. In Italy current routine screening program includes testing for toxoplasmosis. It is not recommended in all countries.

Toxoplasma gondii is an obligate intracellular protozoan. It can take several different forms: the oocyst, the tachyzoite and the cyst. Cats are definitive hosts of *T. gondii*. Human beings can be infected by ingestion or handling of undercooked or raw meat containing tissue cysts or water or food or soil containing oocysts excreted in the faeces of infected cats.

Infection can go unnoticed or could cause signs or symptoms that vary depending on the immune status of the patient and the clinical setting.

In about 10% it causes a self-limited and non-specific illness that rarely needs treatment. The most typical clinical manifestation is isolated cervical or occipital lymphadenopathy. Acute toxoplasma infection during pregnancy is asymptomatic in most women. Reinfection can happen but does not seem to result in disease or congenital transmission of the parasite.

Serological diagnosis

Within two weeks after infection IgG, IgM, IgA and IgE antibodies against many *T. gondii* proteins can be detected.

IgG antibodies to *T. gondii* persist for the individual's lifetime. Absence before or early in pregnancy allows identification of women at risk of acquiring the infection.

Tests for the avidity (functional affinity) of the IgG antibodies have become standard to discriminate between recently acquired infection and those obtained in the more distant past. Presence of high avidity antibodies essentially rule out infection acquired in the recent 3-4 months. Low avidity antibodies can persist beyond 3 months of infection.

IgM antibodies arise within the first week of infection, rapidly increase, and thereafter decline and disappear at highly variable rates. False positive results and persistence of positive titres even years after initial infection can occur with rate as high as 60%. The greatest value of testing for IgM lies in the fact that a negative test essentially rules out recently acquired infection. The confirmatory serological test should be done at a reference laboratory with correct interpretation by an expert. It can reduce the rate of unnecessary abortions by about 50% in women with positive IgM toxoplasma test results reported by outside laboratories.

Congenital toxoplasmosis

After maternal acquisition of *T. gondii* for the first time during gestation, the parasite enters the fetal circulation by infection of the placenta. The birth prevalence of congenital toxoplasmosis ranges from one to ten per 10.000 livebirths. Maternal infection acquired before gestation poses little or no risk to the fetus except in women who become infected a few months before conception. Frequency of congenital transmission varies considerably according to the time during gestation that the mother became infected. Frequency of transmission and severity of disease are inversely related. Maternal infection during first and second trimester may result in severe congenital toxoplasmosis and can result in death of the fetus in utero and spontaneous abortion. By contrast late maternal infection usually results in normal appearing newborns. The overall frequency of subclinical infection in newborns with congenital toxoplasmosis is as high as 85%. Infection initially goes unnoticed, but if it is not treated babies, can later develop chorioretinitis or growth can be delayed in the second or third decade of life.

Diagnosis

The major diagnostic issues related to congenital toxoplasmosis are: i) identification and establishment of infection in a pregnant woman; ii) determination of whether infection has taken place prior to birth; iii) proof of congenital infection after birth.

Fetuses with congenital toxoplasmosis usually look

normal on prenatal ultrasound. If present ultrasonographic findings suggestive of congenital disease include intracranial calcifications, ventricular dilatation, hepatic enlargement, ascites and increased placental thickness, similar to other caused by various pathogens including cytomegalovirus, herpes simplex virus, rubella and syphilis. Isolated ventriculomegaly is the most common sonographic finding in utero. Unfortunately the sensitivity of ultrasound scan in detecting fetal infections is only 20%.

PCR has revolutionised prenatal diagnosis of congenital toxoplasmosis by enabling early diagnosis, thereby avoiding use of more invasive procedures on the fetus, diminishing the risk for pregnancy loss. Moreover the diagnosis of fetal *T.gondii* infection before 22 weeks using fetal blood specimens is not possible because fetal IgM or IgA may not be produced before 22 weeks' gestation. Tests for the detection of IgA antibodies are more sensitive than those for detection of IgM antibodies in the fetus and newborn. Presence of IgG antibodies in fetal's serum could be their own or their mother's antibodies.

Given rapidity and accuracy PCR amplification of the 35-fold repetitive B1 gene for detection of *T. gondii* DNA has successfully been used to diagnose congenital toxoplasmosis. If contamination is not an issue, specificity and positive predictive value of PCR results approach 100%. This test have an overall reported sensitivity of 64-98%. Sensitivity varied greatly according to gestational age and is significantly higher for maternal infections that arose between 17 and 21 weeks of gestation. Amniotic fluid PCR undertaken before week 18 is probably less reliable and tests done after this time and has not been systematically studied.

Management and therapy

Management of maternal and fetal infection varies considerably between different countries and centres within the same country.

The antibody status of a pregnant woman should be obtained before or early in pregnancy. Negative tests for IgM antibodies during the first two trimesters essentially rule out recently acquired infection. Positive IgM test results should always undergo confirmatory tests in a reference laboratory. Definitive diagnosis of acute infection of toxoplasmosis requires demonstration of a rise in titres in serial specimens.

Treatment of the mother during pregnancy is an attempt to reduce the frequency and severity of fetal infection. Spiramycin has been estimated to reduce the incidence of vertical transmission by about 60%. It should be initiated as immediately as feasible after diagnosis of recently acquired maternal infection. Antitoxoplasma treatment should be continued throughout pregnancy.

The design of studies undertaken to date has not permitted a definitive conclusion about efficacy of spiramycin to reduce the congenital transmission of the parasite. Until appropriately studies are done, autho-

rities continue to recommend spiramycin (for the first and early second trimester) or pyrimethamine/sulfadiazine (for late second and third trimester) for women with suspected or confirmed acute *T. gondii* infection acquired during gestation. Since maternal infection does not necessarily result in fetal infection, suspected or established maternal infection acquired during gestation must be confirmed by prenatal diagnosis by PCR of amniotic fluid. However a negative PCR of amniotic fluid cannot rule out congenital infection. Ultrasound should be done at least monthly until term to monitor fetal development.

Toxoplasmosis can be excluded in live-born infants only by postnatal serologic follow-up.

Conclusions

Systematic serological screening of all pregnant women is undertaken only in Italy and few other European countries (France and Austria). Uncertainty about incidence of congenital infection, cost-effectiveness, difficulties with sensitivity and specificity of serological tests and ultrasound scanning, findings suggesting absence of spiramycin effectiveness have hampered attempts to implement screening programs in several countries. Even management and lengths of treatment protocols vary greatly between centres in European countries.

Despite great progress in clinical and basic science research many unresolved issues in toxoplasmosis remain to be addressed. So up to now there is not a well defined and definitive strategy. So that agreement between gynaecologists and neonatologists is fundamental in each centre about management, treatment and follow-up.

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CONNATAL TOXOPLASMOSIS

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The worst type of the toxoplasmic disease is the congenital one. The incidence of connatal toxoplasmosis in Europe is equal to 1-10 cases/ 10000 new born; in the U.S.A. we think 400-4000 cases / year. Every year in Europe 3100 babies run the risk of heavy cerebral malformations; also 3100 pregnant women and 2800 babies escape a diagnosis. In Italy the incidence of infection during pregnancy is about 6.4 / 1000.

The risk that the *Toxoplasma gondii* gets over the placental barrier and parasitizes the fetus is strictly related to the pregnancy period in which the mother's infection is registered: it is about 17% in the first trimester, 45% in the second trimester and 65% in the third trimester but it reaches 90% during the last weeks of pregnancy. Vice versa, the seriousness of the possible embryo-fetal damage is inversely related to the pregnancy period in which the infection is registered: high in the first weeks, whereas, the infection is often in subclinic form during the last weeks. This means that at least 80-90% of congenital toxoplasmosis taken in the third quarter of pregnancy are clinically non-apparent. They can be diagnosed only by indirect and/or direct studies aiming to the isolation of the parasite and by instrumental exams which point out its effects on the target organs.

In the newborn baby these organs are represented by eye (chorioretinitis, glaucoma, cataracts) and brain (hydrocephalus, calcifications, microcephaly, mental retardation and convulsions). Sometimes other clinical non-specific manifestations are registered: fever, maculo-papular rash, hepatosplenomegaly, jaundice, thrombocytopenia and general lymphadenomegaly. The classical triad of the congenital toxoplasmosis is very rarely registered: hydrocephalus, chorioretinitis and intracranial calcifications. A clinical, serological and instrumental follow-up is therefore strictly required especially in those cases where the mother's infection is not clearly documented. This is also necessary for babies born by women who acquired infection during the second and third pregnancy trimester with high risk of transmission but almost always clinically asymptomatic at the time of birth.

Babies with negative reports at the fetal diagnosis, normal clinical examination, serological test on mother and baby almost superimposable concerning specific IgG, but with negative IgM and IgA in the baby, proceed to cerebral ultrasound to be repeated at three months and fundus oculi to be repeated every six months in the first six years and every year in the following ten years.

Our patients do not execute routine auditory brain stem evoked responses, but Boel Test at the 9th month.

Babies with documented maternal infection in the first and second trimester and with doubtful ultrasound images are sent to cranium CAT scan.

In case of documented fetal infection or maternal infection at the third trimester, with negative prenatal diagnosis and babies apparently in good health at the time of birth, we send babies to a cranium CAT scan, spinal fluid and research of the parasite by means of PCR.

Unfortunately a precise diagnosis of the gestational period in the right moment of the infection is only possible in infrequent cases. Therefore it is extremely difficult to come to an unambiguous or excluded diagnosis of congenital toxoplasmosis in the first month of life. Considering that the IgG antibodies pass through the placenta, it is necessary to proceed to IgM antibodies sequenced samples and specific IgG at the time of birth and when the baby is 1-2-3-4-5-6-9-12 months old. The infected baby shows IgG upgrading times exceptionally low when he/she is 3-4 months old but they can be high at the age of 6 months.

The diagnosis of connatal infection in newborn babies is determined on fulfilment of one or more of the following criteria:

1. Positivity of the specific IgM antibodies in peripheral blood within the first month of life;
2. Failed maternal IgG antibodies decrease in at least three successive serological checks at the above-mentioned ages;
3. Presence of specific IgG after the 12th month.

These criteria help us to define a precise diagnosis but they are not very helpful for the immediate management of the postnatal period. In particular they do not allow us to identify which newborn babies to send to therapy with the aim to avoid the occurrence of sequels (tertiary prevention).

The traditional treatment alternates 30 days cycles of PIRIMETAMINE, SULFADIAZINE and FOLIC ACID with 30 days cycles of SPIRAMYCIN.

In case of undoubted or most probable maternal infection we always administer the first two cycles of therapy and then we estimate the need to continue or discontinue on the basis of the results of the specific IgG antibodies.

If we notice increasing or stable IgG on the third check, it is necessary to resume the therapy and continue it for the first year of life.

If IgG are clearly decreasing we go on with the checks and if the serologic negativity is confirmed at the 12th month, the connatal infection is excluded.

Summing up: the wrong interpretation of the maternal serologic exams with a subsequent incorrect definition of the gestational period when the infection occurred prevent a timely assessment of the clinical and contagious risk in a lot of newborn babies, thus exposing them to a long and sometimes unjustified follow-up. However it is fundamental to prevent delays in establishing the tertiary prevention in those infected babies with risks of sequels.

Therefore it is advisable that pregnant women and their babies are addressed to specialised centres of excellence providing advanced resources for pre and post-natal diagnosis.

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RETINOPATHY OF PREMATURITY (ROP) TODAY

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Retinopathy of prematurity (ROP) is a potentially blinding eye disorder that primarily affects premature infants weighing about 1250 grams or less that are born before 31 weeks of gestation (see 1 for a review). The smaller a baby is at birth, the more likely that baby is to develop ROP. More than 80% of premature babies who weigh less than 1000 grams develop ROP. Fielder (2) studied infants weighing less than 1700 g and noted development of ROP in 51%. In general, more than 50% of premature infants weighing less than 1250 g at birth show evidence of ROP, and about 10% of the infants develop stage 3 ROP. The incidence of ROP is rising because of the medical profession's ability to improve survival rates among the most premature babies. On average, 500-700 children become blind because of ROP in the United States annually. Several complex factors may be responsible for the development of ROP. In addition to birth weight and how early a baby is born, other factors contributing to the risk of ROP include supplemental oxygen use, anemia, blood transfusions, respiratory distress, breathing difficulties, and the overall health of the infant. The eye starts to develop at about 16 weeks of pregnancy, when the blood vessels of the retina begin to form at the optic nerve in the back of the eye. The blood vessels grow gradually toward the edges of the

developing retina, supplying oxygen and nutrients. During the last 12 weeks of a pregnancy, the eye develops rapidly. When a baby is born full-term, the retinal blood vessel growth is mostly complete (the retina usually finishes growing a few weeks to a month after birth). But if a baby is born prematurely, before these blood vessels have reached the edges of the retina, normal vessel growth may stop. The edges of the retina—the periphery—may not get enough oxygen and nutrients. Probably the periphery of the retina then sends out signals to other areas of the retina for nourishment. As a result, new abnormal vessels begin to grow. Pathologic angiogenesis is linked with upregulation of vascular endothelial growth factor (VEGF) and its second receptor, VEGFR-2. The disease improves and leaves no permanent damage in milder cases of ROP. About 90 percent of all infants with ROP are in the milder category and do not need treatment. However these infants with ROP are considered to be at higher risk for developing certain eye problems later in life, such as retinal detachment, myopia (nearsightedness), strabismus (crossed eyes), amblyopia (lazy eye), and glaucoma. In many cases, these eye problems can be treated or controlled.

In infants with more severe disease, the retina neovascularization is fragile and weak and can bleed, leading to retinal scarring. When these scars shrink, they pull on the retina, causing it to detach from the back of the eye, causing impaired vision or even blindness.

ROP is classified in five stages, ranging from mild (stage 0) to severe (stage 5). Following a series of consensus meetings in 1984, ROP was newly classified according to:

- location (zone) – (Fig. 1)
- extent
- severity (stage)
- the presence or absence of plus disease, (vascular decompensation manifest as vessel dilatation and tortuosity).

Location

The area of the retina affected by ROP is divided into three zones (Fig. 1). Zone 1 is the most posterior retina, that contains the optic nerve and the macula (zone of central vision). ROP develops in this zone if the retina in this area is most underdeveloped. Disease in zone 1 is more severe compared with disease limited to zones 2 or 3. Any disease in zone 1 (even stage 0, immature) is critical and must be monitored closely. Zone 2 is the intermediate zone where blood vessels often stop in ROP. Zone 3 is the peripheral zone of the retina, where vessels are absent in ROP, but present in normal eyes.

Extent. Is calculated as clock hours of eye fundus extension of the disease (Fig. 1).

Severity of Disease

Stage 0: This is the mildest form of ROP. It is immature retinal vasculature. No clear demarcation of vascu-

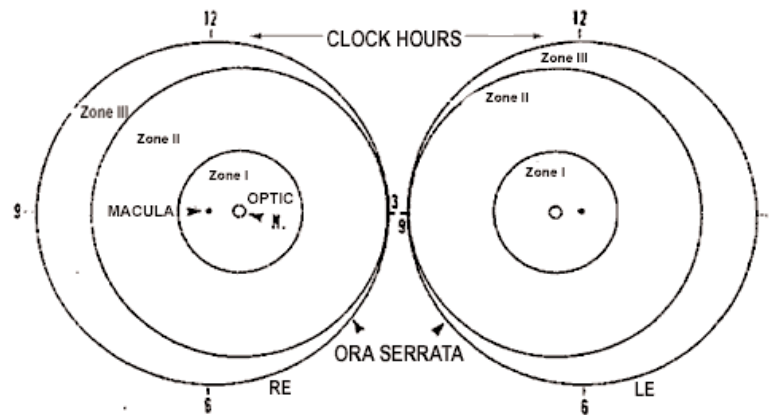


Fig. 1
 Schematic drawing of the eye fundus showing the division in zones and clock hours. RE right eye, LE left eye

larized and nonvascularized retina is present. Only a suggestion of the border is noted on examination.

Stage 1. Mildly abnormal neovascularization. A **demarcation line** is seen at the edge of vessels, dividing the vascular from the avascular retina. Many children who develop stage 1 improve with no treatment and eventually develop normal vision. The disease resolves on its own without further progression.

Stage 2 Moderately abnormal neovascularization. The line structure of stage 2 acquires a volume to form a **ridge** with height and width. Many children who develop stage 2 improve with no treatment and eventually develop normal vision. The disease resolves on its own without further progression.

Stage 3 Severely abnormal neovascularization. The abnormal blood vessels grow toward the center of the eye instead of following their normal growth pattern along the surface of the retina. The ridge of stage 3 develops more volume and there is extra-retinal fibrovascular proliferation into the vitreous. This stage is further subdivided into mild, moderate and severe, depending on the amount of fibrovascular proliferation. Some infants who develop stage 3 improve with no treatment and eventually develop normal vision. However, when infants have a certain degree of Stage 3 and "plus disease" develops, treatment is considered. The ROP in stage 3 that requires treatment is generally called threshold disease. The Cryotherapy for Retinopathy of Prematurity Cooperative Group (CRYO-ROP) study described *threshold disease* as 5 contiguous or 8 noncontiguous hours of neovascularization with plus disease in zone 1 or 2. Of threshold eyes left untreated, 50% would develop adverse structural outcomes (eg, retinal detachment) after 12 months.

Stage 4a Subtotal retinal detachment not involving the macula. Traction from the scar produced by bleeding, abnormal vessels pulls the retina away from the wall of the eye.

Stage 4b Subtotal retinal detachment involving the macula. With this stage onwards prognosis for vision

becomes poor.

Stage 5 Total retinal detachment. Completely detached retina is always funnel-shaped and represents the end stage of the disease. If the eye is left alone at this stage, the baby can have severe visual impairment and even blindness.

Plus Disease. Another indicator of severity which reflects progressive vascular incompetence seen clinically as (a) posterior polar abnormal dilatation of retinal veins (compared with a standard fundus photograph) with florid abnormal new vessels (b) pupillary rigidity (c) vitreous haze is called Plus disease.

Currently, treatment relies on the destruction of the areas of the retina that are without blood vessels, slowing or reversing the abnormal growth of blood vessels (3-5). The destruction is achieved by cryotherapy or photocoagulation with laser energy. This becomes necessary only if the disease has reached a certain, well defined stage, particularly stage III with "plus disease", as the milder forms often regress spontaneously. Timing is one of the important factors that make the treatment successful in ROP, because the disease can advance very quickly and delayed treatment often reduces the chances of success. The rapidly progressing ROP is called *Rush disease*, and it is usually associated with very extensive or aggressive growth of abnormal blood vessels. If the center of the retina or the entire retina detaches, central vision is threatened, and surgery may be recommended to reattach the retina, and treatment options include:

- **Scleral buckle.** This involves placing a silicone band around the eye and tightening it. This keeps the vitreous gel from pulling on the scar tissue and allows the retina to flatten back down onto the wall of the eye. Infants who have had a sclera buckle need to have the band removed months or years later, since the eye continues to grow; otherwise they will become nearsighted.
- **Vitreotomy.** Vitreotomy involves removing the vitreous and replacing it with a saline solution. After the vitreous has been removed, the scar tissue on the

retina can be peeled back or cut away, allowing the retina to relax and lay back down against the eye wall. Vitrectomy is performed only at stage V.

There are no symptoms of ROP and infants must be screened by an ophthalmologist. Screening is recommended for a) all premature infants (born at less than 35 weeks gestation or who weighed less than 1800 grams) who received supplemental oxygen, and b) infants born at less than 30 weeks gestation or who weighed less than 1300 grams, whether or not they received supplemental oxygen (6-8).

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ROP TODAY

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ROP is growing cause of blindness or severe visual outcome.

The greatest single risk factor for developing ROP is being born prematurely especially extremely low born (23-25 G.A.).

Risk factor

The retinal damage occurs in two phases:

- a) hyperoxia → vasoconstriction
- b) hypoxia → neovascularization initiated by release of vascular endothelial growth factor (VEGF)

Causes

OXIGEN

Oxygen fluctuations cause ROP more than hyperoxia. Free Radicals release cause oxidative damage in pre-term infants without defensive enzyme: this situation is more severe in extremely low G.A.

More recently, in current neonatal medical practice, with the aid of transcutaneous oxygen monitoring , studies have found that oxygen variability , even within clinically predetermined acceptable ranges, was associated with ROP.

A recent study reported a lower incidence of threshold ROP in premature infants nursed in slightly lower oxygen saturation levels than that recommended in current guide lines.

LIGHT

Light was proposed as causative factor in ROP because light leads to a release of free radicals that can cause oxidative damage and release angiogenic compounds. Expression of tumor necrosis factor- α and VEGF during retinal neovascularization can be initiated by lipid hydroperoxides.

GROWTH FACTORS

Growth factors typically act as a signaling molecules between cells. They often promote cell differentiation and maturation, which varies between growth factors: vascular endothelial growth factors stimulate blood vessel differentiation. It is involved in ROP vasculogenesis and angiogenesis.

These factors are produced after tissutal hypoxia.

What to do to prevent ROP

Reduce in premature infants the amount of oxygen
In infants at-risk reanimation is reasonable in room air instead of oxygen

Use low oxygen arterial saturations in extreme pretermatures

Avoid oxygen fluctuations

Vitamin E and SOD are the anti-oxidant agents to use

in the future, but need further studies.

Conclusions

Retrospective studies have supported managing of infants in lower levels of oxygen or with reduced variability in oxygen. Both of these parameters were tested in a small clinical trial with excellent results and low pulmonary or neurologic sequelae, supporting plans to develop a multicenter trial. However although these studies may reduce the incidence of ROP, long term follow up is essential to determine possible morbidity from pulmonary and neurologic problems associated with managing infants in lower oxygen. Future studies will test low oxygen saturations from shortly after birth up to 32 weeks PMA and its effect on the development of prethreshold ROP.

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WHEN AND HOW TO TREAT THE PATENT DUCTUS ARTERIOSUS

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The ductus arteriosus, a large channel normally found in all mammalian fetuses, develops from the distal portion of the left sixth aortic arch and connects the main pulmonary trunk with the descending aorta, 5 to 10 mm distal to the origin of the left subclavian artery.

The attention which has been paid to this structure over the last several decades is due to its importance in fetal and neonatal circulatory physiology.

The first surgical ligation of patent ductus arteriosus (PDA) by Dr. Gross at the Children Hospital in Boston in 1938 (1) signalled the beginning of the modern era of diagnosis and treatment of heart disease in children. In 1939, Burnard (2) noted that murmurs were more commonly observed in infants with respiratory distress and suggested that the ductus arteriosus might be patent in these infants. In 1961, Rudolph (3) demonstrated with catheterization the association between patent ductus arteriosus (PDA) and respiratory distress syndrome in premature infants. In the following years, the observations of the PDA in premature infants increased, in concomitance with improvement in survival of these patients (4). Substantial left-to-right shunting through the ductus may increase the risk of intravascular hemorrhage, necrotizing enterocolitis, bronchopulmonary dysplasia, and death. Therefore, closure of the duct is indicated.

Incidence

Closure of the ductus is often delayed in premature infants (5) and the problem is inversely related to the grade of maturity. Its increasing incidence is principally related to the increased survival of premature infants, delayed ductal closure being extremely common in these infants with an incidence varying from 20% in infants greater than 32 weeks, to 60% in those less than 28 weeks gestation. PDA accounts for about 10% of congenital heart diseases in term newborn, while the commonest setting in which a PDA plays an important clinical role is usually that of ventilatory-bound premature baby. In particular the advent of newborn intensive care in the late 1960' resulted in survival of a large number of small premature infants in whom PDA became manifest clinically

Diagnosis

The clinical features are different, depending on the gestational age of the patient. Prolonged ductal patency has its most dramatic effects in the low birth weight premature infants with respiratory distress syndrome. It has been argued that a patent ductus arteriosus with a left-to-right shunt is universal in the initial respiratory disease in infant less than 1000 grams and may play a role in the pathophysiology of

the condition as important as surfactant insufficiency. These effects are multi-system and are the results of the mechanical effects on the heart of a large left-to-right shunt, low diastolic aortic pressure and the state of immaturity of the myocardium and the pulmonary arterioles.

A large left-to-right shunt at ductus level determines a "diastolic steal" from the abdominal organs which could explain the association between PDA and necrotizing enterocolitis (6). Increased pulmonary blood flow exacerbates respiratory distress syndrome, and the redistribution of cardiac output could explain other complications such as intracranial hemorrhage, and retinopathy of prematurity. The limited left ventricular response to volume overload in the premature infant further compromises cardiovascular function and organ perfusion.

Echocardiography

Color Doppler echocardiography represents the best diagnostic exam for the study of PDA. This technique allows accurate evaluation of ductal size and ductal shunting. The internal ductal diameter, the maximal shunting velocity, the ratio of the diameter of the left atrium to that of the aortic root (left atrium-to-aortic ratio) and the degree of shunting must be assessed. Shunting is graded as moderate if a diastolic flow is

easily detected in the main pulmonary artery and if there is a diastolic backflow in the aorta immediately beneath the ductus arteriosus and a forward flow above the ductal insertion. It is graded as severe if a diastolic backflow is detected in the pulmonary trunk and if a dilation of the left atrium is present with a left atrium/aortic root ratio above 1.6.

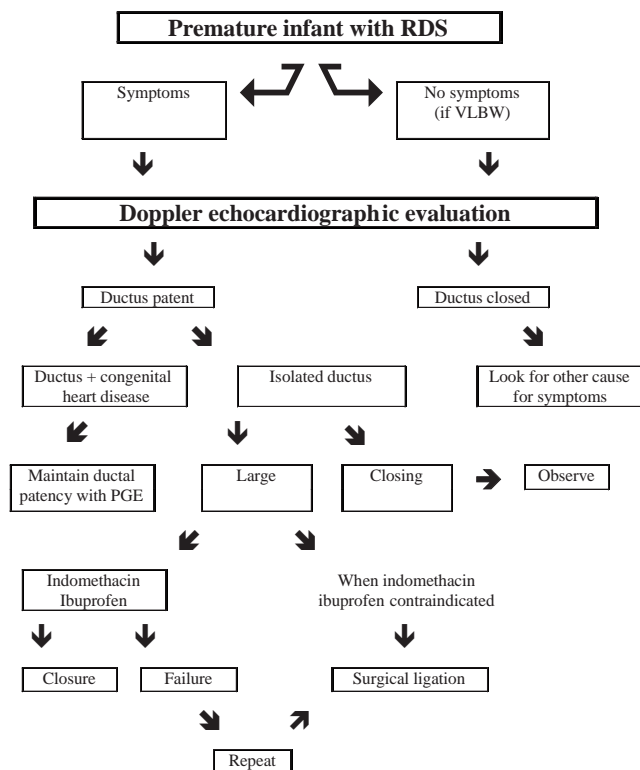
Clinical features

The clinical features of PDA in the newborn are different, depending on gestational age and the presence of lung disease.

The very low birth weight infant (VLBW), corresponding to <28 weeks of gestation or a birth weight <1200 g, present clinical evidence of ductal patency within the first three to four days of life and often not before the first week. The classical signs consist of tachycardia, precordial hyperactivity, and bounding peripheral pulses. Respiratory parameters usually deteriorate with tachypnea, increasing respiratory distress, rising oxygen requirement and apnoea. These symptoms may mimic sepsis or intracranial haemorrhage. Occasionally the classical signs, like murmur or bounding pulses are absent, the so-called "silent ductus" (7). Thus in VLBW infants, some patients may not have clinical signs clearly indicative of ductal patency. The low birth weight preterm infant (LWB) comprises

Table I

Flow diagram for management of the premature infant with respiratory distress syndrome and suspected patent ductus arteriosus



those newborn weighing between 1200 and 2500 g, ranging in gestational age from 28 to 32 weeks. The classical signs are found in this group most often around day 3 or 4, usually with a sudden deterioration of respiratory distress, consisting in increasing tachypnea, retractions, and oxygen requirements. In the term infants, prolonged ductal patency is unusual and, when present, is often clinically silent.

Therapy

Treatment in preterm infants

Definitive treatment of a PDA is closure of the ductus arteriosus. Some conservative measures have been advocated in order to avoid therapeutic interventions, like fluid restriction, diuretics and digitalis. All of them usually only delay, rather than prevent, the ultimate need for PDA closure. An important consideration in treating a premature infant with a PDA is maintenance of an adequate hematocrit and haemoglobin.

In many centers, indomethacin is the first line of therapy when a hemodynamically significant ductus is documented. The treatment consists in three dosages of 0.2 mg/kg at 12-hour intervals.

In infants weighing less than 1000 g the use of indomethacin before signs of cardiovascular compromise appear is associated with a better outcome (8). In contrast, in those weighing over 1000 g, early use of indomethacin appears not to improve outcome. These infants may therefore be safely treated once signs of hemodynamically significant shunting have developed.

Glucocorticoids administered postnatally have been reported to results unsuccessful ductal closure. Indeed combined therapy with indomethacin and betamethasone has been experimentally shown to cause increased ductal constriction in animals. However its use needs further investigation and clinical trials.

Indomethacin has many complications and contraindications, well documented by an extensive literature. Among the significant complications are an increased tendency of bleeding related to platelet dysfunction, renal dysfunction resulting in decreased urine output, necrotizing enterocolitis and intestinal perforation, intracranial hemorrhage and transient cardiac diastolic dysfunction of uncertain clinical significance.

Ibuprofen (three dosages: the first of 10 mg/kg followed by two doses of 5 mg/kg at 24 hours intervals), appears as effective as indomethacin in promoting ductal closure in premature infant. A recent large prospective, multicenter trial (9) showed that it appears to be very effective, without reducing mesenteric, renal or cerebral blood flow. In particular it is significantly less likely to induce oliguria. These data suggest that ibuprofen could be the preferred medical treatment for PDA.

Surgical ligation is a safe and efficacy procedure (10) and is usually reserved for those premature infants who have failed an adequate course or courses of medical therapy, or where contraindications such as intracranial hemorrhage, thrombocytopenia, necrotizing ente-

rocolitis or poor renal function are present.

Table I presents a suggested algorithm for the evaluation and management of ductal shunting in the premature infant.

Treatment in full-term infant

A patent ductus arteriosus in a full-term infant rarely causes significant symptoms. However, closure is recommended if the ductus remains patent past infancy. Outside the neonatal period, the ductus arteriosus is not responsive to indomethacin, and either a surgical or catheter-based approach to closure is necessary. Occlusion of the ductus in the catheterization laboratory has become commonplace and is generally recommended as the treatment of choice. At present this approach is not technically feasible for small, premature infants.

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WHEN TO TREAT AND HOW TO TREAT PATENT DUCTUS ARTERIOSUS?

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After birth ductus arteriosus (DA) closes in nearly all term infants by 3 days of age, while in preterm infants the closure takes longer and is less predictable, depending on the presence of concomitant respiratory diseases, the sensitivity of the ductus to oxygen, the level of circulating prostaglandin E₂, ductus' tissue sensitivity to prostaglandin E₂ and nitric oxide. Patent ductus arteriosus (PDA) is the most frequent cardiovascular abnormality in preterm low birth weight neonates. Its incidence is inversely related to gestational age at birth, with values varying from 20% in preterm infants >32 weeks gestation up to 60% in those <28 weeks¹. In the largest Italian epidemiological study on very preterm infants performed so far (ACTION study) the overall incidence of PDA in newborn infants <32 weeks gestation was 40%, with decreasing values from 56% in subjects of 23-25 weeks to 30% in those of 30-31 weeks (1). In 2003, in the Vermont Oxford Network database the incidence of PDA by birth weight was 36% in neonates <1501 g (52% under 1001 g and 24% between 1001-1500 g); the frequency of surgical ligation of PDA in the same group of infants was 8% (2).

Although the wide spread use of echocardiography has made the detection of a PDA very simple, it is difficult to establish when a PDA can be harmful, since ductal patency does not equate to hemodynamic significance in any case. Significant left to right shunting through the PDA modifies the flow pattern in the aorta with the development of a diastolic steal; in these circumstances, retrograde diastolic flow has been documented in cerebral, renal and gastrointestinal blood vessels, suggesting that hemodynamically significant PDA might be associated with an increased risk of intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC) and bronchopulmonary dysplasia (BPD). Indomethacin, an inhibitor of prostaglandin synthesis, was introduced to treat PDA in 1976 (3). Due to the high incidence of PDA in preterm neonates with low gestational age, prophylaxis with Indomethacin has been proposed in neonates with gestational age <32 weeks. However, the use of this drug has been associated with potentially harmful side effects such as reduction in cerebral blood flow velocity (4) and reduction in cerebral oxygen delivery (5). Ibuprofen, another cyclooxygenase blocker, does not seem to affect cerebral and renal blood flow velocities (6), although a higher frequency of BPD has been found in association with its use, and three cases of pulmonary hypertension have been described following its administration in the first days of life.

Prophylaxis both with Indomethacin or Ibuprofen has been widely investigated in randomized trials and found to be effective in closing PDA. However, PDA

can close spontaneously, such as recently reported by Koch et al. who have demonstrated that this happens in 34% of extremely low birth weight neonates by 8 days of life (7). This means that prophylaxis exposes a large number of neonates to a drug that they would not need. Furthermore, prophylaxis studies have shown some short term benefits, including a decreased risk of IVH with the use of Indomethacin, but no evidence of reduction in long term morbidity and/or mortality. It is therefore necessary to balance the benefits and the risks of an early attempt to close pharmacologically a PDA.

The results of a large series of randomized trials have proven that Indomethacin is effective in decreasing the need for surgery when used therapeutically to close a hemodynamically significant PDA, both in a pre-symptomatic and symptomatic phase, however without modifying the risk of mortality, BPD, retinopathy of prematurity (ROP) and necrotizing enterocolitis (NEC). On the other hand, potential side effects of Indomethacin are cerebral and gastrointestinal vasoconstriction, transient renal impairment, thrombocytopenia, and pulmonary and gastrointestinal hemorrhage. Ibuprofen has been found to be as effective as Indomethacin in closing a PDA, but with lower incidence of decreased urine output and lower creatinine concentrations in blood. However, the observation of some cases of pulmonary hypertension and the possible increased risk of BPD associated with Ibuprofen have raised concerns about its use. Some authors therefore think that, despite theoretical advantages, there is no clinical evidence to support the use of Ibuprofen in place of Indomethacin.

Despite the enthusiasm for routine closure of PDA in the last years, it is to consider that there are no randomized trials designed to compare the pharmacological treatment of PDA with no treatment at all; or to compare the benefit of surgical closure, the standard treatment outside the pharmacological approach, with medical management (8). All trials include a 'backup' treatment, namely surgery, in case of persistence of a PDA after the administration of the drug under study or of the placebo. Furthermore the results of studies aimed at investigating the relationships between PDA and a series of neonatal diseases such as IVH, NEC and, most of all, BPD are inconsistent and can possibly suggest only an associative but not a causal implication with PDA (9). Moreover, there is a lack of extensive long-term follow-up data, especially regarding treatment with Ibuprofen.

The aim of neonatologists should therefore be to develop new tools to predict which neonates are at highest risk for developing a hemodynamically significant PDA and to treat only these patients. It is assumed that clinical signs such as a murmur, tachycardia, bounding pulses, overactive precordium and hepatomegaly, are not predictive of the relevance of the left to right shunt. In fact some infants with a large left to right ductal shunt have no clinical signs related to shunting, not even an important murmur. A strategy based on echo-

cardiographic features could help in discriminating which neonates to treat, particularly in presence of a "pulsatile" or "growing" flow pattern, that is associated with a low probability of spontaneous closure; in this way, the unnecessary exposure of a large number of neonates to the potential side effects of drugs can be avoided, according to the findings of Su et al (10).

If pharmacological therapy is ineffective or contraindicated, it seems advisable to proceed with the surgical ligation of the ductus, although also this approach has potential side effects such as vocal cord paralysis, hemorrhage, air leaks, wound infection, and, in some rare cases, also the inadvertent ligation of the left pulmonary artery.

In conclusion, it is probably prudent to treat PDA aggressively in very preterm babies when there is a large left to right shunt and poor pulmonary function. The approach for larger babies or in mild cases is less clear and not supported by the scientific evidence. For these reasons, routine closure of a PDA should not be considered "standard of care".

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