

Selected Lectures of the 12th International Workshop on Neonatology

10 P PEDIATRICS: NOTES FOR THE FUTURE

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LECT 1

NEONATAL CONGENITAL HEART DISEASE: WHAT'S NEW?

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Congenital heart disease (CHD) is the most common birth defect and the leading non-infectious cause of death in the newborn, affecting 19-75 per 1,000 live births. Since CHD can be associated with prenatal lethality, its actual incidence may be underestimated. Undoubtedly, CHD covers a large set of morpho-functional defects that arise during embryogenesis, inclusive of mild lesions, that are clinically quiescent for decades such as bicuspid aortic valve. Current improvements in pre- and neonatal diagnosis as well as in surgical procedures have reduced morbidity and mortality for many CHDs, but not for the most severe forms of CHD. Fetal echocardiogram is a primary tool for the evaluation and diagnosis of fetal cardiovascular pathology. Prenatal discovery of CHD may improve pregnancy outcome of fetuses with specific types of cardiac lesions, offering potential clinical benefit with regards to infant outcome. Initially, fetal echocardiography used to include only a 4-chamber view of the heart, then outflow tract view (OTV) and 3-vessels trachea view (3VTV) were added to enhance precision of fetal echocardiography. More recently, in addition to the 4-chamber view, the right ventricular outflow tract, the left ventricular outflow tract, and the main pulmonary artery and its branches are included in order to identify some minimal defects *in utero* and give more exhaustive data on suspicious fetal heart. A management plan for a pregnancy with CHD requires a multidisciplinary approach to improve perinatal outcome of the newborn. In fact, in our Center (Regional Reference Center for Congenital Heart Disease in Child and Adult), pregnant women and fetuses with CHD follow a specific diagnostic-therapeutic care pathway from the

moment of the diagnosis to the end of pregnancy. The care pathway involves different professionals such as a Fetal Cardiologists, Neonatologists, Surgeons, Gynecologists and Obstetricians. The network of clinical teams involved defines the modality of care on the basis of the pathology, evaluating the necessity of further clinical and instrumental assessment, monitoring the pregnancy during its duration and choosing the “safest way of delivery” for the fetus and the mother.

LECT 2

THE PRETERM NEWBORN INFANT WITH PATENT DUCTUS ARTERIOSUS: TO TREAT IT? HOW TO MANAGE IT? WHEN TO TREAT IT?

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The patency of the ductus arteriosus (PDA) in the newborn is common in Neonatology and Pediatric Cardiology since the 1970s. Despite this, no international protocols for standardized treatment have so far been validated. Even if the ductus arteriosus (DA) is expected to be functionally closed at 72 hours of life, it remains open in a significant number of preterm infants [1].

TO TREAT OR NOT TO TREAT?

In recent years, concerns have emerged on whether or not forcing the closure of a PDA is always the good choice for a preterm baby. Thus criteria have emerged to evaluate when the risk/benefit ratio of closing a PDA could be higher than leaving it to a managed course. The DA is positioned between the pulmonary and the systemic circulation, which must both severely change in the very first days of life (dol), with a reverse relationship between severity of PDA and relevance of these modifications and the gestational age of the infant. Hemodynamics of the DA can affect both the systemic perfusion and the pulmonary function, with oxygenation depending upon the direction of the shunt (left-to-right, right-to-left or bidirectional). However, the expected effects of the hemodynamic impact of a DA can differ from the real occurrence of complications [2]. For these reasons, proposals for tailored approaches have emerged after a period in which treating seemed to be the preferable approach (1995-2005).

HOW TO MANAGE IT?

In the last 15 years Neonatologists have increasingly shown interest in the management of hemody-

namics in newborns and preterms. Thus a new tool, TnECHO (Targeted Neonatal Echocardiography), has emerged improving the cooperation between Neonatologists and Pediatric Cardiologists in the management of preterms (Fig. 1). As a matter of fact the management of PDA begins with a tailored ventilatory support (the less invasive support the best), fluid restriction (≤ 135 ml/kg/24 h beyond 4 days of life), avoiding “free water” overload (diuretics), appropriate caloric and protein supply (anabolism). Nowadays a strict echocardiographic-based hemodynamic approach as suggested since the end of the 1990s is no more the best approach available. Above all, the definition of a hemodynamically significant DA is difficult to be achieved and variable in the literature [3]. Moreover, differences between a conservative approach to PDA and an aggressive treatment (either prophylactic or early) are not statistically relevant [4]. Most Authors suggest performing the first evaluation by TnECHO within 2 dol and a daily check afterwards. A thorough evaluation of cardiac function is the approach to monitor the ongoing clinical situation. Eventually, recent flow-charts combine both clinical and echocardiographic patterns to allow decision-making. They could represent a modern approach [5]. When PDA must be treated, i.v. ibuprofen or indomethacin are the first standard choice with similar

efficacy. A second cycle is performed in case of PDA still relevant. Further cycles can be considered in a non-standard approach. More recently, paracetamol has emerged as an alternative but studies on long-lasting effects are lacking so far [6]. At present, surgical ligation is mostly considered only after pharmacologic therapy failed.

WHEN TO TREAT IT?

Prophylactic protocols provide an early treatment > 6 hours of life and < 1 dol. Conversely, in a standard decision process the decision to treat or not a PDA occurs within the 5-7 dol, even if later approaches are reported. A surgical ligation most frequently occurs around 14-16 dol. It is possible that the timing will be modified if mixed approaches will be validated in the future.

DISCUSSION

Several approaches have been proposed to manage PDA in preterms, but none has been identified as the best available so far. The modern evaluation of a PDA must take care of all these parameters: hemodynamics, ventilation and organ perfusion. All these aspects must be a prerogative for Neonatologists, in order to involve other Professionals for a multidisciplinary approach aiming to reach an individualized treatment.

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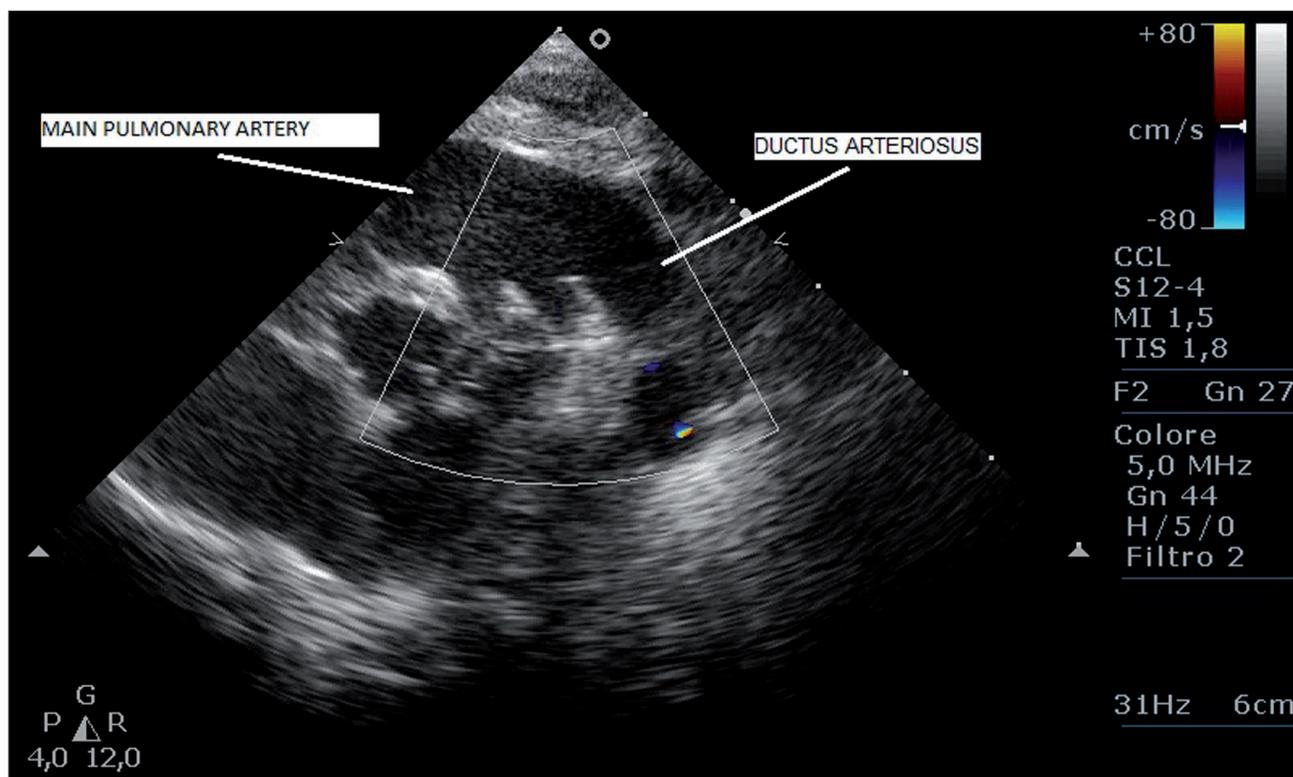


Figure 1 (LECT 2). TnECHO (Targeted Neonatal Echocardiography) in a newborn.

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LECT 3

CONGENITAL CARDIOPATHIES IN CHILDREN WITH DOWN SYNDROME

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INTRODUCTION

Between 44% and 56% of children with Down syndrome present isolated or complex cardiac defects with a variety of pathological conditions, from simple growth retardation to congestive heart failure and pulmonary vasculopathy when they are not properly treated [1]. Congenital cardiopathies are the most common cause of death in children with Down syndrome in the first two years of life [2, 3]. The atrioventricular canal (AVC) and the interventricular defect (IVD) have been reported as the most common congenital cardiopathies, constituting respectively 45% and 35% of the congenital cardiopathies associated with the Down syndrome. Furthermore, the interatrial defect (IAD) ostium secundum type, the patent ductus arteriosus (PDA) and the tetralogy of Fallot (TOF) are present in 8%, 7% and 4% of cases, respectively. The remaining 1% of the observed congenital cardiopathies includes aortic arch anomalies (right aortic arch, aberrant left subclavian artery). On the other hand, heterotaxy syndrome, aortic

coarctation or the transposition of the great vessels are rare among patients with trisomy 21. In addition, there is an increase in pulmonary arterial hypertension (PAH) in children with Down syndrome compared to the general population despite the absence of structural cardiopathies [4].

AIMS OF THE STUDY

The aims of the study were the following: to define the incidence and the type of congenital cardiopathies; to evaluate the presence of pulmonary hypertension in newborns with Down syndrome and then to establish a potential impact of these conditions on specific neonatal factors such as birth weight, Apgar score and metabolic alterations including hypoglycemia and hypocalcemia.

PATIENTS AND METHODS

This is a descriptive observational and retrospective study that included all children born alive between January 1st 2006 and December 31st 2015 with pre- and post-natal diagnosis of Down syndrome. Children with a karyotype analysis that showed a free or complete trisomy 21 or even a mosaicism or a Robertsonian translocation were recruited. The study sample included 56 patients admitted in the Neonatal Pathology and Neonatal Section, University of Cagliari. An expert pediatric cardiologist performed an echocardiographic examination within the first week of life in all children; the device used was a VIVID 7 GE Ultrasonic that allowed a bi-dimensional study and a deepening with color-Doppler. The following data were analyzed: age of the mother, sex of the neonate, gestational age, birth weight, presence or absence of congenital cardiopathy, type of congenital cardiopathy when present, presence or absence of pulmonary hypertension, presence of metabolic alterations such as hypoglycemia and hypocalcemia and presence of cardiac malformations.

RESULTS

The patients that presented a congenital cardiopathy were 39; 23 cases showed an isolated form and 16 cases showed more associated cardiac deficiencies. The most frequent congenital cardiopathy was AVC, that was found in 16 of these patients. The second most common cardiac defect was IVD that occurred in 11 of the 39 children in whom the perimembranosus was the most frequent: in 8 cases it was present in isolated form, while in 2 cases the defect was associated with an IAD ostium secundum type and in 1 case it was associated with PDA. Pulmonary hypertension was present in 18 of the 56 children with Down syndrome, and 5 of them didn't develop any cardiopathy. The congenital cardiopathy mostly associated with pulmonary hypertension was AVC, that was found in 12

patients as a result of altered hemodynamic due to the heart defect.

DISCUSSION

This is the first study ever performed in Cagliari concerning the distribution of congenital cardiopathies in children with Down syndrome. The prevalence of congenital cardiopathy was found to be 70% of patients; this is slightly higher than the data reported in literature, where they state prevalence between 44% and 58% [5-11]. AVC is the most frequent congenital cardiopathy (41%) in children with Down syndrome, followed by IVD (28%) and PDA (23%). In this study, another significant clinical expression was found: the presence of pulmonary hypertension in 32% of cases. In fact, 18 children showed pulmonary hypertension and 12 of them showed AVC (66.7%), while only 1 case (5.6%) showed a wide IVD and 5 cases (27.8%) did not show any congenital cardiopathy (**Tab. 1**).

Table 1 (LECT 3). Pulmonary hypertension (n = 18) in children with Down syndrome.

Congenital cardiopathy	Number	%
AVC	12	66.7%
IVD	1	5.6%
No congenital cardiopathy	5	27.8%
Total	18	100%

AVC: atrioventricular canal; IVD: interventricular defect.

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LECT 4

FROM FETUS TO ADULTHOOD: THE YOUNG ADULT WITH CONGENITAL HEART DISEASE (ACHD)

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During the last 40 years, we assisted to a significant improvement in life expectancy and quality of life of patients born with a heart defect. Over 90% of these patients are now expected to survive to adulthood in West countries. This improvement was due to major advances in diagnostic methods and in surgical and medical management. The 32nd Bethesda Conference report in 2001 estimated that there were approximately 2,800 adults with congenital heart disease (ACHD) per one million populations; recently, Marelli et al. reported a prevalence of 4,090 per one million adults in Quebec for the year 2000, with 380 having severe disease. In Europe, we estimate an ACHD population of around 2.3 million. The Italian ACHD population is around 100,000 patients. With few exceptions, both reparative surgery and interventional procedures are followed by residua and sequelae that require long-term if not life-long surveillance. This has led to establish an increasing number of ACHD programs, to publish management guidelines and to establish the proper organization of an ACHD center. The starting point of a successful ACHD program is a tight collaboration with the Pediatric Cardiology Center, that can be accomplished by setting a Transition Clinic. This Clinic should avoid discontinuity of care during the handoff between pediatric to adult providers, usually due to insufficient patient and family preparation, cognitive or psychosocial impairment, inadequate program integration, and poor access to adult specialty care. Discontinuity of care threatens long-term patient outcomes and increases cost through duplication

of procedures and lost opportunities for strategic intervention. By doing so, both fields are enriched. Pediatric cardiologists receive feedback about long-term outcome and adult cardiologists become better informed about the ever-changing cohort of patients who are accessing their services. Last but not least, these patients have very special needs and therefore physicians responsible for their care need specific expertise and training. Many European national cardiology-training guidelines recognize the need for specific training in congenital heart disease, but most cardiologists have little experience or understanding of how to manage these patients. Conversely, pediatric cardiologists have a great understanding of congenital heart disease, but a limited knowledge of the long-term course in adults and the acquired diseases they may develop over time. Specific formal training in ACHD is essential for both adult and pediatric cardiologists in order for them to gain the necessary knowledge and experience for providing best care for these patients. In conclusion, we succeed improving survival of our patients, now we should aim to help them reach their life potential creating well-organized Centers and well trained professionals.

LECT 5

RESEARCH NEWS IN CARDIOLOGY: FROM FETUS TO ADULT PATIENT

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Over the last few decades it has been demonstrated that prematurity at birth and intrauterine growth restriction, the latter expressed as low birth weight, are capable to influence cardiovascular health even in adulthood [1]. For example, myocardial infarction mortality rate is higher in subjects born preterm and/or with low birth weight than in those born at term [2]. This condition is the so-called cardiovascular perinatal programming [3]. Contrary to what has been hypothesized in the past, genetic (hereditary) factors seem to be more important in influencing the future cardiovascular destiny during the vulnerable period of fetal development than epigenetic (environmental) factors [4, 5]. Recent research reports in cardiology have highlighted that low birth weight, intrauterine growth restriction, and decreased gestational age are able to increase susceptibility to atherosclerosis, atrial and ventricular arrhythmias, high blood pressure, and cardio-renal syndrome. Mechanisms

underlying this influence are still uncertain, but increased release of asymmetric dimethylarginine – a strong inhibitor of nitric oxide – atrial and ventricular remodeling, interrupted elastin synthesis as well as reduced nephrons number in the kidneys are hypothesized to be responsible [6]. Specifically, it has been demonstrated in young adults born preterm that blood levels of asymmetric dimethylarginine are increased and significantly inversely related with gestational age and birth weight [7]. This is probably due to the fact that asymmetric dimethylarginine renal catabolism by its specific enzyme dimethylarginine-dimethylaminohydrolase is significantly decreased in these individuals [8]. These findings suggest the onset of an early circulatory dysfunction predictive of increased cardiovascular risk in subjects born preterm. Furthermore, a statistically significant correlation has been discovered between asymmetric dimethylarginine and urinary neutrophil gelatinase-associated lipocalin (NGAL), a well-established biomarker of kidney injury. These preliminary results support the hypothesis that, in subjects born with intrauterine growth restriction, the development of an early chronic kidney disease contributes to induce an increase in the atherosclerotic process and to increase the risk of future adverse cardiovascular events. By means of metabolomics, a technique that allows the systematic study of the complete set of metabolites in a biological sample, a relationship has been shown between the urinary metabolic profile of these individuals and their blood asymmetric dimethylarginine levels, thus confirming the presence of a subclinical cardio-renal involvement in these subjects [9, 10]. Young adults born with a low weight owing to prematurity at birth show an increase in their systemic blood pressure and arterial stiffness, which seem to be mainly related with interrupted elastin synthesis as well as reduced nephrons number in their kidneys [11]. In addition, those born with low birth weight are at high risk of developing supraventricular and ventricular arrhythmias as well. Regarding supraventricular arrhythmias, in this at risk population atrial fibrillation is more common than in subjects born full term. A pathophysiological explanation of these findings probably rely on the fact that prematurity at birth and low birth weight are able to lead to atrial remodeling probably induced by the presence of a marked difference in pressure between the two atria, such as in subjects born preterm with a long time patency of ductus arteriosus and/or the presence of severe respiratory distress [12, 13]. The electrophysiological remodeling of the neonatal heart in newborns born with intrauterine growth retardation

involves their ventricles as well. This is evident in the marked QT dispersion at basal electrocardiogram in these newborns, which might predispose to future adverse arrhythmic events [14]. As pioneer in this field, our research group had already demonstrated a similar alteration also in young adults who were born extremely preterm. In fact, their QT tract corrected for heart rate (QTc) has been found to be longer than in counterparts born at term [2]. This implies that the potentially arrhythmogenic condition described persists as time goes by. Taking into account all the above-described alterations, by practical point of view cardiologists should consider prematurity and/or intrauterine growth restriction as a novel risk factor for cardiovascular diseases even in adulthood.

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LECT 6

ADVANCED SIMULATION IN NEONATOLOGY, MARGIN NOTES ON A METICULOUS TAILORING JOB

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The Neonatal Unit is a battlefield where there are the most trained professionals, with the most advanced and aggressive therapies and techniques. However, it is also a place where blunt weapons are used, where we fight against antibiotic-resistant, selected microorganisms. The Neonatal Unit offers a setting to test teamwork, as well as the use of the guidelines, protocols and checklists. Variables such as the turnover of the staff on duty can cause latent mistakes. High-fidelity simulation plays a very important role both for extraordinary events and for ordinary ones. Technical skills must be used frequently in daily work as well as during simulation. Teamwork usually works, but it is necessary to repeatedly simulate stress situations during ordinary operations, or during extraordinary operations, where a high number of variables interplay. Guidelines suit the Neonatal Unit like a ready-made dress. The national and international scientific community validates them. In a different way, in every operative unit, protocols and checklists become refined tailor-made suits. Once a problem is identified, it is necessary to generate a simulation to study how to solve it. The simulation itself becomes an objective analysis of the problem, including the mechanism of its construction, and the characteristics that compose it, from the emotional sphere to the cognitive one. Anything that can be experienced in this way, as an approach of cognitive-formative training, can and should be thought to be subsequently shared and applied to the related centers. To achieve this, we prepare small groups of trainers mentally equipped to process the events. They are able to train their cooperators in the most efficient way,

through the creation of simulations, to face these events. The preparation goes beyond the classic criteria of the standard didactic, and it dresses a bespoke suit that is shaped itself on the models of the interactive communication and on an andragogical approach. The Train the Trainer path puts into effect the skills of the trainers that build the nodes of the Italian Simulation Network. A study group is dedicated to the “Clinical Risk and High Fidelity Simulation” to maintain its scientific orthodoxy. Advanced simulation, thought in this way, uses a new approach, which is closer to the operative realities and uses words of the daily communication. This new way to communicate is compatible with the new multimedia instruments available. This instrument also allows practicing simulation remotely, through the web, and it allows to follow someone that is simulating in streaming. Even debriefing and analysis benefit from the new technologies, which allow us to analyze them later. Research in the field of Humanities goes well with the most advanced technological research. The study of new simulators should be built around the needs of the operators, who should be able to test them. For example, in our center, bio-robotic engineers and neonatologists work together in this field of research. This research, funded by public and private entities, will produce transferable results in the practice of the daily care of the patient. If well planned and well managed, Advanced Simulation can therefore make the difference. This kind of approach lead us to redefine the importance of an equipped simulation center compared to another, more important element: an equipped mind, a *forma mentis* that allows trainers to educate professionals to operate efficiently at any level, without any limits of context restriction. This also shifts the idea of “equipped Center” to a different “equipped Mentality” for the Advanced Simulation in the Neonatology Unit.

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LECT 7

THORACIC ULTRASOUND IN NEONATOLOGY: PRESENT AND FUTURE

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Lung ultrasound has become a powerful tool for critical care. It has the advantages to be a quick, radiation-free, suitable, reproducible and noninvasive technique. In the last years a huge amount of literature has been produced demonstrating its clinical usefulness and clarifying its relatively simple semiology. Lung ultrasound has also a steep learning curve and this has facilitated its introduction. For its characteristics, lung ultrasound is particularly useful in critical care where decisions should be quick and in neonatal critical care, since patients are small, fragile and unstable. Our group has extensively studied the applications of lung ultrasound in neonatology and produced specific scores to monitor mechanical ventilation and the surfactant therapy. The diagnostic signs of meconium aspiration and other rare diseases have also been clarified. Finally, the impact of lung ultrasound in terms of radiation exposure has been analyzed. We will present these data and the basic ones regarding the use of lung ultrasound to diagnose RDS, transient tachypnea of the neonate and pneumothorax also giving an overview of the ultrasound semiology. Finally, new research lines will be presented and our lung ultrasound-training course in Paris will be advertised.

LECT 8

MAGNETIC RESONANCE IMAGING IN NEONATAL ASPHYXIA

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We want to define the role of the conventional magnetic resonance imaging (MRI) combined with advanced functional imaging diffusion weighted imaging (DWI), diffusion tensor imaging (DTI) and MR spectroscopy (MRS) in the diagnosis and the prognosis of hypoxic-ischemic encephalopathy (HIE), and in assessing the effectiveness of hypothermic treatment. HIE is due to brain hypoperfusion or prolonged hypoxia with an incidence of 1-6/1,000 live births; it is associated in the 10% of cases to severe disabilities, and in 25-50% of cases to learning disabilities. Brain damage and MRI alterations in HIE depend on the magnitude and duration of the insult and on the gestational age, in relation to the degree of maturation of the central nervous system (CNS) (**Tab. 1**). The most vulnerable areas of the brain are the most metabolically active, where synaptogenesis and neuronal activity are higher. In early hours of hypoxic-ischemic insult, anaerobic metabolism with reduced energy production, increase of lactate and tissue necrosis are present; six hours after the release of free radicals, the inflammatory response and the loss of self-regulation of cerebral blood flow lead to secondary damage with cell apoptosis and irreversible damage. Ultrasound (US) and conventional MRI combined with DWI-DTI-MRS are the main neuroimaging techniques used to study ischemic and post asphyxia brain damage in the neonatal age. US is the first-choice exam in neonatal CNS evaluation, especially in unstable patients, for cost, availability, absence of X-rays, repeatability, although its lower sensitivity and specificity compared to MRI. In preterm (24-36 weeks of gestational age) and term (37-41 weeks of gestational age) infants MRI-DWI-DTI-MRS show higher sensitivity in the detection of HIE lesions; the damage is well defined with greater anatomical detail by combined-MRI. The limits of MRI are the higher costs, the lower availability and the need of sedation. The use of MRI sequences, conventional MRI-advanced MRI, depends on the time of the lesion

Table 1 (LECT 8). Brain damage in preterm and term infants.

Preterm infants	Term infants
Postero-ventral-lateral nucleus of thalamus	Parasagittal region
Germinal matrix hemorrhage	Subcortical leukomalacia
Intraventricular hemorrhage	Cortical ischemia
	Mesencephalic ischemia
	Basal ganglia and thalamus ischemia

(**Fig. 1**). MRI findings evolve over several weeks; in the first day of injury conventional MRI findings are negative or minimal; conventional MRI performed about 3-4 weeks is useful in evaluation of outcomes. White matter is most vulnerable in preterms; the hypoxic-ischemic damage leads to periventricular leukoencephalopathy (PVL) with different MRI findings depending on the stage of the investigation and on the lesion's degree. In hyperacute and acute stages of PVL the sensitivity of US is poor, although it remains the first choice examination in critical patients. MRI can also be performed during subacute and chronic phases to evaluate the extent and degree of damage in stabilized patients. The combination of MRI with DTI, mean diffusivity (Dm) and fractional anisotropy (FA) allows to detect the microstructure of white matter; this can be useful in mild HIE, which is the most difficult form to identify with conventional MRI. HIE injury may be selective in term infants, with involvement of grey matter, or diffuse. MRI is

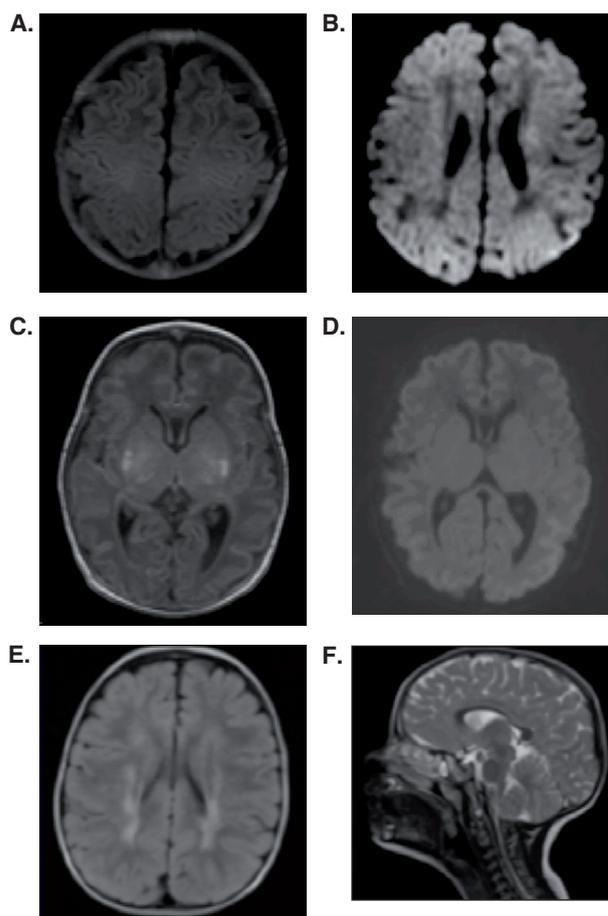


Figure 1 (LECT 8). Examples of MRI findings in neonatal asphyxia in different periods of life. **A-B.** Acute HIE left corona radiata in term infant on T1W and DWI. **C-D.** Another term infant with subacute HIE bilateral lenticular nucleus on T1W and DWI. **E-F.** PVL in a 4-year-old boy on FLAIR and T2W.

the first-choice examination, whereas conventional MRI combined with advanced imaging offers important prognostic information from the first days of life. Within 24 hours DWI is able to highlight the widespread damage and selective damage. A pseudo-normalization of DWI can be visible approximately 6-7 days after injury, when conventional MRI becomes positive. The severity and the extent of injury could be underestimated in the first 48 hours; MRI-DWI must be performed between the 2nd and 4th day after the damage; in fact, in that period the extent of the injury is well related with the final damage. MRS can also show high diagnostic sensitivity in the hyper acute stage, with the appearance of lactate peak, marker of anaerobic metabolism; DTI can provide valuable prognostic information in the early evolution of HIE. MRI evaluation is indicative within 3-4 days from the damage in untreated cases. Combined MRI should be performed within 4-5 days in patients treated with hypothermia. MRI combined with advanced techniques may provide valuable diagnostic and prognostic information. Moreover, advanced imaging DWI, MRS and DTI may increase the specificity and sensitivity in predicting the neurological outcome. US still remains the first-level examination in unstable preterm-term infants; combined MRI is the first level examination in stabilized newborns, although in the early stages (24-48 hours) it can underestimate the damage.

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LECT 9

INTRAOSSEOUS VASCULAR ACCESS IN PRETERM NEONATES

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During the resuscitation in patients in cardiac arrest, severe state of shock, and in all situations where the infusion of fluid or administration of drugs are essential for the success of therapy, adequate vascular access is of paramount importance. In these situations, it is often difficult to obtain a peripheral vein and the attempts of a central vein cannulation can be risky and too time-consuming, as well as forcing the interruption of resuscitation. Intraosseous infusion (IO) is a fast access to the venous circulation, it is easy to perform and effective, and it was already used in children in the 1920s and spread to the end of the 40s, when peripheral venous cannulas have started to be used. In the 1980s, after the publication of numerous clinical reports of its effective use in children, the practice of IO increased. A particular and common clinical context relates to preterm children who, despite having already placed a small caliber (1 Fr) peripherally inserted central catheter (PICC), require rapid infusions of medication or fluids for cardiopulmonary resuscitation or blood products that, by their nature, tend to occlude the lumen of catheter. The IO access is a rapid and safe method to obtain vascular access and it is successfully used in critically ill adults and children, but little is known in neonatology, especially in premature babies. Nevertheless, in these critical patients, accessing a rapid and stable conventional vascular access in emergency situations could be challenging. We present two cases of extremely

premature newborn babies in whom conventional venous access had failed and who underwent the positioning of a successful intraosseous line. In both cases previously the following contraindications have been excluded: fractures, osteogenesis imperfecta, previous attempt made in the same bone. No major complications were observed. M.F. a male preterm with gestational age of 24⁺² weeks, was born after a natural childbirth for preterm labor, birth weight 645 g (56th percentile); the Apgar scores recorded at 1, 5 and 10 minutes were 2, 5 and 7 respectively. The patient was intubated at birth and he received one dose of surfactant therapy because of severe RDS. In the first day of life an umbilical venous catheter was placed; it was removed on the fifth day of life and soon after a 1Fr PICC was positioned. At the age of 24 days his Hb was 8 g/dl and he needed an urgent blood transfusion, but several attempts to find a conventional venous access failed. For that reason, an intraosseous access (butterfly needle, 25 G, 10 mm) was inserted under aseptic conditions at the upper right tibial level. Correct placement was confirmed by aspiration of bone marrow and it was removed after the transfusion (approximately 12 hours) without any complications. P.M., a girl, was born at 23⁺¹ weeks of gestation by vaginal delivery with weight 510 g (39th percentile). The 1, 5 and 10 min Apgar scores were 1, 4 and 6 respectively. She was resuscitated and intubated at birth and she received ventilatory support and surfactant therapy (one dose). At two weeks of life her general condition worsened and she needed a blood transfusion (Hb 10.9 g/dl); at that time, she had a 1Fr PICC which could not be used for the administration of blood. After several attempts to find an alternative vascular access an IO access line was placed (butterfly needle Pic Venogliss, 25 G, 10 mm) in the left upper right tibia level, but in few minutes it was blocked due to an attempt to obtain blood sampling and withdrawn; soon after another access was successfully positioned in the right tibia level just below the tibia superior tuberosity and used to infuse blood (**Fig. 1**); it was left in place for 24 hours, then after that it was dislocated and then removed. Battery-powered intraosseous driver and needle sets are available only to children weighing more than three kilos. Other types of needles may be used; these include bone marrow needles, styletted needles, and spinal needles. Manual IO needles are less expensive and are more widely available than other devices. Observational studies suggest that the success rates for manual placement in infants is high, and the technique can be easily learned by a variety of health care providers. Although IO cannulation by manual



Figure 1 (LECT 9). Intraosseous line *in situ*.

insertion becomes progressively more difficult as children age, because the cortex of bone becomes thicker and the tibia bone marrow cavity becomes smaller, in preterm babies it is relatively easy and it is a safe alternative, which may be lifesaving in an emergency situation when other methods fail. Although standard hypodermic needles are generally not recommended, since they can become clogged with bone and bone marrow, we have used them without problems. In compromised neonates, or when intravenous access is impossible, intraosseous infusion is quick, safe, and effective; immediate vascular access can be safely and rapidly established through intraosseous lines. However, they must be replaced with intravenous lines as soon as the infant stabilizes. We also suggest that in every delivery room and neonatal intensive care unit the emergency set be equipped with intraosseous needles.

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LECT 10

THE EARLY GUT COLONIZATION IN NEWBORNS

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Although it has been generally assumed that newborns are born germ free and that initial gut colonization occurs during birth, more recent studies suggest that fetal colonization begins prior to birth. Besides a possible prenatal transfer of maternal bacteria to fetus, other major determinants for neonatal gut colonization are mode of delivery, mode of feeding and perinatal antibiotic exposure. Several studies have shown effects of delivery mode on the gut microbiota composition of newborns. Generally, vaginally born infants are first colonized by bacteria from the maternal vagina, mainly characterized by a prevalence of *Prevotella*, *Sneathia*, and *Lactobacillus* genera, also including bacteria present in the maternal gut, while the gut microbiota of infants born by Cesarean (C)-section more often resembles maternal skin and oral microbiota, with a prevalence of *Propionibacterium spp.*, *Corynebacterium spp.*, and *Streptococcus spp.* Moreover, infants born by C-section have delayed colonization of *Bacteroides spp.*, and lower microbial diversity throughout the first 2 years of life. In a previous study, we also have evaluated the relation between intestinal ecosystem of the newborn and mode of delivery by means of a molecular biology approach, collecting fecal samples on day 3 of life in 23 infants born vaginally and in 23 infants delivered by C-section. The intestinal microbiota of neonates delivered by C-section actually appeared to be less diverse, in terms of bacteria species, than the microbiota of vaginally

delivered infants, being characterized by an absence of *Bifidobacteria spp.* Conversely, vaginally delivered neonates, although showing individual microbial profiles, were characterized by predominant groups such as *B. longum* and *B. catenulatum*. However, the effects on species diversity between different modes of delivery are reported to progressively disappear by the first year of life, when infant microbiome becomes more similar to the maternal one. It has been suggested that the early gut colonization may have long-term medical consequences: indeed, C-section delivered babies seems to display higher incidence of celiac disease, obesity and asthma, with some implications on the maturation of the immune system, in terms of lower blood levels of T-helper cell-related chemokines, possibly due to the reduced gut colonization of *Bacteroides* genus. Perinatal antibiotic exposure is another major determinant of early gut microbial composition in newborns. Thanks to new molecular techniques currently available, we now have proof of antibiotic-induced intestinal dysbiosis, in turn associated with intestinal and plasma lipid profile alterations. Several studies have also demonstrated that antibiotic exposure in early infancy is associated with increased risk of developing overweight/obesity, as well as asthma, wheezing and inflammatory bowel disease later in life. There has been accumulating evidence that intestinal microbiota play a key role in modulating the cross-talk between brain and gut (the so called “brain-gut-enteric microbiota axis”), by means of the synthesis of many neuroactive molecules such as serotonin, melatonin, adrenaline, dopamine, acetylcholine and GABA. Finally, mode of feeding also plays an important role in influencing early intestinal microbiota. Breastfeeding is undoubtedly the best way to promote the healthy development of human offspring as it is considered to be the optimal source for all the nutritional and functional factors that infants need. Several studies have recently proven that human milk is not sterile and it is the predominant source for establishing a “healthy microbiome” in the newborn. Milk microbial composition changes over the lactation period, being colostrum predominantly colonized by *Staphylococci spp.*, *Streptococci spp.*, and *Lactococci spp.*, whereas milk samples collected later on harbor oral cavity related bacteria, perhaps due to frequent interaction with the infant’s oral microbiota. The milk microbial colonization is likely derived from mother’s gut and the elevation of progesterone levels seems to be a major cause for increasing gut permeability, thus facilitating the bacterial passage to the bloodstream and then to mammary glands. The infant’s early gut colonization is therefore modulated both by human

milk microbiome and by other unique nutritional components of human milk, such as oligosaccharides and lactoferrin, also known as prebiotic or bifidogenic factors. As a consequence, there are significant differences in the gut microbiota composition of breast-fed versus formula-fed infants. Several studies have pointed out that Bifidobacteria are the most abundant organisms in breast-fed infant guts, whereas the gut microbiota of formula-fed infants is dominated by *Enterococci spp.* and *Clostridia spp.*, with more species diversity. In conclusion, although the complex interaction between host and intestinal microbiota is not fully clarified yet, neonatal early gut microbial colonization seems to be a crucial step at a critical age for modulating infant's healthy immunological, hormonal and metabolic development.

LECT 11

MICROBIOTA AND PROBIOTICS

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It has been recently estimated that the human body contains 4×10^{13} colonizing bacteria and 3×10^{13} own cells, a ratio roughly equal one to one. The collection of microorganisms coexisting in peacefully with their human host is defined microbiota. The microbiota colonizes virtually every surface of the human body that is exposed to the external environment. Gut microbiota is the complex community of microorganisms that lives in the digestive tract. The human intestinal microbiota contains more than 10^{13} microbes subdivided into different species of bacteria, with large inter-individual variability. Although more than 50 bacterial phyla have been described to date, only two are prevailing: the Bacteroidetes and the Firmicutes. Most of the bacteria reside in the lower part of the digestive system, especially in the large intestine. In fact, in the proximal tract the gastric juice, bile and pancreatic secretions are toxic or not favorable for the growth of most microorganisms. After birth, the composition of microbiota of newborns is initially constituted by Bacteroidetes derived from the maternal microbiota colonizing the urogenital tract and the skin, and also by other environmental factors, such as diet (formula versus breastfeeding). The mode of delivery and subsequent environmental exposures also influence the composition of microbiota in the infant. After the initial establishment of the intestinal microbiota and

during the first year of life, the microbial composition of the mammalian gut is relatively simple and varies widely between different individuals. After the first year of age the intestinal microbiota undergoes a second transformation until it is fairly stabilized in its composition, which is very similar to that of the adult. The constant interaction between the host and the resident microbes influences the health of the host, with a combination of bacterial species that favors non-pathogenic symbiotics. The probiotics with prebiotics, symbiotics and postbiotics are considered biomodulators of the intestinal microbiota. It has been suggested that the increase of some bacterial species of intestinal microbiota considered "favorable" to the health of the organism, such as *Bifidobacterium spp.* and *Lactobacillus spp.*, is correlated with a reduction in the incidence and severity of different disorders of the gastrointestinal tract. Probiotics are defined as live non-pathogenic microorganisms (bacteria or yeasts) that, when administered in adequate amounts, can replicate and colonize the gastrointestinal tract in sufficient numbers and may confer health benefits of the host. Microorganisms, in order to be defined as probiotic, have to be of human origin, resist to the gastric acid pH, the bile and survive in the gastrointestinal tract by adhering to the intestinal mucosa. They have to be able to replicate into the gastrointestinal tract and must be tolerated by the intestinal immune system; in addition, they have to have beneficial effects on health, antagonizing pathogenic microorganisms and producing antimicrobial molecules. The therapeutic efficacy of probiotics has been evaluated in randomized controlled trials for various gastrointestinal diseases in both children and adults. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) has validated the effectiveness of *L. rhamnosus* GG and *S. boulardii* in acute diarrhea. A 2011 Cochrane review acknowledged to the *L. rhamnosus* GG and *S. boulardii* a preventive role in diarrhea associated with antibiotic therapy. A Cochrane review of 2013 recognized the ability of probiotics to prevent diarrhea caused by *C. difficile*, but not in reducing its incidence. RCTs and meta-analyses have shown effectiveness of *L. rhamnosus* GG in reducing the frequency and severity of abdominal pain in children with irritable bowel syndrome. The probiotic VSL#3 has shown efficacy in the induction and maintenance of remission of ulcerative colitis. A 2014 Cochrane review concluded that in preterm infants supplementation with probiotics prevents severe forms of NEC, although there was no conclusive evidence on the strain and on

the optimal dose. A recent meta-analysis concluded that probiotic supplementation reduces the risk of late onset sepsis in preterm infants. According to the guidelines of the WAO (World Allergy Organization) produced with GRADE methodology, probiotics are effective in the prevention of atopic dermatitis. The effectiveness of a probiotic treatment is affected by many factors including: bacterial strain, duration of administration, disease and age. Not all products marketed as probiotics provide the same safety and efficacy. In conclusion, since there is insufficient data with regard to the benefits and potential adverse effects of probiotics, comparative studies are mandatory to assess the most effective formulations, timing and the optimal length of therapy.

LECT 12

ORGANIZATION, TRAINING AND CHARACTERISTICS OF COTTOLENGO VOLUNTEERING

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Our volunteering association has been helping the Little House of Divine Providence, also briefly called Cottolengo, substantially since 1997. In that year, a spontaneous group of volunteers was formed to provide professional support to the Mission Cottolengo Chaaria (Kenya), where some religious brothers and sisters of Little House of Cottolengo had been working for a long time. In 2004, this spontaneous group of about 15 people obtained legal recognition as Non-profit Association. Currently, our group is composed of 280 volunteers (**Tab. 1**). We are inspired by St. Cottolengo's motto: *Caritas Christi urget nos*. He urged us to focus on helping the poor with enthusiasm, trusting Providence to achieve extraordinary results. Basically, putting his words into practice means being able to see the needs of others; avoiding vainglory;

Table 1 (LECT 12). Composition of the Non-profit Association of volunteers that provide professional support to the Mission Cottolengo Chaaria (Kenya).

Profession	Age				Total	%
	30-40	40-50	50-60	> 60		
Company administrator		1	2	1	4	1.43
Anatomical pathologist		2			2	0.71
Anesthetist	8	1		1	10	3.57
Lawyer		3			3	1.07
Biologist	3	1		2	6	2.14
Surgeon	8	5	8	5	26	9.29
Business consultant		2			2	0.71
Gastroenterologist	1				1	0.36
Physiotherapist	8		1	1	10	3.57
Geriatrician		1	1		2	0.71
Gynecologist	4	1		1	6	2.14
Journalist		1			1	0.36
Office worker	5	2	4	1	12	4.29
Nurse	41	9	2		52	18.57
Infectious diseases specialist				2	2	0.71
Engineer	1		1	1	3	1.07
Teacher	1	1		1	3	1.07
Internist	13	2	4	2	21	7.50
Medical jobs	1		1		2	0.71
General Medicine specialist	1		1		2	0.71
Radiotherapy specialist				1	1	0.36
Odontologist	9	1	1	1	12	4.29
Midwife	11				11	3.93
Healthcare worker	3		1		4	1.43
Pediatrician	3	1		1	5	1.79
Pensioner			3	10	13	4.64
Pulmonologist	2				2	0.71
Psychiatrist		1		1	2	0.71
Psychologist	1				1	0.36
Urologist				1	1	0.36
Various	32	9	8	9	58	20.71
Total	156	44	38	42	280	100
%	55.71	15.71	13.57	15.00	100.00	

solving difficulties; offering solutions; respecting different cultures; pursuing continuous training; thinking before acting. In general, a volunteer must have the following features: ability to work in teams, determination, competence, lucidity and balance, transparency, concreteness. In particular, a volunteer association must always consider three aspects: the goal, the result and its own characteristics, that are the human and professional characteristics of the group of volunteers. Every action becomes effective if supported by a serious training to understand our origins, the context in which the volunteers will work and the kind of help needed. Goals are achieved with discipline, method and humility.

LECT 13

HEALTH SITUATION AND EPIDEMIOLOGY IN THE COTTOLENGO'S MISSIONS

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The Cottolengo's missions are widespread in Europe, Africa, Asia and America. The focus of this presentation is the health condition of Kenya. Kenya is an African country that is in a phase of economic development resulting in improved health conditions of the population. In fact, by the late 1980s Kenya had more than quadrupled the number of health facilities serving its growing population, it had extended life expectancy from 40 years to 62 years and improved child survival rates. But despite this, there are still many health problems, particularly affecting people living in rural areas where poor and dirty roads, rainy seasons, great distances to health centers, paid health services are barriers to health. Furthermore, services in rural areas are provided by health centers and dispensing clinics, though these often lack facilities and trained staff. Kenya is classified by United Nations as a chronically water-scarce country, resulting in illnesses caused by intake of contaminated water and worsening health conditions especially in children under 5 years. Major risk factors for health are: sexually transmitted diseases (29.7%); contaminated water and lack of sewage system and sanitation (5.3%); inadequate breastfeeding (4.1%); malnutrition and underweight (3.5%); environmental pollution in dwellings (3.2%); alcoholism (2.6%); lack of vitamin A (2.1%); diabetes (1.8%); hypertension (1.6%). In Kenya the leading cause of mortality are communicable diseases with maternal, prenatal and nutritional conditions. In fact

the main causes of death are: HIV/AIDS (15%), lower respiratory infections (12.3%), diarrheal diseases (6.3%), protein-energy malnutrition (4.1%), birth asphyxia and birth trauma 4%), stroke (4%), preterm birth complication (3.7%), malaria (3.2%), tuberculosis (2.5%), ischemic heart disease (2.5%). In particular the main causes of death in children under 5 years are acute respiratory infections, birth asphyxia, prematurity, diarrhea, injuries, neonatal sepsis, congenital anomalies, HIV/AIDS, malaria and measles. Infant mortality rate is 39/1,000 live births. HIV in Kenya is an epidemic. The number of people living with HIV was 1.5 million people in 2015. In the same year roughly 36,000 people died from AIDS-related illnesses. There are now 660,000 children orphaned by AIDS (aged by 0 to 17 years). Children aged 0 to 14 living with HIV are 98,000. Reported confirmed cases of malaria in 2015 were 2,808,931, with 472 deaths. Maternal mortality ratio is still elevated (510:100,000). Tuberculosis is considered as one of the three pandemics of low-and middle-income countries. The prevalence of tuberculosis is 223 per 100,000 populations. Kenya has adopted a number of strategies to fight against these problems, also with the help of international organisms. However, there are still many interventions that could be implemented to promote substantive public health advances, particular to prevent, detect and respond communicable diseases. In this situation, The Little House of Divine Providence, through the Kenya Cottolengo Society, tries to help the poor with some activities: centers for children affected by HIV and disabilities, health centers and an hospital. The mission of these centers is to take care of poor people in all the dimensions (physical and spiritual) with a spirit of family to promote the dignity of every people (**Fig. 1**).



Figure 1 (LECT 13). Mukothima, 30 minutes by car distance from the Mission, a daily work of a Mobile Clinic, where children are weighed and vaccinated.

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LECT 14

MATERNAL AND CHILD SERVICES AND CARING NEEDS

I. Farnetani

L.U.de.S. H.E.I., Malta

In this contribution I describe the situation of children and adolescents in rural areas of the Republic of Kenya, by analyzing aspects of the missions of the sisters of Saint Joseph Benedict Cottolengo as a point of observation and analysis. The objective is to improve the services provided in the missions to achieve greater health promotion. The sanitary situation in Kenya presents health risks and macroscopic deficiencies which are not comparable to the reality in the Northern hemisphere of the world. In Kenya, diagnostic, therapeutic or pharmacological resources that come from the most industrialized countries are often unused or poorly used and allocated, often without a rational and scientific organization and distribution. It is urgent to activate mechanisms of help and international cooperation, giving priority to local staff training, with the objective to rationalize and optimize available resources. Our observations and proposals are schematized in ten points: the first five represent health emergencies; organizational gaps are reported from point six to point eight; while environmental problems are listed in the last two points.

HEALTH EMERGENCIES

1. Pregnancy

Besides complications of pregnancy, pregnant women in Kenya face additional risk factors. In fact, they continue to perform heavy work, especially in the fields and in the domestic environment. In addition, they often live far from medical centers, so they cannot be subjected to periodic inspections and investigations. This is also an issue at the time of labor: some deliveries take place at home, and in other cases (**Fig. 1**) pregnant women must walk many kilometers on foot to get to the medical center. Pregnant women with greater economic means can afford to reach medical centers by a motorcycle taxi, which is a cross bike, and travel very bumpy roads.

2. Childbirth

Childbirth often happens in an unprotected, promiscuous environment, without assistance of obstetric specialists. In many cases, women tend to stay at home as much as possible or to return home as soon as the birth took place, even against the advice of health professionals.

3. Neonatal period

This is one of the most critical issues, because there is a lack of neonatologists and critical preterm babies at birth often do not have the possibility to be immediately stabilized or receive the normal neonatal intensive cares in the immediate hours and days after birth, despite the availability of suitable equipment such as incubators or respirators. The picture described in the previous three points determines high rates of stillbirth, neonatal suffering and infant mortality. Of the three issues described,



Figure 1 (LECT 14). A SUV was provisionally transformed into ambulance to transport a woman who needed a caesarean section. The hospital was located at a distance of two-hour drive. The delivery took place during the journey. On the right, the newborn.

this one is the most neglected and facing the heaviest shortage of medical and nursing care, either because the needs of the newborn are placed after those of the parents, or because there is a lack of perception of neonatal health risks.

4. Endemic malaria

60% of infectious diseases contracted by children younger than six years of age are represented by malaria, which is often contracted repeatedly, causing serious complications.

5. Sexually transmitted diseases

AIDS represents the main type of sexually transmitted diseases. It is noted that there are numerous cases of congenital infections. This situation is aggravated by the lack of prophylaxis.

ORGANIZATIONAL CRITICALITIES

6. Training

In the various missions and hospitals people are animated by good will, aptitude for study and compassion, but they lack specific knowledge, either from the point of view of organizational management, or professional services.

7. Medical and paramedical staff

One of the greatest deficiencies existing in Kenyan health facilities is the availability of doctors. In fact, treatments are assigned, often for a few hours per week, to clinical officers, operators with a reduced academic course, and sometimes even to nursing staff. This leads to an inevitable reduction of the level of assistance provided. This model of care organization explains the improper consumption of drugs and the large number of diagnostic tests. Because of the uncertainty of the diagnosis, there is a significant use of diagnostic laboratory tests, or an excessive use of drugs, which are sometimes not given in an appropriate and targeted way. For example, groups of drugs are administered to have a wider coverage, or drugs, parapharmaceutical and childcare products are sometimes used without justification or scientific indication. Another fact to be taken into account is also the trend of clinical officers and nurses to leave their work in the missions in the beginning of their profession and to move later to more central locations, where they can earn higher profits.

8. Lack of information

Even if information material is available in medical centers, popular beliefs often prevail. This situation determines deficiencies in health care, such as, for example, nutritional errors or lack of prevention.

ENVIRONMENTAL DIFFICULTIES

9. Distance

The distance of housing from medical centers causes a lower use of health services with the risk of lack of

prevention, follow-up, late diagnosis or inadequate or insufficient care.

10. Transport difficulties

Even large distances are travelled by foot. Concerning the use of motorcycle taxi, people use it sporadically because of the cost. Travelling is even more difficult during the rainy season, when the roads become impracticable in many cases. Therefore, the result is that the population often reaches health centers when travelling is required for other needs, such as reaching the market.

CONCLUSIONS

Each of the ten points presented above requires one single resolution. In general, the priorities are, first of all, the training of personnel involved at birth (steps 2 and 3). It is therefore essential to collaborate with the Universities for neonatologists to reach Kenya and train local personnel. It is important to urgently check malaria prophylactics (point 4), to ensure that every family is provided with suitable and sufficient mosquito nets, as well as to make available the most effective antimalarial drugs with fewer side effects. To compensate environmental difficulties characterized by houses scattered in the territory, described in points 1, 9, 10, mobile clinic services should be improved. These are carried out by periodically sending medical staff in the most distant and deprived areas, to perform clinical visits, diagnostic tests and vaccinations to people that otherwise could not access to health services. In the context of a better health care organization, it is also useful also to make the pharmaceutical service and the analysis laboratory more efficient and organized.

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LECT 15

GIVING BIRTH IN RURAL AFRICA: CHALLENGES AND ADVANCES IN A REFERRAL SITE

G. Gaido

Cottolengo Mission Hospital, Chaaria, Meru, Kenya

At Chaaria Mission Hospital in Meru, Kenya, we started up from a dispensary in 1998, without any inpatient facilities, and through the years with the help of many volunteers and donors we managed to gradually build up a hospital. Chaaria hospital is still growing larger in size and capacities, with a current inpatient capacity of > 180 beds and about 300 to 400 outpatients per day. Since we represent one of the main hospitals within a quite large rural area, we have been taking care of an increasing number of severely ill patients over the years. Our patients often need urgent care, including pregnancy complications, vaginal and cesarean deliveries, and maternal and neonatal conditions. These main indications keep our medical and nursing staff busy for the majority of the day, in addition to trauma and severe infections. On average, at the beginning we started recording 400 deliveries/year and we have been recording > 1,200 deliveries per year for the last 10 years. Over half of the deliveries (approximately 700 cases/year) need cesarean section. As we cover a quite large area, a substantial proportion of cases come to our attention already with complications, which are often related to long travel in uncomfortable conditions of transportation, including walking long distances, sometimes requiring days of travel. We also represent the only facility with a functioning surgery theatre within a wide area, therefore we often receive a large amount of complicated labors referred to us by many rural maternity units not equipped for more advanced surgery. Despite > 1,200 deliveries per year, we have recorded only 6 maternal deaths since 1998 (**Tab. 1**); in particular we had no maternal deaths over the last 5 years. Starting as early as in 1998, we set up a delivery room – which we separated and isolated from the surgery room. However, the initial conditions were still unsatisfactory, as we had to use the delivery room also to visit and examine female outpatients for diagnostic purposes, and this of course did not favor an optimal microbiologic isolation. Since 2012, we managed to build a proper and modern surgery theatre, addressing the most stringent

and up to date sterilization requirements: these also included a separate path to surgery in order to prevent any infectious disease transmission. In the same year, we created a new maternal unit with delivery room in aseptic conditions, where we have now three adjustable mechanic delivery stretchers, four incubators, oxygen access, and trail for neonatal resuscitation. There are separate departments for antenatal and post-natal patients; there is also a sterile nursery with 4 incubators for preterm babies. Finally, we also have a consultation room for new admissions, to keep them in a separate site from the delivery room. Over the years we have been able to observe different diseases and complications, often related to maternal or neonatal and infant periods. Amongst delivery complications, we list cephalopelvic disproportion, specifically in teenager mothers, generally due to immature pelvic bones; obstructed labor, due most often to the previously quoted condition and to the fact that the very young mother may have tried to deliver at home. Delivering at home may include several hours of painful labor, rupture of the uterus due to cephalopelvic disproportion and a long labor time, often with the support of traditional birth attendants, not well trained and too keen to perform fundal pressure (sitting or pressing on the uterine fundus, with the goal to trigger uterine contractions, favor the descent of the presenting part and facilitate delivery). We also observed vesicovaginal fistula, which fortunately are in steady decline (at Chaaria Hospital in < 0.5% of our patients). Approximately 50% of our young mothers are circumcised: Muslim mothers are infibulated with Female Genital Mutilation (FGM) type 1, whereas non-Muslims have a FGM type 2 with excision of the clitoris and labia minora. This, together with the pelvis not fully developed at a younger age,

Table 1 (LECT 15). Maternal death in Chaaria since 1998.

Year of occurrence	Age	Estimated week of pregnancy	Reason for death
1998	22	40	Eclampsia
2000	18	39	Post partum hemorrhage
2001	18	42	Rupture of spleen
2003	23	42	Rupture of uterus
2004	20	40	Rupture of uterus
2006	24	38	Respiratory arrest after spinal anesthesia in C/S

Table 2 (LECT 15). Evolution of HIV prevention of mother to child transmission (PMTCT).

Therapeutic schema	Mother (dose)	Child (dose)	Year
Nevirapine alone	200 mg – single dose Before delivery	5 mg/kg at birth o.d. for 2 weeks	1998-2007
Dual/Triple ART	AZT 300 mg + 3TC 150 mg from week 14 and until 1 week after delivery <i>and</i> one additional dose of AZT 600 mg + 3TC 200 mg + Nevirapine 200 mg (beginning of labor)	5 mg/kg Nevirapine o.d. until the completion of breastfeeding	2007-2012
Triple ART	TDF, 3TC, Efavirenz From beginning of pregnancy, irrespective of CD4 count or WHO staging. Trimetoprim + Sulfametoaxazolo for PCP prevention. Lifelong ART	5 mg/kg Nevirapine o.d. until week 12 of age, if mother in ART. Then PCR and serology controls	2012-ongoing

ART: (maternal) antiretroviral treatment; AZT: azidothymidine; 3TC: lamivudine; TDF: tenofovir disoproxil fumarate; PCP: *P. carinii* pneumonia.

leads to a very high rate of perineal lacerations, sometimes very serious (3rd degree ones), where there may be involvement of the anal sphincter, with rectovaginal fistula. The fetal mortality rate remains still much higher than maternal one, the main cause being fetal distress from prolonged labor, and meconium induced pneumonia. Thanks to a long-standing action of counseling and prevention, the HIV prevalence is currently down to < 2% in our antenatal clinic, where we test 100% of our women. Once we identify serum-positive pregnant women, we offer them triple therapy with anti-retroviral treatment, starting after the 1st trimester. The maternal antiretroviral treatment (ART), together with perinatal ART to the babies and strict rules about exclusive breastfeeding for 6 months only, have greatly contributed to prevention of mother to child transmission of HIV. Back in 1998, we had a mother to child HIV transmission rate of 15%, reflecting elective cesarean section only, without ART prophylaxis. Since the year 2000, vertical HIV transmission started to decline, as we began administering nevirapine single dose peripartum to the mothers and as neonatal prophylaxis. This was made possible through the Esther project supported also by the Italian Ministry of Health (**Tab. 2**). Since 2007, WHO started recommending dual anti-HIV treatment therapy to infected mothers, starting from the 28th gestational week – this helped reduce substantially the vertical transmission rate to approximately 2%. The downside of both approaches was that the regimens were potentially triggering resistance, when later on the woman would start proper ART. Currently we have no cases of vertical transmission out of 55 infected mothers since 2014 (**Tab. 3**), in keeping with the fact that all the seropositive newborns were already PCR negative at birth and remained negative at 6 months.

Table 3 (LECT 15). Data from seropositive newborns (years 2014-ongoing).

Year	Seropositive women (antenatal clinic)	Maternal start of ART (trimester)	Child PCR positive at 6 weeks
2014	24	second	0
2015	22	second	0
2016	9	second	0

We test infants at 6 weeks of age by PCR, in case of positive result we start ART (antiretroviral treatment), if negative we perform serology testing at 9 months – if the latter is negative, we consider the baby to be negative for HIV. In case of positive serology at 9 months, we repeat the PCR at 18 months.

LECT 16

COTTOLENGO MISSION HOSPITAL CHAARIA (KENYA): BORN IN A BUSH HOSPITAL

R. Cavallini

Sardinian Volunteers Group Karibu Africa, Cagliari, Italy

The Cottolengo Mission Hospital Chaaria belongs to the Congregation of the Holy Giuseppe Cottolengo Brothers. In support of this small but important bush hospital, an association of physician and nurses, named “Gruppo Volontari Sardi Karibu Africa”, was founded in Cagliari. Geographically this hospital is located about 400 km North of Nairobi and serves an extensive area that includes the districts of Chaaria, Tharaca, Meru Central, Meru North and Isiolo. Mothers and newborns occupy a prominent place in the admissions and in the care delivered by the staff at this hospital. Next to the old operating theatre, a new and modern operating room was inaugurated in 2014, allowing the performance of any surgical operation, including

many caesarean sections, under secure conditions. In 2015 a new and very comfortable Maternity ward was inaugurated for mothers and newborns with delivery rooms (**Tab. 1**). There are neonatal beds and incubators. Nurses are very kind and valid and encourage the kangaroo care. Breastfeeding with maternal milk is favored for all infants including premature infants. To better assist the newborns after delivery, there are two “neonatal islands”: they are illuminated and heated, equipped with oxygen, neonatal suction machine, bag-valve-mask, laryngoscope, endotracheal tubes and all instruments for good and proper care and neonatal resuscitation (**Fig. 1**). A respiratory support on mechanical ventilation is not possible now, but maybe will be available in the future. Phototherapy is available. Mothers of premature infants receive at last one dose of antenatal steroids. The main causes of neonatal deaths are the following: premature births, perinatal asphyxia and meconium aspiration syndrome. The causes of so many cesarean sections are often due to a history of previous caesareans. Very often the women arrive exhausted after a long journey on foots from their huts or rural maternity centers devoid of surgical services, often after attempting to give birth at home. The pediatric clinic follow-up, the

Table 1 (LECT 16). Chaaria’s Maternity department (Kenya): data of 2015.

Total births number	1,400
Spontaneous births	700
Caesarean sections	700
Neonatal deaths	45 (3.2% ^a)

^a7.7% in Kenya.



Figure 1 (LECT 16). Chaaria’s Maternity (Kenya): a voluntary nurse helps the mother of a premature infant (30 weeks).

services for immunization, the outpatient’s department for prevention and treatment of TBC and HIV with prenatal and postnatal check-ups and laboratory tests, for the prevention of cervical cancer and for the prevention and screening for diabetes are all important. An ultrasound service and an efficient laboratory complete this hospital. The service of care of HIV and TBC also operate with “mobile clinics” in the villages for the education, prevention, diagnosis and therapy offering examinations, medicines and food for free. This hospital is open day and night, always ready and willing to welcome the poor and needy people of every ethnic group and religion.

LECT 17

DENTISTRY IN KENYA, THE STORY OF CHAARIA

G. Farnese

Volunteers Association, Cottolengo Missions, Turin, Italy

The aim of this presentation is to describe the benefits of volunteer work in the Chaaria Mission of the Little House of the Divine Providence (Cottolengo) to the population of Meru, a dramatically poor region in equator zone. This region is plagued by drought, famine and very bad general hygienic conditions. The general situation in worsened by the lack of affordable medical facilities and the absence of transportations: patients often have to walk very long distances, which would be unthinkable in the Europeans context and therefore many patients cannot reach medical structures. Equator zone is characterized by high incidence and prevalence of dental decays, due to bad general hygienic conditions, lack of medical culture and sugar cane diffusion, which is highly cariogenic. The absence of structures for dental care makes tooth extraction the main therapy for dental decays. This determines a high percentage of mutilated occlusions since childhood. I still remember when during my first voluntary work experience in Chaaria I first met Philip, who at the time was 14. I treated 23 decays in his mouth and had to reluctantly extract 11 dental roots. It took me about 10 sessions to relief his pain. At the end of my work, I had actually maimed his dentition. Dentistry service in Chaaria was born in the 1980s. At the beginning it started as a sort of pharmacy and afterward, in 1998, the work of the volunteers transformed it into a real dental office that worked as an emergency room. In 1998, dental volunteers began to teach dental prevention among the children

of the local schools. In 2011, Cottolengo Mission Hospital Chaaria Non-profit Association financed the renovation of the office that today is arranged with two dental chairs, a sterilization room and local staff. This evolution made it possible to reduce the percentage of extractions from the initial 98% to 45%, when working with Italian volunteer dentists. Chaaria dental office provides at present around 30 restorative treatments every day. The work of the mission has allowed to improve dental conditions of local young and adult population through primary and secondary prevention and to dramatically reduce the incidence of mutilated dentitions in youth. Today, people in the Meru region are aware of the importance of dental prevention: patients do know that teeth can (and have to) be cured with home and professional hygiene procedures and dental conservative treatments, and do know that teeth extractions can often be avoided. The success of Chaaria project encouraged the Little House of the God Providence to create a second, similar institution in an even warmer, drier and poorer region: Gatunga. To make Gatunga a new Chaaria, the help of many people will be necessary. Today in Chaaria and tomorrow in Gatunga the help of skilled and willing volunteers will be fundamental.

LECT 18

VOLUNTEERS NURSES IN CHAARIA

S. Ferrante

Niguarda Ca' Granda Hospital, Milan, Italy

I am a nurse working in Neurointensive Care Unit, Niguarda Ca' Granda Hospital in Milan. I presented my master's dissertation about the role of nurses in international cooperation, conducting a research on what contribution can nurses bring in developing countries with several voluntary associations working around the world. I discovered what being a volunteer means: understanding the culture of the country of destination; training local people to do their work independently and with their own resources (which can be achieved only in time and not immediately); following the management of the hospital and those who manage the association, because they know what is the best way to work in that context. Being a good volunteer is not easy because there are different and unexpected difficulties coming between ideals, as it is all very different from here, and to our eyes it seems that everything is missing. At the beginning we are full of initiatives and proposals that actually seem impossible

to achieve. We think we have to do so much, but it seems to have done nothing instead. Sometimes we find us complaining and make criticisms that prevent us from living a good experience, even for those who are around us and it is not useful to anyone. You have to think that local staff learn to do things according to their own criteria, which are different from ours and that they were born and grew up in a different place than ours. At first it is important to have confidence in those who know the place. It is normal that the houses are made of mud, that there is not the water and the bathroom inside, that children grow up without shoes and that men still work with their arms. For example, the sewers are open channels and this obviously creates unbearable smell, high degree of dirt, and spread of diseases. It can also happen to encounter animals not living in Italy, sometimes even tarantulas and snakes (e.g. black and green mamba). Working in the hospital of Chaaria is hard because unfortunately there is not yet a real organization of volunteers, so volunteers are expected to work with strangers in an unknown environment without a true coaching. This creates confusion and disorientation. Local nurses work very differently from us. They follow the Anglo-Saxon model, that is based more on management than on practice, and they have a training course different from ours (e.g. global health of patients, personal hygiene, prevention of diseases), but they work in their environment. In addition, the turnover is high, some nurses are just out of school, some others are working there in the hope to move to bigger hospitals, others are still students. Another point relates to materials. There is no large choice as we have in Italy, so you always have to use the same disinfectant, bandages, gloves, drainages, sheets and soaps. However, we have to think that they make the hospital work in a good way with what they have, so with their materials we should create continuity in order to make nurses more autonomous. A good thing is teaching them the scientific principle. The important thing is that, for example, a wound is clean and in order. We must also consider the priorities of the day. Clean bed sheets are not always ready in the morning as the gauze or surgical instruments, and then you have to think which patients need more in that day. The relationship with patients and communication in Chaaria is another problem: they speak only English, Swahili and Kimeru and sometimes our body language is different from theirs. We are often discouraged in the approach with the patient because if we do not find someone who can translate to them we are powerless so that sometimes we would feel like giving up. Many other reasons cause us difficulty: flies, heat and some smells are

unbearable, the beds are low and uncomfortable for us, the patients are too many, sometimes two in a bed, sometimes in the corridors. Some wounds are very large, some necrotic, some smelly, some infected with maggots, some with pus, some with tunnel from knee to ankle, some putrefied. For this reason, the volunteers are often tested physically and mentally, and they feel a bit useless and not satisfied. However, volunteers usually go where people need help: if Chaaria Hospital was perfect, why would we be there? If local nurses knew everything, what should we do there?

TIPS

Assume an attitude of humility, help and cooperation with locals, trying to be accepted. Only after this we can try to teach our techniques hoping that the Kenyan nurses accept and appreciate that. Work with them, become their friend, lead by example and wait for them to ask you questions on your way to work.

TO AVOID

Attitudes of superiority and criticism of the African staff, because we are exposed to the risk of being rejected by our staff of Chaaria that would feel not appreciated and create division and isolation of the new volunteer.

LECT 19

SURGERY IN THIRD WORLD COUNTRIES: THE EXPERIENCE OF THE COTTOLENGO MISSION HOSPITAL

V. Russo

Volunteers Association, Cottolengo Missions, Catania, Italy

It has been over a decade that the Cottolengo Missions Volunteer Association constantly supports several humanitarian organizations, which operate in Africa and Ecuador. A role of key importance in this association is represented by the Chaaria Mission Hospital, which is a rural hospital in central Kenya. Despite being a small rural hospital with about 160 beds on paper, it represents a very important reference point for the population of central Kenya and of the border between Kenya, Ethiopia and Somalia, especially for the poor and needy. In Kenya, up to now, the health care is a luxury that most of the population cannot afford. In fact, medical care and admissions are payment services and have to be paid for. Chaaria Mission Hospital, exclusively financed by both Italian as well as foreign volunteer organizations, provides health care at significantly lower costs compared to other clinics in the country and, if

necessary, does not hesitate to offer free care to those who cannot afford to pay even the very low bill. This medical facility was born with the aim to offer medications and assistance to mentally disabled people, and during the years it had to face rising requests for health care. Initially the hospital had to assist women during labor, but later patient with more and more complex pathologies started to arrive. Br. Beppe Gaido had a vital part in the development of this medical facility. He is a laic friar and a medical doctor, specialist in infectious disease, who was sent to Africa from the Little House of the Divine Providence founded by Saint Joseph Benedict Cottolengo. He had to meet the challenges of increasing requests for medical service. Thank to his willpower, learning ability, faith and, last but not least, to the support he got from Italian volunteer doctors, he could learn how to handle many different aspects of the medical practice/profession, many of which were very far from his specialization. The medical facility started growing and led to the organization of a real hospital with medical equipment and beds. A small operating room was equipped for urgent surgery. It was the first surgical unit in Chaaria. The first surgical procedures performed were cesarean sections, later on minor abdominal surgery and eventually acute abdominal surgery. Today the boasts a complex organizations which includes departments for men and women, one for women in labor and one for children, a laboratory for blood tests, a digestive endoscopic center and an ambulatory care clinic. The staff is composed of Br. Beppe, which supervises the nursing staff and performs most of the surgery procedures, and a local doctor. The nursing staff is composed of local people, and in line with the British healthcare model active in Kenya, it includes healthcare assistants and clinical officers. The latter are receptionists of the patients and in some cases they can also perform small surgery or issue prescriptions. Conversely, the anesthesia clinical officers perform anesthesia in the operating room and take care of pre- and post-surgery assistance. Thanks to the help of the volunteering organizations, the hospital facility has been growing during the years, and it has now new equipment and a second, bigger operating room. There are two operating rooms. The smaller one is used for C-sections and for small surgery. Conversely, more complex surgical procedures are performed in the newer and more modern one, such as general surgery, orthopedic, plastic and pediatric surgery. The hospital is a facility that, despite being fully functional, has limited funds available, leading to the necessity to carefully decide how to invest them. Over the course of the years, the number

of surgeries increased from 2,887 in 2012 to 4,030 in 2016. Moreover, the variety and complexity of the surgical procedure are now significantly wider. This is also due to the improved ability of the paraneurologist, who can now relatively easily manage complex pathologies in risky patients. The most commonly requested surgical procedures include the following: gynecological pathology (myomectomy, hysterectomy, ovarian cysts, ectopic pregnancy, post partum metrorrhagia, plastic tubal, pelvic inflammatory disease [PID]); urological disorders (benign prostatic hypertrophy [BPH], prostate cancer, testicular tumors, phimosis, hydrocele); general surgical diseases (hernias, incisional hernias, masses [inflammatory, infectious and neoplastic], thyroidectomy, gastrostomy); acute abdomen (appendicitis, cholecystitis, perforated ulcer, intestinal obstruction, digestive hemorrhage); neoplastic diseases; traumatic and orthopedic pathologies (decomposed and inveterate fractures, wounds from blunt weapons); others (pediatric diseases, head injuries, cuts, burns, plastic, rare diseases including tropical diseases). All the above mentioned pathologies are indeed very commonly observed in any Italian hospital. However, limited funds, poor preoperative diagnosis and the type of patients make management of even the easiest surgery cases difficult at the Chaaria Mission Hospital. How should the surgeon behave and what is expected of him? First of all, one needs to be aware of being a guest, in a facility that has an organizational autonomy with its own procedures and protocols, which one may not fully understand nor agree with. According to this mental schema, changing an organizational model already in place is not helpful. In fact, this would disorient and make it difficult to work for the local staff, who has to deal with working with a new team of surgeons with different habits every 30 days. It is thus very important to be able to adapt and to respect the way the local staff handles any situation, in ways sometimes very unusual for us. Before leaving, it is useful to study the therapeutic schemes which are used locally. The main aim of the surgeon is not to change procedures and protocols according to his own way of working, but to adapt and to be helpful within the organization. In case it seems necessary to make any changes to the system, it is possible to discuss them with the staff and to explain motivations in detail, together with any improvements that this could bring to the everyday clinic management. Pre-surgery assistance is very different than in the countries of the EU. In fact, appropriate radiological and clinical laboratory diagnostics are lacking. There is no advanced radiological service at the hospital, and the

only possible service than can be offered is the ultrasound. A thoracic or abdominal X-ray, for example, requires further resources to transfer the patient with an ambulance to the closer medical center (in the city of Meru, about 50 km distance by a partially unpaved road). Even more difficult is to perform a computed axial tomography (CAT), due to waiting time (often unacceptable, considering the clinical conditions of the patient) and costs. It seems therefore necessary to rediscover and restudy the “old clinic diagnosis” and still maintain an “aggressive” attitude in suspecting an urgent surgical pathology and proceed to an urgent surgery. The blood tests laboratory inside the facility is able to provide a complete screening in a very short time, including blood test, physical chemical coagulation and HIV antibodies for all patients. Given the environmental conditions (hot weather, dust, overcrowding, infections), all patients receive antibiotic prophylaxis after surgery for a number of days higher than usually recommended in our protocols (often until the stitches removal): this procedure was associated with a reduction of postoperative infections over the years. Almost all patients have a low hemoglobin value (this is often a chronically and well tolerated condition), but sometimes it is necessary perform a hemotransfusion. In the facility there are limited reserves of blood, which are not sufficient to cover the real need, therefore they need to be carefully used. After all, the low value of hemoglobin and consequently the low hematic viscosity allow for avoiding the preoperative antithrombotic prophylaxis with heparin. In cases of neoplastic disease, it is very useful to have an aggressive attitude during the surgery procedure, since only very few patients will be able to follow postoperative radiotherapy and chemotherapy. These treatments, besides being very pricy for the patients, are actually only available in the capital Nairobi, which is 600 km away. Another very important role of the volunteer surgeon is to tutor the local medical staff. Performing very complex surgery procedures is not as important as being capable of instructing the local staff and help them become independent. The contribution of the surgeon will be way more valuable when he will help the local staff to proceed autonomously and perform surgery by themselves. To conclude this brief discussion, a surgeon willing to go on a volunteer mission will need to offer collaboration to the local staff without conflicts, be able to perform several surgical procedures with poor means available, and teach and render independent the local staff, making sure that they will be able to perform and assist the patients efficiently.

LECT 20

THE HUMAN PLACENTA: THE FLIGHT RECORDER OF PREGNANCY

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The placenta represents a unique interface between mother and fetus, and plays a critical role in fetal development. Fetal growth is closely related to the transport of the proper nutrients and ions. Therefore, regulation of placental nutrient transport plays a critical role in fetal development, down-regulation causing intrauterine growth restriction (IUGR) and up-regulation being associated with fetal overgrowth. This review is focused on the relevant role played by macro- and microscopic examination of the multiple placental compartments in the reconstruction of physiopathology of gestation. In particular, we will try to delineate the most important pathological changes associated with pregnancy complications, allowing perinatal pathologists to give neonatologists the most relevant information regarding the prenatal life experiences of neonates.

THE UMBILICAL CORD

Gross evaluation of the cord represents one of the most important moments in placental examination. A true tight knot with vascular congestion is always associated with IUGR and fetal morbidity. Excessively short and excessively long umbilical cords are often detected in intrauterine growth restricted fetuses [1]. The histological examination of cross sections of the umbilical cord allows the study of umbilical vessels and Wharton jelly: a decrease in the Wharton jelly area, in the vein and in the artery area are frequently found in IUGR [2] (**Fig. 1**). The finding of neutrophils in the wall of the umbilical vein (phlebitis), in the arterial wall (arteritis) and/or in the Wharton jelly (funisitis) is often seen in association with chorioamnionitis and neonatal sepsis. The finding of plasma cells in the umbilical structures is suggestive of chronic inflammation and should raise suspicion of Herpes simplex virus infection (**Fig. 2**). The observation of umbilical vein or arterial thrombosis is often associated with slow blood flow or with a hypercoagulation state in the neonate and,

clinically, with neonatal distress and perinatal death. In short, cord pathology is often associated with IUGR, particularly in pregnancies complicated by tobacco smoking or maternal hypertension [3].

EXTRAPLACENTAL MEMBRANES

At gross evaluation, the amnion, the extension of the fetal skin, normally appears as a thin translucent membrane. A yellow-white opaque appearing amnion is suggestive of chorioamnionitis, whereas a green-brown amnion suggests meconium. At histology, amnion detachment from the underlying chorion and amniocyte necrosis represents the first response to meconium or to amniotic fluid infection. The finding of neutrophils extending from the subchorionic space towards the fibrous chorion and the overlying amniocytes is the hallmark of acute chorioamnionitis (**Fig. 3**). Perl's stain may be useful to detect hemosiderin deposits in the chorion, which are strongly associated with prematurity.

THE CHORIONIC PLATE

Gross examination of the chorionic plate (CP) should first focus on the distribution of vasculature. Central cord insertion is generally associated with an even vascular coverage of the CP, whereas in eccentric cord insertion vessels are evenly less distributed. At histology, CP veins and arteries are indistinguishable, both showing asymmetry of the wall, due to variable thickness of the muscular layer, and myxoid protrusions into the lumen. The finding of neutrophils migrating from the intervillous blood space toward the CP connective tissue may represent one of the first signs of acute chorioamnionitis.

STEM VILLI

Characterized by a dense fibrous stroma containing muscular vessels, stem villi take fetal blood from the chorionic plate to distal villi. On a cut section, the largest stem villi are grossly visible, appearing as white foci centered by a vessel, or as vertical streaks extending from the chorionic plate. Vascular dilatation and thrombosis of stem villi are commonly associated with fetal pathology, whereas cysts are characteristic of placental mesenchymal dysplasia [4]. At histology, the exam of stem villi may give relevant data on placenta development. At term, the abundance of immature intermediate stem villi (characterized by numerous fetal macrophages also known as Hofbauer cells and surrounded by trophoblast and syncytiotrophoblast layers) is considered a typical sign of placental immaturity. The finding of neutrophils underneath the syncytiotrophoblast layer is suggestive of acute villitis, whereas their location in the vascular wall is diagnostic for stem villus vasculitis, which is frequently associated with maternal bacterial sepsis.

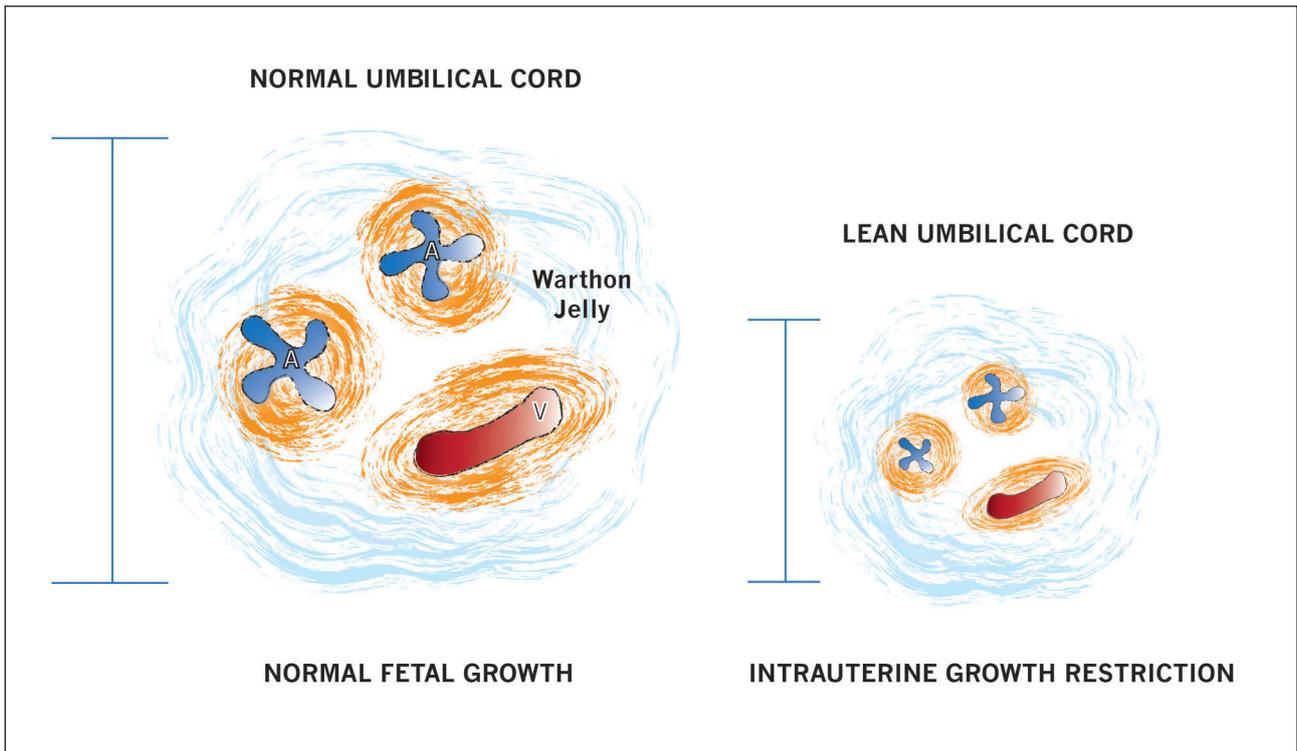


Figure 1 (LECT 20). Lean umbilical cord in intrauterine growth restriction (IUGR): a decrease in the Wharton jelly area, in the vein and in the artery area are frequently found.

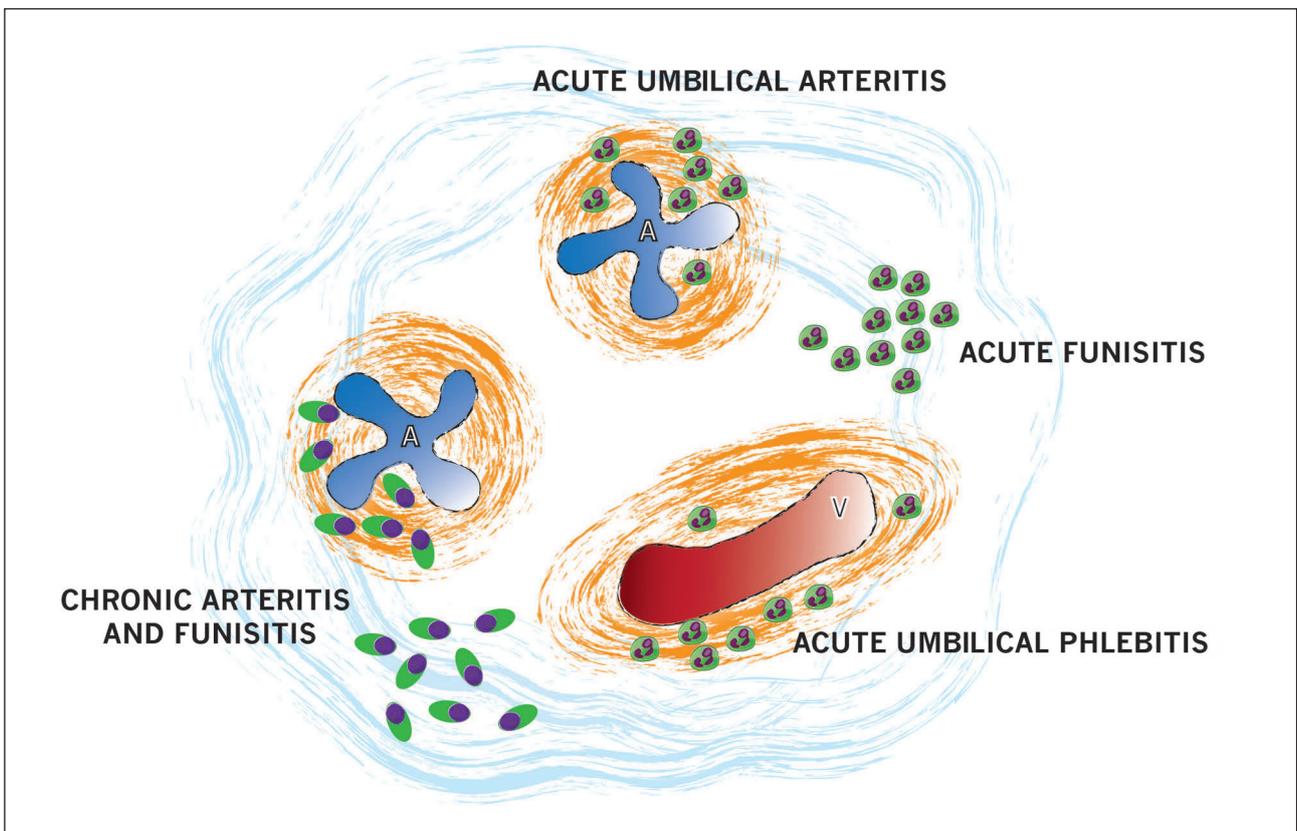


Figure 2 (LECT 20). Schematic picture of acute and chronic inflammation. The finding of neutrophils in the wall of the umbilical vein (phlebitis), in the arterial wall (arteritis) and/or in the Wharton jelly (funisitis) is often seen in association with chorioamnionitis and neonatal sepsis. The finding of plasma cells in the umbilical structures is suggestive of chronic inflammation.

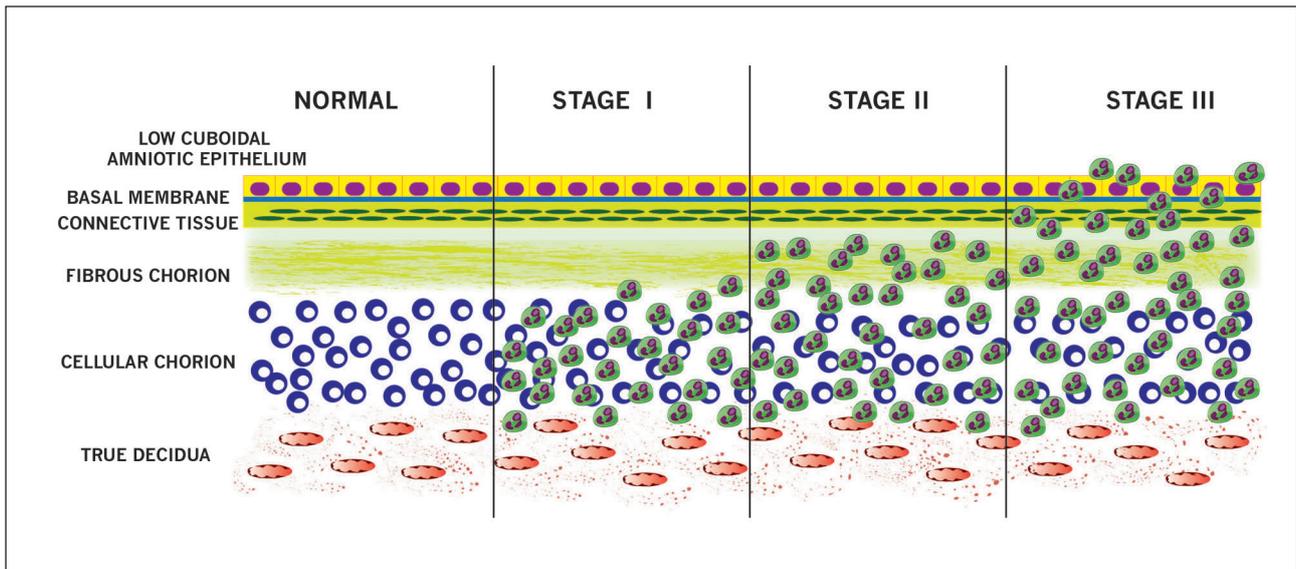


Figure 3 (LECT 20). Staging of acute chorioamnionitis.

CHORIONIC VILLI

At macroscopic examination, the most frequent lesions in the villous parenchyma are infarcts and intervillous thrombi, appearing as tan areas contrasting with the red color of the adjacent parenchyma. Only singular, small (< 1 cm) infarcts located at the disc margin are considered normal in the placenta at term. The histological analysis of chorionic villi is fundamental for the evaluation of the degree of development of the human placenta. At term, distal villi, which have more than 50% of their volume occupied by vascular lumens, represent the vast majority of chorionic villi. Vasculo-syncytial membranes, the essential structures for optimal gas and nutrient exchange, are clearly visible in numbers of 1-6 per villus in term placenta. Syncytiotrophoblast nuclei aggregate to give rise to syncytial knots which, at term, are found in 1 out of 3 villi. At the end of gestation, fibrin replaces syncytiotrophoblasts around larger stem villi. The absence of one or more of these parameters is at the basis of the diagnosis of villous maturation defects. In particular, distal villous hypoplasia, characterized by thin elongated chorionic villi with few capillaries and devoid of syncytiotrophoblast knots, is strongly associated with severe fetal growth restriction. Chorangiomas are at the other extreme of the spectrum, being characterized by hypervascular villi (more than 10 capillaries per villus). Chorangiomas are often seen in diabetic pregnancies [5]. The accurate macroscopic and histological study of human placenta may give relevant data on the physiological development of the fetus, as well as on the pathological history of the entire gestation. A careful examination of the human

placenta may function like a flight recorder, better known as the black box, allowing the reconstruction of the history of the whole gestation, and facilitating the interpretation of “incidents” occurring during the 9 months of intrauterine life. The developmental and pathological data obtained by the analysis of placenta might represent an important source of data for neonatologists, allowing a better comprehension of the health status of neonates. However, in clinical practice, the analysis of the placenta/black box is not taken in the right consideration, and developmental and pathological data obtained with the analysis of placenta tissue are not immediately given to neonatologists. This perplexing phenomenon, recently defined as the “placenta paradox” [6], represents a new challenge for neonatologists and perinatal pathologists, with the hope that a better cooperation between these two specialists might allow a better comprehension of the intrauterine clinical history of every newborn and, consequently, a better sartorial personalized therapy of every neonate admitted to a neonatal intensive care unit. Look for the placenta/black box and rapidly decode the messages recorded in its placental compartments: this is the take home message of this review.

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LECT 21

THE LIMITS OF VIABILITY

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In recent years, the outcome of infants born between 23 and 26 weeks of gestation has dramatically improved. However, every delivery at the threshold of viability remains a great challenge for obstetricians, neonatologists and parents. The critical question remains whether a periviable newborn should be always resuscitated and if so, how aggressively the management should be carried out, particularly in the presence of brain injury. Hence, it is important that the information given to parents regarding survival and long term outcome reflects local data and not general statistics, since outcomes vary tremendously from one center to another and from one country to another. Parental religious and personal beliefs often play an important role in the decision-making process. Physicians can also be biased according to their personal experience. The legal implications of poor communication between parents and physicians are obvious. It is generally accepted that parental decision should be respected when the outcome is ambiguous or uncertain. However, with the dramatic improvement in the survival and outcome of extremely low birth weight infants, physicians have the obligation to protect the life of newborns from unreasonable parental demands. On the other hand, they should take into account the potential long-term implication for both parents and society at large.

LECT 22

THE INFANT BORN TO A DIABETIC MOTHER: EARLY AND LONG TERM CONSEQUENCES

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The frequency of hyperglycemia in pregnancy (HIP) is increasing worldwide, together with that of overweight, obesity and type 2 diabetes (T2D), with devastating effects. Diabetes in pregnancy is still responsible for significant perinatal mortality and morbidity in many countries. Moreover, intra-uterine exposure to altered maternal glucose metabolism is associated with an increased risk of chronic, non-communicable diseases in adulthood.

SHORT-TERM EFFECTS OF HYPERGLYCEMIA IN PREGNANCY ON THE FETUS AND THE NEONATE

Neonatal complications such as birth defects, perinatal deaths or asphyxia, are mainly due to pre-existing, pregestational diabetes. However, complications related to excess fetal nutrient intake occurring later in pregnancy in the context of pure gestational diabetes could be severe, especially when gestational diabetes mellitus (GDM) is recognized late, or is poorly controlled with treatment. Macrosomia and excess fetal growth, together with hypoglycemia, all being due to hyperinsulinism, are the landmarks of neonatal morbidity and mortality due to HIP. However, the role of maternal overweight and obesity, both conditions which *per se* often underlie HIP, is likely to be important.

CONSEQUENCES OF HYPERGLYCEMIA IN PREGNANCY ON THE OFFSPRING IN ADULTHOOD

Increasing knowledge is available on the long-term consequences of gestational diabetes or excess growth during the fetal period. Converging data from clinical and epidemiological research, but also from animal models show that offspring exposed *in utero* to altered maternal metabolic function face an increased risk of developing at adulthood impaired glucose tolerance, hypertension, overweight and obesity and dyslipidemia, as well as other non-communicable diseases. This may be part of a leveraging epidemiologic effect linked to the cycle of reproduction. Indeed HIP is considered a major contributor to the current T2D pandemic, possibly through altered developmental programming and transgenerational epigenetic imprinting mechanisms.

Optimizing diabetic care before and during pregnancy reduces the risk of neonatal complications. Indeed, thanks to considerable advances in the management of diabetes in pregnancy, whether pre-existing (type 1 or

type 2) or purely gestational, nowadays many infants born to diabetic mothers experience a healthy neonatal course in high-income countries. Treatment of GDM has indeed been shown to reduce specific or composite neonatal outcomes. Weight control before pregnancy and control of weight gain during pregnancy, efficient maternal, fetal and neonatal monitoring, HIP treatment, and breastfeeding allow such improved outcomes. However, the implementation of strategies to limit the consequences of diabetes in pregnancy in the offspring is still difficult to achieve in many low and middle-income countries. The initiative on HIP recently taken by the International Federation of Gynecology and Obstetrics (FIGO) may considerably help for the prevention and the early detection of HIP in low and middle resources contexts. Preventative strategies for mothers and neonates would considerably benefit from a better understanding of the mechanisms involved in the impact of HIP on the embryo, the fetus and the neonate, and on the mother in the longer term, as she is exposed to a significantly higher risk of developing T2D in the long run. An extensive and coordinated research policy may help identifying optimal early biomarkers of the risk for GDM and developing personalized therapeutic approaches.

LECT 23

BIOACTIVE SUBSTANCES IN HUMAN MILK

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Bioactive substances in breast milk contribute to its physiological functions and its characterization as “functional food”. We investigated substances associated with a) adaptation to extrauterine life and obesity, b) maternal/neonatal bone metabolism. Irisin, adropin, arginine-vasopressin (AVP) and copeptin are implicated in postpartum adaptation and obesity. Irisin triggers transformation of white adipose tissue to brown adipose tissue, ensuring thermogenesis. Reduced irisin levels exist in obese and diabetic individuals. Adropin promotes postnatal angiogenesis and downregulates genes involved in lipogenesis. AVP and its surrogate marker copeptin are involved in water retention, pulmonary vasodilatation and surfactant secretion. We hypothesized that presence of these substances in colostrum may be critical for postnatal adaptation by regulating the functions described above. We determined these substances in breast milk and

maternal serum of 81 mothers on day 3-4 postpartum by ELISA and investigated their possible association with several perinatal parameters. Irisin concentrations were lower in breast milk than in maternal serum ($p < 0.001$), decreasing with increasing maternal pre-pregnancy BMI ($b = -63.503$, 95% CI: -121.785 to -5.222 , $p = 0.033$), and increasing with maternal age ($b = 0.012$, 95% CI: 0.003 to 0.021 , $p = 0.012$). Adropin concentrations were higher in breast milk ($p < 0.001$) and positively correlated with maternal serum ones ($r = 0.304$, $p = 0.006$). Serum adropin concentrations were lower in smoking ($b = -0.285$, 95% CI: -0.459 to -0.111 , $p = 0.002$) and diabetic mothers ($b = -0.166$, 95% CI: -0.251 to -0.080 , $p < 0.001$). Copeptin concentrations were higher in breast milk ($p < 0.001$) and positively correlated with maternal serum ones ($r = 0.304$, $p = 0.006$). We conclude that irisin presence in early breast milk is possibly implicated in postnatal thermoregulation, as well as protection from obesity. Its lower milk concentrations point to its transfer from the maternal circulation. Its downregulation with increasing BMI emphasizes its inverse association with adverse metabolism, and its increasing production with age possibly counterbalances resistance to irisin. Adropin abundance in human milk plays a crucial role in regulating growth, glucose metabolism, pulmonary adaptation and obesity prevention. Its higher breast milk concentrations and positive correlation with maternal serum ones point to its secretion by breast tissue and maternal circulation. Its downregulation in cases of maternal diabetes and smoking suggests insulin resistance and endothelial dysfunction, respectively. Copeptin abundance in human milk is implicated in the regulation of postnatal adaptation with respect to lung function and water retention, while its higher breast milk concentrations and positive correlation with maternal serum ones reflect its secretion by breast tissue and maternal circulation. Concerning maternal/neonatal bone metabolism, important markers are the following: bone specific alkaline phosphatase (BALP), which is the most reliable marker of bone synthesis, N-telopeptide of type I collagen (NTx), which is the most specific marker of bone catabolism, and the system RANKL/Osteoprotegerin/RANK. RANKL produced by osteoblasts, which is a key factor in osteoclast formation, inducing bone resorption by binding to receptor RANK on osteoclasts. Osteoprotegerin (OPG), secreted by osteoblasts, blocks binding between RANKL and RANK thus inhibiting osteoclastogenesis and bone resorption. We hypothesized that despite its low mineral content, breast milk may have short/long-term benefits for bone health, probably via non-nutrient

effects and that bone biomarkers in breast milk may be implicated in maternal/neonatal bone metabolism. We determined the above markers by ELISA in breast milk and maternal serum from 85 mothers on day 3-4 postpartum and explored their possible correlation with several perinatal parameters. Serum soluble RANKL (sRANKL) was detected for the first time in breast milk but at considerably lower concentrations than in serum ($p < 0.001$). Decreased breast milk sRANKL concentrations were found in maternal diabetes ($b = -0.366$, 95% CI: -0.622 to -0.110 , $p = 0.006$). NTx concentrations were higher in exclusive lactation ($b = 0.269$, 95% CI: 0.014 to 0.524 , $p = 0.039$) and lower in caesarean sections ($b = -0.224$, 95% CI: -0.428 to -0.019 , $p = 0.032$). We conclude that lower sRANKL concentrations in breast milk point to inhibition of osteoclast activity and bone resorption by breast milk. Lower breast milk sRANKL concentrations in diabetes represent a protective mechanism against compromised bone growth and mineralization, characterizing infants of diabetic mothers. Increased breast milk NTx concentrations in exclusive lactation reflect higher maternal skeletal mineral content loss, while decreased breast milk NTx concentrations in caesarean sections signify delayed initiation of lactation and lower early milk production, thus lower early postpartum maternal bone resorption.

LECT 24

PRECISION MEDICINE AND NARRATIVE MEDICINE

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Precision medicine and narrative medicine are clinical and research areas – one that capitalizes on the fundamental aspects of human interaction, and the other that exploits innovative processes of the biomedical sciences – that are both expressions of a person's communicative profiles, whether narrative or omics-based. While the two fields may seem to be diametrically opposed, a complementary synergy between them is necessary for the scientific and healthcare community to emancipate itself from an overly biological and technical approach and embrace a vision of medicine that is simultaneously global

and individual. Both approaches have much to offer to all phases of the care pathway, from prevention to diagnosis to treatment [1, 2], but such a holistic vision of medicine requires a dramatic change in paradigm [3], not only to improve on what has been achieved in the past, but also to guide the future away from a passive subordination to technology. Medicine must free itself from Descartes' error – the dualist separation between mind and body, rationality and emotion – that is still overbearing in medical care, as well as from a consideration of the patient as merely a biological “machine” and not in a holistic, truly systems-based way that includes a consideration of the patients' desires, needs, values, habits, hopes and fears in addition to a quantitative, laboratory-based analysis. It must succeed in doing so from within the confines of the healthcare system, which is organized to obtain objectives in increasingly less time and with lower costs, according to an economic logic that is often at odds with a patient's individual sensitivities. In addition to systemic changes, this change will also require a new approach to training, in which young graduates are not only well-versed in the mechanisms of disease, but are also prepared for all facets of the practice of the art of medicine, in particular those skills needed to effectively interact with patients. The change will also require courage, as the standard procedures and methods established by protocols and guidelines become superseded in part by new experimental concepts such as n-of-1 clinical trials and a willingness to truly tailor the patient experience to the individual. Overcoming the “ideology” of guidelines and protocols based on the results of omics sciences and narrative medicine is a need, but at the same time a challenge for the future of medicine, considered in its entirety.

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LECT 25

CRITICAL QUESTIONS ON NUTRITION OF PRETERM INFANTS

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Preterm birth causes the abrupt interruption of maternal-fetal transfer of macro and micronutrients. Furthermore, infants born preterm may be unable to synthesize the adequate amount of metabolically essential nutrients, such as long chain polyunsaturated fatty acids, due to developmental immaturity. As a result, infants born preterm represent a nutritional emergency that must be addressed immediately in order to avoid/limit the development of nutritional deficiencies that lead to postnatal growth retardation, which is still a relatively common finding in NICUs. When taking care of preterm infants from a nutritional point of view, it must be taken into consideration that promotion of growth is achieved by the accomplishment of their high nutritional needs, that become even more demanding with the occurrence of comorbidities. It has been demonstrated that the resting energy expenditure of healthy preterm infants increases by 140% in the first 6 weeks of postnatal age, whereas that of term infants increases by 47% in the same time frame. However, the need for ventilator support and the development of chronic lung disease raise the energy expenditure by 25% and 20%, respectively. On the basis of these data, it becomes clear that the identification of factors that determine and/or affect nutrient requirements in preterm infants is mandatory. In addition, a full understanding of the most appropriate biological setting that should be used for establishing nutritional requirements in preterm infants is desirable. The latter implies gaining further insight into the role played by the placenta in modulating the maternal-fetal nutrients passage in addition to the identification of the nutrients that could potentially become essential for preterm infants due to their developmental immaturity. It is also essential to consider the way of delivering nutrients; nutrient requirements when using the enteral route differ from nutrients requirements when using the parenteral one due to the lower absorption and the partial utilization of nutrients by the enterocytes. A deeper knowledge with regards to these points would allow for the provision of an appropriate amount of specific essential nutrients, avoiding the under- or overexposure to certain nutrients, and for the individualization of nutritional care of preterm infants. The avoidance of early malnutrition and, hence, the promotion of postnatal growth is of major importance since they been associated with the improvement of later neurodevelopment outcome. Furthermore, the limitation of extrauterine growth re-

striction prevents the need for rapid catch-up growth after discharge that, in turns, has been linked to later adverse metabolic consequences. Increasing evidence has indicated that postnatal growth retardation is accompanied by a fat free mass deficit, probably related to immature metabolic mechanisms, delayed amino acid administration and protein intakes lower than recommendations. The different ways macronutrients are supplied during intrauterine life in comparison to postnatal life, in addition to the different environmental conditions preterm infants are exposed to, may also be partially responsible for the development of an altered body composition at term corrected age. The potential long lasting effects of these body composition modifications on future health, both in terms of neurodevelopment outcome and metabolic risk, are still under investigation. A higher protein-to-caloric ratio, including the provision of fortified human milk, has been demonstrated to be a major contributing factor in increasing fat free mass deposition. Providing optimal nutritional care to preterm infants increases survival and enhances quality of life. Future research needs to focus on the areas of early parenteral nutrition and optimal fortification of human milk, in order to gain further insight with regard to the optimal regimen to improve early postnatal growth and brain development of these infants.

LECT 26

NEWBORNS BORN TO MOTHERS WITH IMMUNE THROMBOCYTOPENIC PURPURA

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The leading cause of moderate or severe thrombocytopenia is immune thrombocytopenia in otherwise healthy appearing neonates. Immune thrombocytopenia in the fetus or newborn may result from platelet alloantibodies against paternal antigens inherited by the fetus (alloimmune thrombocytopenia) or platelet autoantibodies in the mother with immune thrombocytopenic purpura (ITP). Only 10% of human platelet antigen (HPA)-1a negative mothers who are exposed to HPA-1a positive fetal platelets during pregnancy develop HPA-1a alloantibodies, and 30% of fetuses/neonates develop thrombocytopenia, 20% of these cases being severe. The most serious complication of severe fetal and neonatal alloimmune

thrombocytopenia (FNAIT) is intracranial hemorrhage (ICH), which is detected in 10-20% of affected fetuses/neonates, with most cases occurring antenatally, and leads to neurological sequelae in 20% and death in 5-10% of cases. There is no evidence-based optimal treatment strategy. Platelet antibody titration in maternal plasma is not helpful for decision-making. The best indicator for current pregnancy is the outcome of the previous pregnancy. The risk of recurrence among subsequent HPA-positive sibling is close to 100% where the previous sibling was affected with antenatal intracranial ICH. The risk of ICH becomes higher with more severe and earlier onset in each subsequent pregnancy. Serial platelet counts should be obtained for the first 5-7 days of delivery to keep the platelet counts higher than 30,000/ μ L without active bleeding and higher than 50,000-100,000/ μ L with active bleeding. IVIG is not alternative to platelet transfusions, since platelet counts do not rise before 24-48 h. In platelet-transfused patients, IVIG can be given to potentially prolong the survival of the incompatible platelet. ITP during pregnancy is not considered a serious risk of perinatal bleeding, but may cause a moderate thrombocytopenia in neonate. In mothers with ITP, the risk of thrombocytopenia is only 10%, with no more than 1% risk of *in utero* ICH.

LECT 27

GENETIC SURFACTANT DYSFUNCTION

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INTRODUCTION

Severe and progressive respiratory distress syndrome (RDS) in term newborns and interstitial lung disease (ILD) in infants and children may result from mutations in genes encoding surfactant protein B (*SFTPB*), surfactant protein C (*SFTPC*) and *ABCA3* (*ABCA3*) (**Tab. 1**).

PATIENTS AND METHODS

We retrospectively reviewed 391 molecular analysis of SP-B, SP-C and *ABCA3* genes, performed in our laboratory from 2000 to 2015 in term and preterm newborns with severe RDS, infants and children with

Interstitial Lung Disease (ILD), chorionic villi for prenatal diagnosis and parents and siblings of affected infants. Molecular analyses have been performed on genomic DNA extracted from peripheral blood by Sanger sequencing of whole gene coding regions and intron junctions. Histopathologic examination, immunohistochemical analysis and electron microscopy have been performed when lung tissue was available.

RESULTS

Genetic variants of *SFTPB*, *SFTPC*, and *ABCA3* were identified in 74 of 183 (40%) newborns tested for severe and unexplained RDS and in 38 of 74 (51%) infants and children with ILD (**Tab. 2**). Newborns with RDS were divided into 3 groups according to gestational age. *SFTPB* variants were identified in 5 term newborns, in 7 prematures and in 3 children with ILD. 31 term newborns, 12 late preterm newborns, 19 preterm newborns and 25 children with ILD showed mutations in *ABCA3*. Prenatal diagnosis on chorionic villi was performed in 8 women that had previously lost a child to *ABCA3* deficiency. 2 fetuses resulted affected, 5 were carrier and 1 was normal. 38 of 74 (51%) tested infants and children with ILD were affected by surfactant dysfunction: 3 heterozygous mutations were identified in *SFTPB*, 15 in *SFTPC*, and 25 heterozygous mutations in *ABCA3*. In 10 infants, genetic variants were identified in more than one gene. Higher mortality rate was among term newborns with homozygous or compound heterozygous variants in *SFTPB* and in *ABCA3*. Consanguinity was present in 15 cases, and familiarity for pulmonary diseases in 30 cases. Light microscopy was performed in 11 infants, immunohistochemical analysis in 12 and electron microscopy in 8 cases.

DISCUSSION

Surfactant dysfunction was identified in a significant number of newborns with severe, unexplained respiratory failure and children with ILD, indicating the importance of genetic studies in infants and children with this phenotype. *ABCA3* dysfunction was the most frequently identified defect either in newborns with RDS or in children with ILD. In newborns, clinical symptoms of RDS are evident in the first hours or days; this lung disease is progressive, unresponsive to maximal medical treatment and may result in death within few weeks or months after birth. However, partial and transient SP-B deficiency compatible with prolonged survival has been recognized. ILD in older infants and children is caused by mutations in *ABCA3* or in *SFTPC*, with a phenotype highly variable regarding severity of disease and age of onset. While SP-B, SP-C and *ABCA3* deficiency are rare, carriers for mutant alleles in these genes may be at risk for respiratory

Table 1 (LECT 27). Genes involved in neonatal and pediatric surfactant dysfunction.

Protein (Gene)	Inheritance	Mechanism	Clinical presentation/ Diagnosis	Onset/Disease	Outcome
Surfactant Protein B (<i>SFTPB</i>)	AR	Loss of function	RDS, hypoxic respiratory failure. Radiographic opacification typical of RDS	Newborn infants during the first hours or days	Fatal
Surfactant Protein C (<i>SFTPC</i>)	AD	Gain of toxic function or dominant negative	Variable respiratory symptoms in neonatal and pediatric age. Radiographic diffuse alveolar damage, interstitial feature of inflammation typical of ILD	Newborns, infants and children	Variable
Adenosine triphosphate (ATP)-binding cassette transporter A3 (<i>ABCA3</i>)	AR	Loss of function	RDS, hypoxic respiratory failure in newborns. ILD in infants and children. Radiographic finding of alveolar disease.	Newborns, infants and children	Severe, fatal, variable

AR: Autosomal Recessive; AD: Autosomal Dominant; RDS: Respiratory Distress Syndrome; ILD: Interstitial Lung Disease.

Table 2 (LECT 27). Characteristics of *ABCA3*, *SFTPB* and *SFTPC* cases.

Clinical presentation	Tested infants	Affected infants	Infants with <i>ABCA3</i> variants	Infants with <i>SFTPB</i> variants	Infants with <i>SFTPC</i> variants	Infants with variants in more than 1 gene	Dead
RDS < 32 w	70	27	19	7	2	1	10
RDS 33-36 w	31	12	12	0	2	2	4
RDS > 37 w	82	35	32	5	0	2	20
ILD	74	38	25	3	15	5	6

RDS: Respiratory Distress Syndrome; ILD: Interstitial Lung Disease.

failure if born prematurely or if other environmental factors impair gene expression. We identified *SFTPB*, *SFTPC* and *ABCA3* heterozygous mutations in 27 of 70 tested premature newborns with 24 w to 32 w gestation with a particularly severe course of RDS. Molecular analysis is a noninvasive test and should be considered promptly in term newborns who develop progressive hypoxemic respiratory failure. A history of unexplained respiratory failure in a previous child should further strengthen the suspicion. In prematures, testing for surfactant dysfunction should be performed in infants with unusually severe RDS, prolonged ventilatory support, difficulty of extubation, prolonged oxygen dependency. When the onset of lung disease is in the neonatal period, *ABCA3* and *SFTPB* should be analyzed first; if negative, *SFTPC* analysis should be performed. In families with identified *SFTPB* or *ABCA3* mutations, prenatal diagnosis can be proposed, allowing families to orient therapeutic options in advance. When the onset of respiratory failure manifests after neonatal age with a slowly progressive course and clinical characteristics of ILD, *ABCA3* and *SFTPC* should be analyzed first. Lung tissue analysis (histopathology, immunohistochemistry, electron

microscopy) may aid in the diagnosis. No specific therapies have demonstrated their effectiveness in the treatment of surfactant dysfunction. With rare exceptions, SP-B deficiency remains a fatal disease and lung transplantation is the only therapeutic option, whereas mutations in *ABCA3* or *SFTPC* lead to a more variable disease and a more favorable prognosis. Supportive and preventive care of infants with ILD includes treatment of hypoxemia, nutritional failure, prevention of infections. Steroids, hydroxychloroquine and azithromycin have been used, but not in controlled trials. Gene therapy is a promising treatment, but still experimental. While actually treatment is primarily supportive, early identification is important to establish appropriate management and evaluation of treatment options, and to offer genetic counseling and prenatal diagnosis, where applicable.

LECT 28

NON-INVASIVE VENTILATION

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Nasal continuous positive airway pressure (NCPAP) was the earliest form of non-invasive respiratory support used in infants with respiratory failure. Its use has become standard practice in order to avoid invasive mechanical ventilation (MV) and to facilitate weaning from the ventilator. However, despite early NCPAP and intubation-surfactant-extubation (INSURE) technique, a very high percentage of very low birth weight (VLBW) infants develops respiratory failure and needs intubation and MV or fails extubation. These high failure rates, inversely correlated to the gestational age (GA) of the neonate, prompted the use of more effective non-invasive techniques of respiratory support. Nasal Intermittent Positive Pressure Ventilation (NIPPV) or Nasal Intermittent Mandatory Ventilation (NIMV) are non-invasive modes of ventilation that provide NCPAP plus superimposed ventilator mandatory breaths and are identified as SNIPPV/SNIMV when the ventilator pressure waves are synchronized with the spontaneous efforts of the patient. These techniques are being increasingly used in preterm infants with respiratory failure and several trials seem to demonstrate that NIPPV and SNIPPV are more effective than NCPAP in reducing extubation failure [1, 2]. Their effects include a higher mean airway pressure (MAP), a washout of the anatomical dead space in the upper airways and a stimulatory effect on the respiratory drive. With SNIPPV the benefits are more consistent due to the positive effects of synchronized mechanical breaths in reducing thoracoabdominal asynchrony, inspiratory effort, breathing frequency and work of breathing (WOB) and at the same time in increasing tidal volume (V_t) and minute volume (V_e), gas exchange and respiratory drive [1, 2]. Different modes of synchronization have been reported [1, 2]. SNIPPV was initially performed by a capsule (Graseby capsule) placed on the baby's abdomen, which detects the increase of the pressure due to the contraction of the diaphragm, but this device has several disadvantages. Although it is a relatively simple device, accuracy is limited by position and fixation, movement is often misinterpreted as breathing and at higher spontaneous breath rates its response is less consistent. Neurally adjusted ventilatory assist (NAVA), which uses electrical activity of the diaphragm (Edi) to trigger the ventilator, has been more recently developed.

However, it has the disadvantage of being invasive and costly, as a dedicated electrode-equipped catheter to detect Edi is required and to date there are few data on clinical outcomes. To overcome all these disadvantages our team decided to create a flow-sensor, a simpler differential pressure transducer interposed between the nasal prongs and the Y piece [1, 2]. Using this device, we were able to demonstrate in a randomized controlled trial that flow-SNIPPV was more effective than conventional NCPAP in decreasing extubation failure in 63 preterm infants who had been ventilated for RDS [3]. The success rate of extubation was significantly higher in the flow-SNIPPV group (90%) compared to the NCPAP group (61%). Flow-SNIPPV stimulated breathing, as demonstrated by the absence of respiratory acidosis and apneic episodes as causes of failure. Afterward our group conducted a new clinical study using flow-SNIPPV as the primary mode of ventilatory support in preterm infants < 32 weeks' gestation with RDS [4]. The aim of the trial was to evaluate whether flow-SNIPPV used immediately after INSURE technique was effective in further reducing the incidence of MV when compared to the conventional INSURE/NCPAP treatment. We found the following statistically significant results: 11 out of 31 (35.5%) infants in the NCPAP group and 2 out of 33 (6.1%) infants in the flow-SNIPPV group failed the INSURE approach and underwent MV. Fewer infants in the INSURE/flow-SNIPPV group needed a second dose of surfactant and a high caffeine maintenance dose. Differences in O_2 dependency at 28 days and 36 weeks of postmenstrual age were at the limit of statistical significance in favor of flow-SNIPPV treated infants. More recently we also successfully applied flow-SNIPPV to treat apnea of prematurity (AOP) [5]. Nineteen infants with a mean GA of 30 weeks suffering from severe apneic spells were enrolled in a randomized controlled trial with a crossover design. They received flow-SNIPPV, NIPPV and NCPAP for 4 hours each. Cardiorespiratory recordings showed a significantly lower incidence of desaturations, bradycardia and central apnea episodes in preterm infants during flow-SNIPPV compared with NCPAP or NIPPV, while these events did not differ between NCPAP and NIPPV. In agreement with our previous trials, these findings seem to indicate that, compared to NCPAP and NIPPV, flow-SNIPPV might have a greater efficacy in augmenting and stimulating spontaneous breathing in preterm infants. These clinical observations show that the flow-sensor is a reliable device and that flow-SNIPPV seems more effective than NCPAP in reducing the need for intubation among infants suffering from RDS, in

improving the success of extubation and in treating apnea, with a reassuring absence of side effects.

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LECT 29

THE RESPIRATORY DISTRESS SYNDROME (RDS) IN LATE PRETERM INFANTS

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Late preterm infants (34⁺⁰ to 36⁺⁶ weeks' gestation) represent a significant percentage of all neonates and their frequency has remained stable in the last years [1]. They account for 8% of all newborns in the USA [1], and for 6% in Italy [2]. Infants born late preterm are at increased risk for morbidities in the immediate newborn period including a higher rate of respiratory failure, whose occurrence ranges from 4% to 29% [3]. Its etiology is represented mainly by respiratory distress syndrome (RDS), transient tachypnea of the newborn (TTN), pneumonia, air leak, and apnea [2, 4]. In fact, alveolarization and surfactant synthesis in the human lung occurs in the third trimester of gestation, and preterm delivery may therefore affect lung structure, development, and function [5]. In particular, the lung pool size of surfactant is expected to be lower than that of term infants, and McEvoy et al. demonstrated that late preterm infants without clinical respiratory disease have significantly higher

respiratory resistance, lower respiratory compliance and expiratory flow ratio when compared with term infants [5]. Non-invasive and invasive respiratory supports and surfactant treatment are commonly used in the management of respiratory failure in late preterm infants, however, the frequency of this use is not well known. Non-invasive ventilation rate ranged from 3.3% in infants born at 36 weeks of gestation to 12.9% in infants born at 34 weeks of gestation, while mechanical ventilation (MV) rate ranged from 1.6% in infants born at 36 weeks of gestation to 6.5% in infants born at 34 weeks of gestation [2, 4]. Similarly, surfactant treatment was more frequent in infants born at 34 weeks of gestation (range 4.0-8.5%) than in infants born at 35 (range 2.9-4.3%) or 36 (range 0.9-2.2%) weeks of gestation [2, 4]. Overall, literature reports that 3.9% of late preterm infant are treated with surfactant for RDS [2, 4, 6, 7], and it is interesting that although the occurrence of respiratory morbidity in late preterm infants varies among studies, the surfactant treatment rate remains similar, ranging from 2% to 4% [2, 4, 6, 7]. On the basis of the previous considerations, it can be assumed that about 30,000 late preterm infants are born every year in Italy and that from 600 to 1,200 of them are treated with surfactant, although its effectiveness has never been investigated. Therefore, we decided to investigate the effects of surfactant in late preterm infants with RDS and the need and duration of non-invasive respiratory support and MV, as well as NICU and hospital stay duration in surfactant-treated or untreated infants with RDS. We carried out a multicenter retrospective study in seven perinatal centers that enrolled a large cohort of infants born between the ages of 34⁺⁰ weeks and 36⁺⁶ weeks at the time of birth who were admitted to the neonatal care units for respiratory failure from January 2010 to December 2014. The occurrence of respiratory failure, RDS, and surfactant treatment was similar to that previously reported [4], supporting the accuracy of the collected data. Our data suggest that surfactant therapy improves respiratory function in late preterm infants with RDS, although we could not demonstrate a positive effect also in the short-term outcomes in our population. This probably occurred because other factors play a relevant role on these variables. Caution is necessary in interpreting these results due to the retrospective design of our study, confirming the need of randomized controlled studies on this topic.

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LECT 30

RESPIRATORY SYNCYTIAL VIRUS PROPHYLAXIS IN SPECIAL POPULATIONS

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Respiratory Syncytial Virus (RSV) is a ubiquitous RNA virus of the Pneumovirus genus and Paramyxoviridae family and is the most important cause of lower respiratory tract infection (LRTI) in infancy. RSV accounts for approximately 70% of hospitalizations for bronchiolitis, up to 40% of pneumonia and up to 30% of tracheobronchitis cases. RSV is known to cause severe LRTI in preterm infants, children with bronchopulmonary dysplasia and children with congenital heart disease. Additionally, there is a growing evidence of severe RSV disease in infants and children with cystic fibrosis, neuromuscular impairment, Down syndrome, multiple congenital anomalies and immunodeficiency. A large proportion of these high-risk infants and children hospitalized with RSV infection require admission to an intensive care unit and mechanical ventilation, and even die. Beyond the substantial disease burden during acute infection, evidence suggests that RSV bronchiolitis plays a causal part in the development of recurrent wheeze and is associated with the development of asthma and subsequent morbidity. Due to a lack of effective treatment strategies, prevention remains the

most effective approach against severe RSV infection in infants and young children. In addition to basic hygienic measures, although no vaccination strategy is yet available, palivizumab, a humanized murine monoclonal antibody produced by recombinant technology and directed against the surface RSV fusion protein, was shown to reduce hospitalizations and the clinical severity of RSV in high-risk infants. The definition of high-risk is yet controversial: although the pharmacotherapeutic profile of licensed indications of palivizumab are well defined, especially prematurity, bronchopulmonary dysplasia and/or hemodynamically significant congenital heart disease, there is some heterogeneity for the use of this drug in other "special" high risk populations group, excluded by indication from clinical trials. In such children, risk is not associated with immaturity of the respiratory tract but rather with a presence of specific anatomical, functional, immune and pathophysiological conditions such as to generate major risk of severe disease in presence of bronchiolitis. The Italian intersociety consensus and the most recent technical report issued by American Academy of Pediatrics concerning indications to palivizumab prophylaxis, agree that a conclusive determination of the possible worsening of the underlying disease represent a true dilemma in these categories of patients. Unfortunately, methodologically valid scientific documentation supporting safety and efficacy of anti-RSV prophylaxis with palivizumab in other "special" high-risk populations is still lacking. Therefore, larger prospective studies are urgently needed to demonstrate the efficacy and safety of palivizumab and its effects on hospitalizations in these groups of infants.

LECT 31

SPINAL MUSCULAR ATROPHIES

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Spinal muscular atrophies (SMAs) are autosomal recessive neuromuscular disorders involving motor neurons of the ventral horn of the spinal cord and motor nuclei from the brainstem. It is the second most common, potentially fatal autosomal recessive disorder. These disorders affect from 1 in 6 to 1 in 8 live-born infants. The gene of SMA is located on chromosome 5q13. Gene abnormalities are present in > 95% of patients with SMA. The severity of

weakness generally correlates with the age of onset. Weakness evolves within the first few months of life. SMA disorders are classified into three forms, based on the age at onset.

SMA type 1 (Werdnig-Hoffmann disease) is a progressive disease usually fatal in infancy. The onset of symptoms occurs before 6 months of age with severe hypotonia, diffuse muscle weakness, absence of reflexes: patients can never roll, sit, or walk. SMA type 0 is the most severe form of SMA type 1, diagnosed in babies that are born so weak that they can survive only a few weeks, even with intensive respiratory support. On the basis of severity, SMA type 1 can be defined as “true”, when the onset of clinical symptoms is before 3 months of age and floppy children are not able to raise their head, or “intermediate”, when the onset of symptoms is after 3 months of age and the ability to raise the head is preserved. The severity of this condition can be classified as “severe 1A”, “typical 1A” or “mild 1B” on the basis of the age of the child at first episode of respiratory decompensation.

SMA type 2 is a disease with onset of symptoms between 6 months and 18 months; patients never walk but are able to sit independently for some period of time.

In *SMA type 3* (Kugelberg-Welander disease) patients develop the ability to walk autonomously for some period of time.

In SMA types 1 and 2, pulmonary disease is the major cause of morbidity and mortality. A combination of inspiratory and expiratory muscle weakness can lead to a respiratory pattern with low tidal volumes and superficial breathing. The respiratory consequences are the following: hypoventilation, upper airway obstruction, aspiration lung disease, secretion retention and lower airway infection. They are not mutually exclusive and often coexist, leading to progressive respiratory insufficiency. Respiratory failure is the most common cause of mortality in patients with SMA. Forced vital capacity (FVC) in decubitus may be abnormally low respect to the FVC in sitting position (> 25%), indicating significant in the diaphragmatic weakness and nocturnal hypoxemia. Currently the approach to assisted ventilation in SMA type 1 is very variable. The evolution and development of non-invasive ventilation (NIV) had a major impact on the natural history of SMA, as respiratory failure is one of the most common causes of early death. Survival and quality of life have considerably improved since the introduction of non-invasive treatment of respiratory complications with ventilatory support in the management of patients with SMA. NIV stabilizes vital capacity, increasing PaO₂ decreasing PaCO₂ and

improving the quality of sleep. NIV can be used as a routine therapy or as a palliative tool. A key goal is to prevent pediatric intensive care unit stays and avoid tracheotomy if possible. Tracheotomy is performed when both NIV and ineffectiveness of coughing aids fail or bulbar involvement reaches a critical point. It can cause some complications due to traumatic suctioning, overinflation of the balloon or contact with the distal end of the cannula. Effective cough is a protective mechanism against respiratory tract infections; peak cough flow (PCF) measured with a peak flow meter or pneumotachograph after a vigorous cough effort evaluates cough strength, in terms of the ability to expel secretions (normal values > 270 l/min). An inability to generate effective expiratory peaks in the expiratory flow trace indicates a poor prognosis. We can consider two types of action: preventive and active rehabilitation program (RP). The aim of preventive RP is to maintain thoracic and lung compliance and to avoid the appearance of microatelectasis. Maintaining normal bulbar function to allows closure of the glottis at the end of insufflation is critical for patients. Mechanically assisted coughing with insufflation-exsufflation device finds an indication when manual techniques are not enough to achieve effective cough. This can be delivered via a nasobuccal mask or tracheal tube and pressures > 30 cm H₂O for both insufflation and exsufflation are recommended. The NIV Protocol known as Dr. Bach's Non-Invasive Protocol is a respiratory protocol using a breathing machine called “BiLevel” and a machine called a “Cough Assist” to help manage the respiratory issues that are core problems with SMA. Impairment of the oropharyngeal muscles and ineffective cough will cause phonation and swallowing disorders with a risk of bronchoaspiration and acute respiratory failure. Feeding and swallowing difficulties are common in SMA patients. Videofluoroscopy is considered as the gold standard in the assessment of dysphagia. NIV can be considered, in part, a palliative treatment. Invasive ventilation through the tracheal route is controversial as it increases survival but does not alter the progression of the disease. Patients who do not achieve adequate clinical response with mechanical ventilation (MV) or who cannot tolerate it, palliative treatment (such as oxygen therapy and morphine) should be considered.

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LECT 32

PRETERM DELIVERY: CLINICAL AND BIO-CHEMICAL ASPECTS

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Preterm birth represents the most important cause of infant morbidity and mortality and complicates about 6-15% of all pregnancies. The risk decreases with gestational weeks, being very important before 27 weeks of gestation. It is defined as delivery occurring before or at 36⁺⁶ gestational weeks and it is further classified by gestational age in moderately preterm (32⁺⁰-36⁺⁶ weeks), very preterm (28⁺⁰-31⁺⁶ weeks) and extremely preterm delivery (< 28 weeks). The etiology of preterm birth is not completely understood, but it is known to be multifactorial, even if most of the cases are not related to any of this causes. Maternal factors include acute and chronic diseases, such as essential hypertension, pregnancy induced hypertension (PIH), cardiopathies, nephropathies, gestational diabetes, hyperthyroidism, urinary and genital infections, sideropenic anemia, prior spontaneous preterm delivery, polyhydramnios, premature rupture of membrane (PROM), placental abruption and chorioamnionitis. Lifestyle factors like cigarette smoking, alcohol, use of substances and an inadequate maternal weight gain have an important role in both the incidence and outcome of low birth-weight neonates. Further, several previous studies have showed that a low socioeconomic and educational status were associated with a higher risk of preterm delivery. It has recently been suggested that gene-environment interactions could play a significant role in determining the risk of preterm birth. Among all causes, inflammation plays a critical role in modulating the pathophysiological mechanism

leading to spontaneous preterm delivery. In fact, high levels of pro-inflammatory cytokines have been shown to stimulate cervical prostaglandin secretion and uterine contractility in different ways: maternal and fetal stress, with high levels of CRF, cortisol, catecholamines, vasopressin, oxytocin, can stimulate fetal cortisol and prostaglandin secretion; decidual infections and chorioamnionitis, usually secondary to ascending genital infection of several microorganisms, induce the release of pro-inflammatory cytokines, i.e. interleukin-1 (IL-1) and tumor necrosis factor (TNF) by macrophages, amnion, decidua and myometrium, and the release of high concentrations of elastase with the reduction of the extracellular matrix, possible cause of premature rupture of membranes. A wide range of morbidities are significantly more common in infants born before 37 weeks' gestation compared with those delivered at term. Development of neonatal intensive care units, use of antenatal corticosteroids and post-natal treatment with surfactant and nitric oxide have permitted to improve the survival of preterm infants, above all those delivered after 33 weeks. However, in infants born before 28 weeks of gestation these factors do not preclude serious developmental impairment such as IVH (intraventricular hemorrhage), respiratory distress syndrome, necrotizing enterocolitis and retinopathy of prematurity. The following therapy strategies are currently used: tocolytic agents, cervical cerclage, and medical drugs, such as progesterone, even if no human deficiency syndrome has ever been proven. The recent OPPTIMUM trial, which tested the effect of progesterone prophylaxis in pregnancies at high risk of preterm birth, has recently been reported. This trial showed that progesterone had no effect on the three primary outcomes: birth before 34 weeks of gestation; neonatal death or short-term morbidity; 2-year cognitive score in the progesterone group versus the placebo group. New therapeutic strategies are needed, and an omics or immunological approach could open a new intervention plan.

LECT 33

PREMEDICATION FOR ENDOTRACHEAL INTUBATION IN THE NEWBORN INFANT

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Endotracheal intubation is a common procedure in newborn care, and it is often associated with

pain and cardiorespiratory instability. Invasive procedures have been shown to elicit adverse physiological responses, even in extremely preterm babies, like systemic and pulmonary hypertension, bradycardia, hypoxia and intracranial hypertension, with a potential risk of intracranial hemorrhage. The administration of vagolytic, muscle relaxants, analgesics and pre-oxygenation can reduce or eliminate the physiological responses to intubation, at the same time decreasing pain and discomfort associated with the procedure. Over recent years, use of premedication before intubation has been increasing, with both single center results and consensus statements considering this as standard management. As an exception, intubation without premedication may be acceptable during resuscitation and acute deterioration, or in infants with severely abnormal airways. In fact, in the latter case, infants' own respiratory effort may be essential for maintaining an open airway, particularly if intubation attempts are unsuccessful and the use of laryngeal mask airway or bronchoscopic intubation may become necessary. Several studies evaluating the success rate of endotracheal intubations have reported that many failed attempts can be attributed to suboptimal intubating circumstances. Indeed, the use of premedication for intubation may significantly improve intubating conditions, decreasing time and number of attempts needed to complete the procedure, and minimizing the potential for intubation-related airway trauma. Despite the growing evidence and wider acceptance for the routine use of premedication during neonatal intubation attempts, its adoption is still not universal. Furthermore, there appears to be little consensus among different centers and countries as to which medication or combination of medications is preferable. In fact, the choice of premedication regimen may markedly vary between units and over time. Ideally, any strategy for premedication before intubation should aim to effectively eliminate pain, discomfort, and physiologic abnormalities linked to the procedure, help to carry out intubation expeditiously, minimize the chance for traumatic injury to the newborn and have no adverse effects. Of note, concern of potential adverse effects has been a barrier to implementing premedication for intubation, even though most reports and randomized controlled trials have not demonstrated serious events related to premedication. A popular approach includes pre-oxygenation via a properly sized facemask, then a vagolytic agent, followed by analgesic medications and infusion of a muscle relaxant. Vagolytic drugs may blunt any

bradycardic stimulus; analgesic agents can manage the procedure-related pain and decrease adverse hemodynamic responses to laryngoscopy; muscle relaxants give the best intubating conditions. The use of sedatives alone, such as benzodiazepines without analgesic agents, should be avoided. Medications with rapid onset and short duration of action are preferable. Each procedure should be performed or supervised by individuals with adequate training and experience in airway management, both in term and preterm newborns. Propofol, a hypnotic anesthetic, has been recently proposed as an alternative regimen in some centers; it has a rapid onset of action and relaxes the oropharyngeal musculature, facilitating laryngoscopy and tracheal intubation. Moreover, apnea episodes associated with propofol seem to be rare at low dosages. However, there is still a limited and somewhat conflicting literature. Actually, doses have been empirically extrapolated from the pediatric experience, and further studies are needed to determine the best dose requirements for induction of anesthesia and tracheal intubation. In addition, several cases of hypotension after a bolus of propofol have been reported in neonates. Interestingly, nowadays the most frequent reason for intubation in preterm neonates is surfactant replacement therapy for acute respiratory distress syndrome, usually by means of the INSURE (Intubation, SURfactant therapy, Extubation) technique. Rapid recovery of the newborn's respiratory drive is essential for the success of such procedure, which implies immediate or early extubation after surfactant instillation. At least in theory, given their very short onset and duration of action, both propofol and remifentanyl appear to be the most suitable agents for premedication during the INSURE procedure. In conclusion, optimal pharmaceutical regimens and appropriate doses of available drugs for premedication before neonatal endotracheal intubation remain still unknown. In particular, further work is needed to better clarify the pharmacokinetics and pharmacodynamics of these agents in the neonatal population. Future studies will have to establish the ideal combinations and sequence of premedication drugs, balancing their expected short and long-term benefits with the risk of potential adverse effects.

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LECT 34

BRAIN MRI: WHAT'S NEW IN PRETERM INFANTS

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There are multiple lines of neurological research using magnetic resonance imaging (MRI) with different purposes in order to improve clinical practice. We know already that MRI with all its growing potentials has been widely used to promote the earliest possible diagnosis of certain perinatally acquired diseases (i.e. diffusion weighted imaging to detect arterial stroke), to improve understanding of different steps of a disease pathophysiology (i.e. 3D or 4D venography in venous thrombosis), to assess a more accurate prognostic values of the preterm brain thanks to very recent MRI applications like Diffusion Tensor Imaging (DTI). DTI is increasingly used to understand how the microstructure of white matter has changed in very preterm babies at term equivalent age, even in those without any brain lesion, and therefore to better predict long-term outcome. A new clinical way of using MRI as an equivalent of short outcome is also very challenging, like in those cases where we need to assess the efficacy of a treatment (i.e. hypothermia and reduction of brain lesions following asphyxia). In our opinion the definition of “what’s new” on neonatal brain MRI should include only those very sophisticated software techniques that have shown robust and reproducible clinical applications. In addition we should also use old techniques in a “new context”, with different eyes, thanks to the changing scenario and the changing knowledge on that subject, such as we did with conventional imaging looking for incidental findings in preterm babies scanned at term equivalent age. The rate of incidental findings was unexpectedly high, like the number of cases in which the clinical outcome could be improved. We will focus on direct clinical experience using certain MRI techniques.

SUSCEPTIBILITY WEIGHTED IMAGING (SWI) TO STUDY BRAIN HEMORRHAGES AND VENOUS STRUCTURE

SWI, a recently developed MRI sequence, is based on high-resolution, three-dimensional, fully velocity-compensated gradient echo sequences using both magnitude and phase images. SWI is very sensitive in detecting magnetic susceptibility changes in tissues, such as blood, iron and calcification. Additionally, it has been shown to be more sensitive than conventional gradient-echo sequence, or CT, in detecting small sized cerebral bleedings. We firstly used SWI in order to assess diagnostic accuracy of cranial ultrasound (CUS) in detecting low-grade Germinal Matrix Hemorrhage – Intraventricular Hemorrhage (GMH-IVH) by comparing CUS to SWI, which was used as the gold-standard technique. Incidence of grade I and grade II GMH-IVH in our very low birth weight (VLBW) population was comparable to that reported in large multicenter studies and in studies investigating the effects of low-grade GMH-IVH on neurodevelopmental outcome. In addition, CUS sensitivity in detecting grade I-II GMH-IVH proved to be surprisingly low, in contrast with specificity. Thanks to these findings we suggest that low-grade GMH-IVH may be under diagnosed in VLBW infants when assessed with CUS alone. In a further study we compared MRI and US to assess diagnostic accuracy of CUS in detecting cerebellar hemorrhage (CBH). The study was performed through different fontanelles, more precisely the anterior fontanelle (AF) and the mastoid fontanelle (MF), which is supposed to be the ideal one in detecting cerebellar hemorrhages in VLBW infants. Massive CBHs seem easily detectable through AF as well as through MF. Conversely, limited cerebellar hemorrhage is better identified through MF. Thus, our results reinforce literature data suggesting that the routine use of the MF is advisable in a clinical setting where bedside ultrasound is available. In addition, it was very interesting to note overall sensitivity of CUS in detecting CBHs, confirmed to be very low when cerebellar micro-hemorrhages were included. In other words, micro-hemorrhages proved to be undetectable by ultrasound in spite of the routine use of MF, as shown by previous studies. We have also used SWI to study the anatomy of the cerebral deep venous system, that includes subependymal veins (SV) that course into the lateral ventricles from the surrounding white matter and drain into the internal cerebral veins. SV may have an important role in the pathogenesis of brain lesions like GMH-IVH and CBH in preterm neonates. We compared the anatomy of SV evaluated on SWI venography in

three groups of neonates with normal brain MRI: 84 early preterms (gestational age [GA] < 30 weeks; 40 females, average gestational age 28 weeks), 31 late preterms (30 ≤ GA < 37 weeks; 19 females, average gestational age 33 weeks) and 50 term neonates (GA ≥ 37 weeks; 13 females, average gestational age 39 weeks). We managed to demonstrate that the deep venous system of the neonatal brain showed an even larger spectrum of anatomical variants. In particular, anatomical variants were more common in early preterm (66%) than in both late preterms (41%) and term neonates (36%). We think this is an interesting finding as the deep venous system of the neonatal brain showed a large spectrum of anatomical variants with different SV pattern distribution in preterm neonates, likely related to the influence of the preterm birth and epigenetic factors on the SV development.

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LECT 35

INTERSTITIAL STEM/PROGENITOR CELLS DURING KIDNEY DEVELOPMENT

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Multiple cell types and several cellular networks have been shown to play structural and functional roles during human kidney development. Previous studies on the morphological and functional properties of renal interstitial stem/progenitor cells during intrauterine life have highlighted their peculiar features involved in

kidney development [1, 2]. Interstitial stem/progenitor cellularity can be dissimilar in different kidneys, being high and complex in some kidneys and low and scattered in others. The most important interstitial stem/progenitor cell compartments of the developing human kidney are the capsular interstitial/stromal cells, cortical interstitial/stromal cells, medullary interstitial/stromal cells, the interstitial/stromal cells inside the renal stem cell niche, hilar interstitial/stromal cells and ureteric interstitial/stromal cells [2]. The kidney capsule is considered an important interstitial stem/progenitor cell niche, containing cells expressing nestin and other multiple mesenchymal stem cell markers, including vimentin, SCA-1, CD29 and nestin [2]. In the prenatal kidney, the sub-capsular zone, also defined as “blue strip” or small undifferentiated stem/progenitor cells zone, represents the major stem cell niche [1]. The kidney capsule has been subdivided into five zones that are structurally linked to the renal stem cell niche. Renal stem cell niche and stromal stem/progenitors residing in its inner side have been revealed to be in strict contact with the renal capsule [3]. Cell projections originating interstitial stem/progenitor cells demonstrate the existence of a structural and functional contact responsible of the exchange of morphogenic information during nephrogenesis [4]. Cortical interstitial/stromal cells play a recently reconsidered role during kidney development, starting from the early phases of nephrogenesis and influencing kidney differentiation [2]. During development, the renal cortex shows plenty of interstitial/stromal cells, giving rise to a different picture from the one observed in the mature kidney. The cortical interstitial/stromal cells are able to generate new tubules [1]. Two distinct populations from a common progenitor are identified. Both are characterized by the expression of *Osr1*: the *Six2*-expressing cells represent the progenitors of the nephron lineage, whereas *Foxd1*-expressing cells represent the non-nephrogenic lineage, that give origin to the interstitial/stromal cells, pericytes and mesangial cells [2]. Medullary interstitial/stromal cells are large cells, often surrounding the developing collecting ducts and the Henle tubules, with large oval nuclei. Medullary interstitial/stromal cells probably originate from the same common interstitial progenitor of cortical interstitial/stromal cells, characterized by the *Foxd1*+/*PAX2*- phenotype. The progressive acquisition of the expression of specific “medullary” markers, such as *FGF7*, *BMP4* and *Pod1*, is observed in the undergoing the medullary interstitial/stromal fate. *Hox10* and *Hox11* expression is required for differentiation and for their integration with collecting tubules and Henle loops. *CD133* is a characteristic marker of renal papilla

stem/progenitor cells [1]. Metanephric mesenchyme-derived medullary interstitial/stromal cells are required to generate important signals for the branching morphogenesis of the tubular structures from ureteric bud [2]. The medullary interstitial/stromal cells show similar morphologic features with capsular interstitial/stromal stem/progenitors [1]. Interstitial/stromal cells inside the renal stem cell niche are necessary for the maintenance of the complex micro-architecture of the renal stem cell niche and renal stem/progenitor cells homeostasis, maintaining stem cells in a quiescent state, or otherwise inducing proliferation and differentiation. Interstitial/stromal cells inside the renal stem cell niche regulate Wnt9b activation in ureteric bud-derived stem/progenitors, allowing proper nephron endowment during kidney development [2]. Hilar interstitial/stromal cells of the fetal kidney and in preterm infants are characterized by an elongated cytoplasm, a large nucleus, show strong Thymosin Beta-4 reactivity and are embedded in a immature loose connective tissue of the renal pelvis [2]. Stem/progenitor cells of the renal pelvis express c-Kit. They progressively inhabit the ureteral wall during kidney organogenesis and are considered as the pacemaker cells that are able to drive the urine from the upper kidney to the lower urinary bladder [1]. Ureteric interstitial/stromal cells might derive from a Tbx18+ precursor of the hilar zone at the onset of kidney development. Tbx18, myocardin, Sox9 and Smad4 are critical for inducing the smooth muscle cell differentiation during kidney development [2]. Differentiation and integration of the interstitial/stromal cells with the epithelial components of the different renal zones represent a complex process. Nephrogenesis relies on the inductive or inhibitory crosstalk between the many cell types playing different roles on the renal stage. The different interstitial stem/progenitor cells in developing kidney are formed by different cell types that characterize different renal stem cell niches and change significantly in function and origin [2]. Further studies are needed to better define the multiple types of renal interstitial/stromal cells and their specific role during nephrogenesis.

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LECT 36

THE RENAL CAPSULE: A NEW STEM CELL NICHE?

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The renal capsule is generally defined as a thin translucent fibrous layer surrounding the outer surface of each kidney, separating it from the neighboring organs, and providing some protection to the renal parenchyma from trauma and injury. While the renal capsule can usually be easily stripped from the rest of the kidney's tissue in the adult kidney, in the fetal and neonatal kidney the capsule adheres more strongly to the renal parenchyma, due to the presence of fibrous connections extending from the capsule to the fetal cortex [1]. In recent years some researchers have reported the occurrence of structural links between the developing ureteric bud ramifications, extending during human fetal development from the hilum towards the periphery of the developing kidney and the renal capsule [2]. Light microscopical analysis of the embryonic cortex of neonatal rabbit kidney showed that ureteric bud tips exhibit linear growth from the medulla to the sub-capsular region, running in parallel and evenly spaced, at an average distance of 20 μ beneath the capsule. This radial directional growth is possibly due to a structural connection between the ureteric bud ampullae and the renal capsule, and in particular to the existence of fibers running from the ampullary tips to the capsule. These fibers have been demonstrated by electron microscopy analyses in the kidney of neonatal rabbit as well as in human embryonic kidney [3]. More recent studies from the group of Minuth and coworkers revealed a strict relationship between the renal capsule and the renal stem cell niche, the latter being connected to the capsule by a peculiar extracellular matrix [4]. All these data taken together highlight a new role for the renal capsule during nephrogenesis: from simple protecting role from neighboring organs, to the director of the radial growth of branching morphogenesis from the medulla to the periphery of the developing kidney. On the basis of these new findings on the role of the renal capsule in nephrogenesis, this work aimed to analyze the renal capsule and the cells homing inside it during different phases of human development, for a better knowledge of this complex structure and its role

in human nephrogenesis. Human renal capsule first revealed the presence of an unexpected huge number of intracapsular cells with marked differences regarding shape, volume and chromatin organization (**Fig. 1A**).

Capsular cells are extended from the capsular region towards the metanephric mesenchyme cells localized in the sub-capsular areas. The strict morphological similarities between a subset of capsular cells and

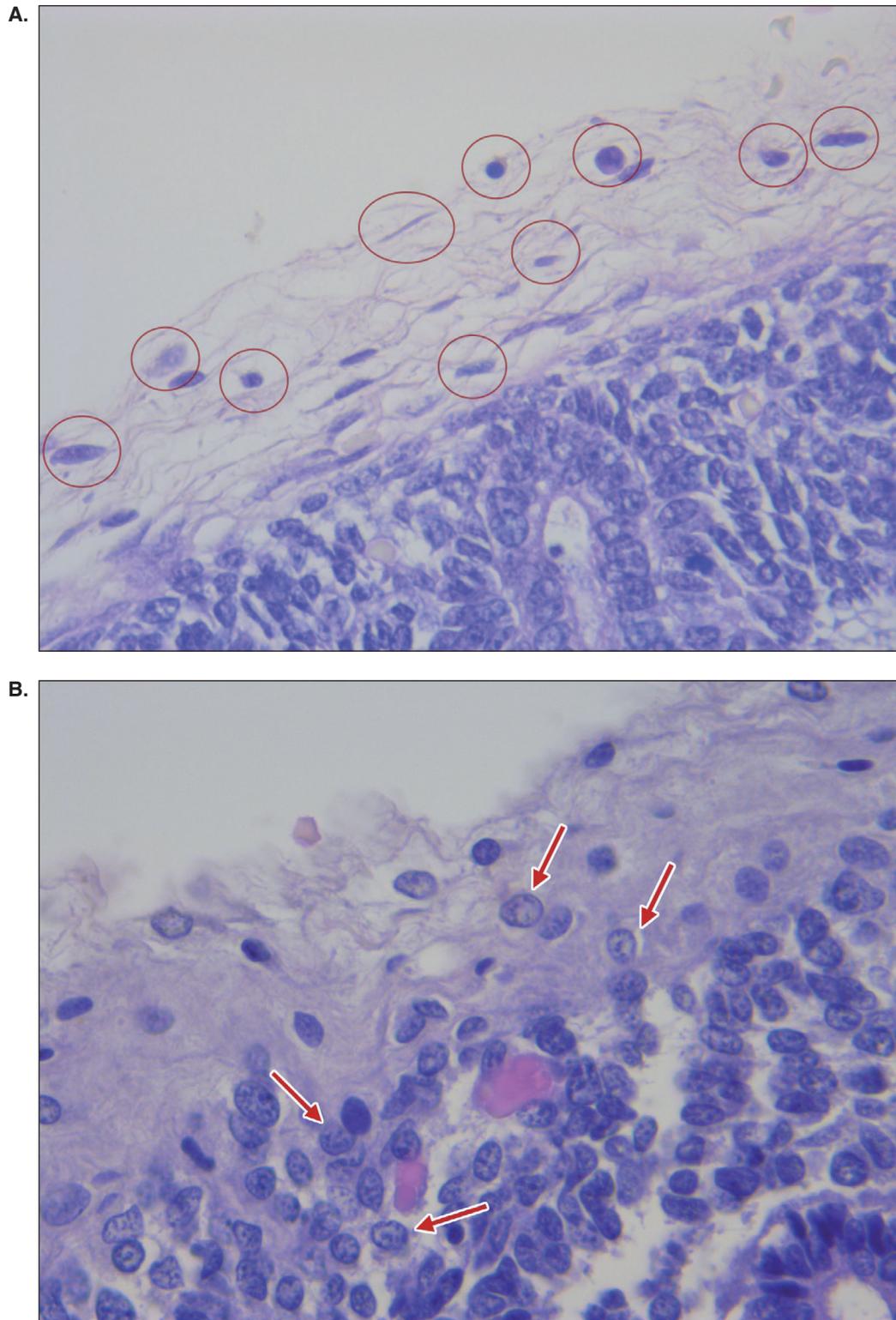


Figure 1 (LECT 36). Renal capsule stem cells. **A.** Intracapsular renal cells showed different morphology, and their nuclei were characterized by marked differences regarding shape, volume and chromatin. **B.** Intracapsular cells showed strict nuclear similarities with metanephric mesenchymal cells in the subcapsular nephrogenic zone.

the metanephric mesenchymal cells localized in the nephrogenic zone suggests that the fetal renal capsule putatively represents the homing for stem/progenitor cells. The finding of capsular cells migrating from the capsule towards the underlying metanephric mesenchymal cells suggests the hypothesis that the renal capsule probably plays a relevant role in human nephrogenesis. Another intriguing finding emerging from our study is the absence of a well-defined border between the capsule and the underlying nephrogenic zone, also known as the blue strip [5, 6]. Capsular cells were frequently observed to extend from the capsular region towards the metanephric mesenchyme cells localized in the sub-capsular areas, suggesting the existence of a strict relationship between these two compartments of the fetal kidney (**Fig. 1B**). These data suggest that renal capsular cells might derive from the outer layers of the metanephric blastema and lay stress on the necessity of giving a new role to the renal capsule: a new stem progenitor stem/progenitor cell niche.

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LECT 37

PODOCYTE CHANGES IN THE EARLY PHASES OF HUMAN KIDNEY DEVELOPMENT SUPPORT THE HYPOTHESIS OF THE PRENATAL ORIGIN OF PODOCYTOPATHIES

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In recent years, podocytes have conquered a relevant position in the pathogenesis of multiple kidney diseases affecting both children and adults. The reason for the critical position of podocytes in renal pathology is probably due to the inability of this glomerular cell type to regenerate after birth. As a consequence, any agent causing podocyte cell death – including drugs, toxicants and infective agents – may result in permanent podocyte depletion, leading to adhesion of the glomerular tuft with parietal cells, ending with crescent formation and glomerular obsolescence [1]. The podocyte depletion hypothesis has encouraged nephropathologists to develop new quantitative methods, able to estimate the podocyte burden in kidney samples, both during the intrauterine renal development and in patients affected by kidney disease. Recently, a study from our group revealed that the median podocyte number per glomerulus decreases during gestation, suggesting a previously unreported plasticity of podocyte precursors in the prenatal phases of nephrogenesis [2]. These findings underline the critical value of a quantitative evaluation of the podocyte number in the evaluation of glomerular function and, in particular, of the susceptibility of a single patient to develop a clinically evident podocytopathy later in life. From a practical point of view, during the last years our group progressively changed the attention on the different renal structures whose change during gestation might represent the *primum movens* responsible for the development of chronic renal disease later in life. First, we focused on nephron number, revealing the existence of a marked interindividual variability in glomerular number among preterms of the same gestational age [3]. Subsequently, we considered that the evaluation of nephron number was not sufficient for an optimal evaluation of kidney fisiopathology, and we focused on the evaluation of the multiple cell types of the glomerulus, including podocytes [4]. In a previous experience of ours [2] 62 fetuses and newborns, ranging in gestational age from 20 up to 41 weeks, without evidence of renal pathology were enrolled in the study. Subjects were subdivided into three groups according with gestational age: group 1 (with gestational age < 24 weeks); group 2 (39 preterm neonates whose gestational age ranged from

25 to 36 weeks); group 3 (18 at term newborns, ranging from 37 up to 41 weeks of gestation). In each kidney section, we analyzed the podocyte number in ten glomeruli. The podocyte number was significantly different in the three groups, progressively decreasing during gestation. In kidneys of group 1, the average podocyte number was $1,908 \pm 645$; in group 2 the mean podocyte number lowered to $1,394 \pm 498$; in group 3, the average podocyte count was $1,126 \pm 256$. Statistical analyses clearly showed that the difference in podocyte number between fetuses (group 1) and at term neonates

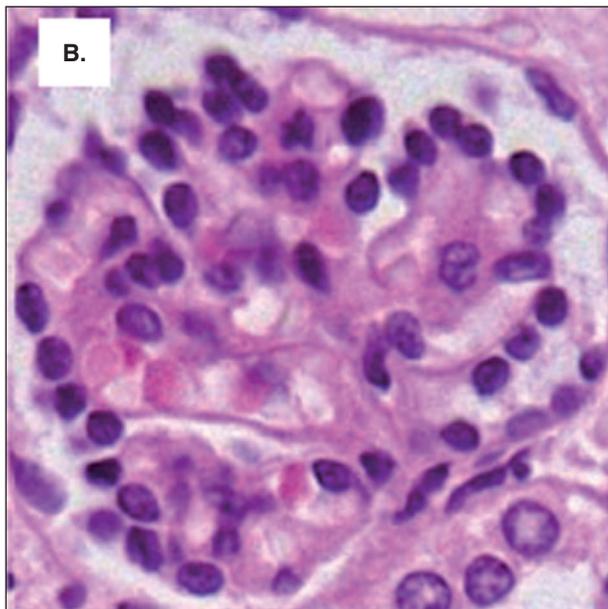
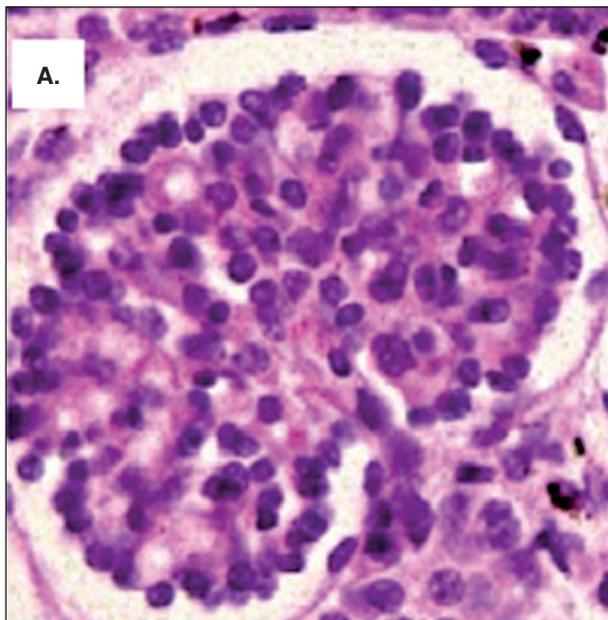


Figure 1 (LECT 37). Glomeruli of subjects at (A) 23rd (fetus) and (B) 41st (at term) week. Podocyte number significantly decreased with increasing gestational age.

(group 3) was significant ($p < 0.001$). No significant correlation was detected between sex and podocyte number. In the early phases of nephrogenesis (group 1) every glomerulus was characterized by a huge amount of podocyte precursors. A percentage of these precursors progressively disappeared with the progression of gestation, ending with the disappearance of about 40% of podocytes at the end of gestation. The mechanisms underlying the selection of podocyte precursors is, at the best of our knowledge, unknown and deserves further studies. Moreover there is a marked variability of podocyte number between subjects of the same gestational age. Differences in podocyte number were detected between subjects of all the three groups, suggesting that epigenetic factors responsible for podocyte loss may act in all the phases of gestation. This finding lays stress on the possibility that even for podocytopathies, the fetal programming hypothesis might represent a key theory, useful for a better understanding of the link between our life as a fetus and the susceptibility to develop chronic kidney disease later in life [5-27].

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LECT 38

METABOLOMICS IN NEPHROLOGY: FROM THE FETUS TO THE ADULT

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Metabolomics is a technology of great interest in the early diagnosis of kidney diseases [1-11] and recently it has been defined as a “new uroscope in town”. We will briefly discuss data obtain in animals, in clinical studies performed in adults, in pediatric nephrology [6] and in amniotic fluid. Experimental studies [6] have been performed mainly in gentamicin-induced damage in rats, in acute kidney injury and in chronic renal failure. In particular, in studies using experimental models of acute kidney injury (AKI), the levels of the following 3 groups of metabolites were found to be markedly increased in rat plasma and brains: 1) 3-indoxyl sulfate, that apparently derives from the tryptophan pathway with the involvement of the intestinal microbiome. It accumulates in the biofluids (blood and urine) of uremic patients due to reduced or absent urinary excretion during AKI and/or chronic kidney disease (CKD); 2) p-cresol sulfate (PCS, deriving from bacterial fermentation of tyrosine in the gut); 3) the metabolites derived by the catabolism of tryptophan (kynurenate, kynurenin, 3-indole lactate). The involvement of indoxyl sulfate and kynurenine, together with xanthurenic acid, suggests a complex dialogue between the gut microbiome. Some of these metabolites are elevated in CKD as well. More studies are needed to unravel the pathogenetic role of the gut microbiota in acute and chronic kidney disease and to evaluate if therapeutic interventions to manipulate the microbiota have a therapeutic potential to correct CKD [3]. In adults, metabolomics has been studied in CKD, autosomal dominant polycystic disease, urinary tract infections, membranous nephropathy, diabetes associated renal dysfunction, rhabdomyolysis. A summary of the main metabolites found in these studies is presented in **Tab. 1** [6]. Considering the urinary tract infections (febrile forms with *E. coli* as the main pathogen) some compounds (e.g., trimethylamine and acetate) are connected with a bacterial infection in the urine, while others (e.g., p-aminohippuric acid [PAH], scyllo-inositol) seem markers of morbidity. Moreover, some metabolites suggest specific pathologies. Another relevant metabolite is indoxyl sulfate, which derives metabolism of enteric gut. Further information could be obtained on geography of damage (**Tab. 2**) [2]: an increase of urinary TMAO could be related to medullary injury; again, an increase of dimethylamine, sorbitol and myo-inositol could indicate the presence of a generalized

tubular disorder, which also includes the kidney papilla. On the other hand, a decrease of citrate, alpha-ketoglutarate and succinate is expression of tubular proximal cells mitochondrial dysfunction, since only these cells can compensate for inhibition of their mitochondrial Krebs cycle. A decrease of the excretion in citrate is indicative of a malfunction of the kidney tubule. About metabolomics in pediatric nephrology, limited data are available in this age group [6, 10, 11]. Early urinary metabolomics (samples obtained 4 and 12 h after surgery) identified homovanillic acid (HVA-SO₄), a metabolite of dopamine, as a new, sensitive, predictive and early AKI biomarker in children after heart surgery. At the moment there is a need to improve the number of studied patients as well as the standardization of methods. In amniotic fluid, high glutamine level, glutamine/glutamate ratios, increase in glycoprotein P1, and lower urea level reflect kidney disorders/underdevelopment. The final goal of metabolomics could be to find specific metabolites for common and rare nephropathies and to prepare easy, quick and reliable tools, like “dipsticks for urine”, able to differentiate patients from controls, or a patient before and after a nutritional or pharmacologic intervention. In few words, we could hope to use the right drug for the right patient (pharmametabolomics): translational medicine from top research to bedside [1, 3]. Surely, we can predict that in the next few years each nephrologist should be extremely expert in the “metabolomics world”.

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Table 1 (LECT 38). Main urinary metabolites found in kidney diseases in humans.

Metabolomics biomarker	Kidney disease in humans
P-aminohippuric acid Scyllo inositol	Urinary tract infection
Fenyl acetyl glutamine	Interstitial cystitis
Metanol	ADPKD
3 indoxyl sulfate	Renal dysfunction (acute kidney injury, chronic kidney injury, diabetes)
P-cresol sulfate	Renal dysfunction (acute kidney injury, chronic kidney injury)
Kynurenate, kynurenine, 3 indol lactate	Acute kidney injury

Table 2 (LECT 38). Geography of damage and metabolomics markers.

Geography of damage	Metabolomics markers
Medullary injury	↑ TMAO (trimethylamino oxyde)
Papillary damage	↑ Dimethyl amine, sorbitol, myo-inositol
Tubular proximal cells mitochondrial dysfunction	↓ Citrate, αketoglutarate, succinate ^a

From: Christians U. et al., 2010 [2], modified.

^a Only proximal tubulus is able to compensate for inhibition of their mitochondrial Krebs cycle.

LECT 39

MICROARCHITECTURE OF THE HUMAN NEPHROGENIC ZONE: AN UPDATE

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On August 2010, the 11th international workshop on developmental nephrology took place in New Paltz, near New York (USA), with the participation of leading researchers involved in the study of nephrogenesis coming from all over the world. Six years later, looking at the handouts of that meeting, it emerges that many progresses have been achieved towards a better understanding of nephrogenesis. The New Paltz meeting is now considered as an important platform for the development of the modern molecular nephrology, mainly based on experiments carried out in multiple animal species and culture systems, that allowed a better understanding of the molecular mechanisms that control renal progenitor cell differentiation pathways during nephrogenesis. What was missing at that meeting was the ability to transfer experimental data to human embryology and in particular to human nephrogenesis, due to the presence of very few pediatric nephrologists, with the exception of one of the authors (VF). Few months later, in October 2010, a new research group founded with the aim of translating the New Paltz meeting data to human nephrology published the first article in which the analysis at autopsy of a large series of human neonatal kidneys revealed the marked variability of nephron number among newborns of the same gestational age, introducing a new role for epigenetic factors acting on the mechanisms regulating nephrogenesis during gestation [1]. By applying the knowledge of the sequence of morpho-molecular events regulating nephrogenesis, acquired by developmental biologists, we were able to look at human embryogenesis with new eyes, and were forced to apply immunohistochemical stains to the interpretation of the neonatal kidney, in order to better characterize the multiple cell/progenitor types involved

in glomerulogenesis and tubulogenesis [2]. One year later, an article from the same group extensively analyzed, for the first time, the molecular mechanisms involved in human kidney development, translating experimental data to human embryology and laying stress on the specificity of human nephrogenesis, both in physiology as well as in pathology [3]. The comprehension of the basic mechanisms regulating nephrogenesis in humans, associated with the knowledge of the consequences of a low nephron number at birth on the susceptibility to develop renal insufficiency later in life, induced our group to move to a new fascinating field: the “physiological” regenerative medicine of preterm infants [4]. Since nephrogenesis in preterm newborns continues for about 4-6 weeks after birth, we hypothesized that a better knowledge of the mechanisms able to accelerate glomerulogenesis might represent a new, powerful tool in the hands of neonatologists. The goal of this new “physiological” regenerative medicine, that should be started immediately after premature birth, would be to prolong nephrogenesis until the 36th week of post-conceptual age, allowing premature babies to escape oligonephronia and susceptibility to develop hypertension and chronic renal failure in adulthood [5]. The presence of a huge amount of stem/progenitor cells with a high nephrogenic potential in the preterm human kidney reinforces our hypothesis that it may be possible to prolong nephrogenesis in preterm babies (Fig. 1). Considering our current knowledge regarding the fetal and perinatal programming, this might represent an important step for the primary prevention of renal failure, by reducing the incidence and global health impact of chronic renal disease due to oligonephronia [6]. In conclusion, the lesson emerging from the experiments carried out on nephrogenesis in zebra fish, as well as in chick embryos and in mice presented at the New Paltz meeting, when translated to human physiopathology, was able to boost a new research team on human nephrology, nowadays aimed to introduce regenerative renal medicine in neonatology. We hope that our work will help premature babies to escape oligonephronia and kidney disease later in life.

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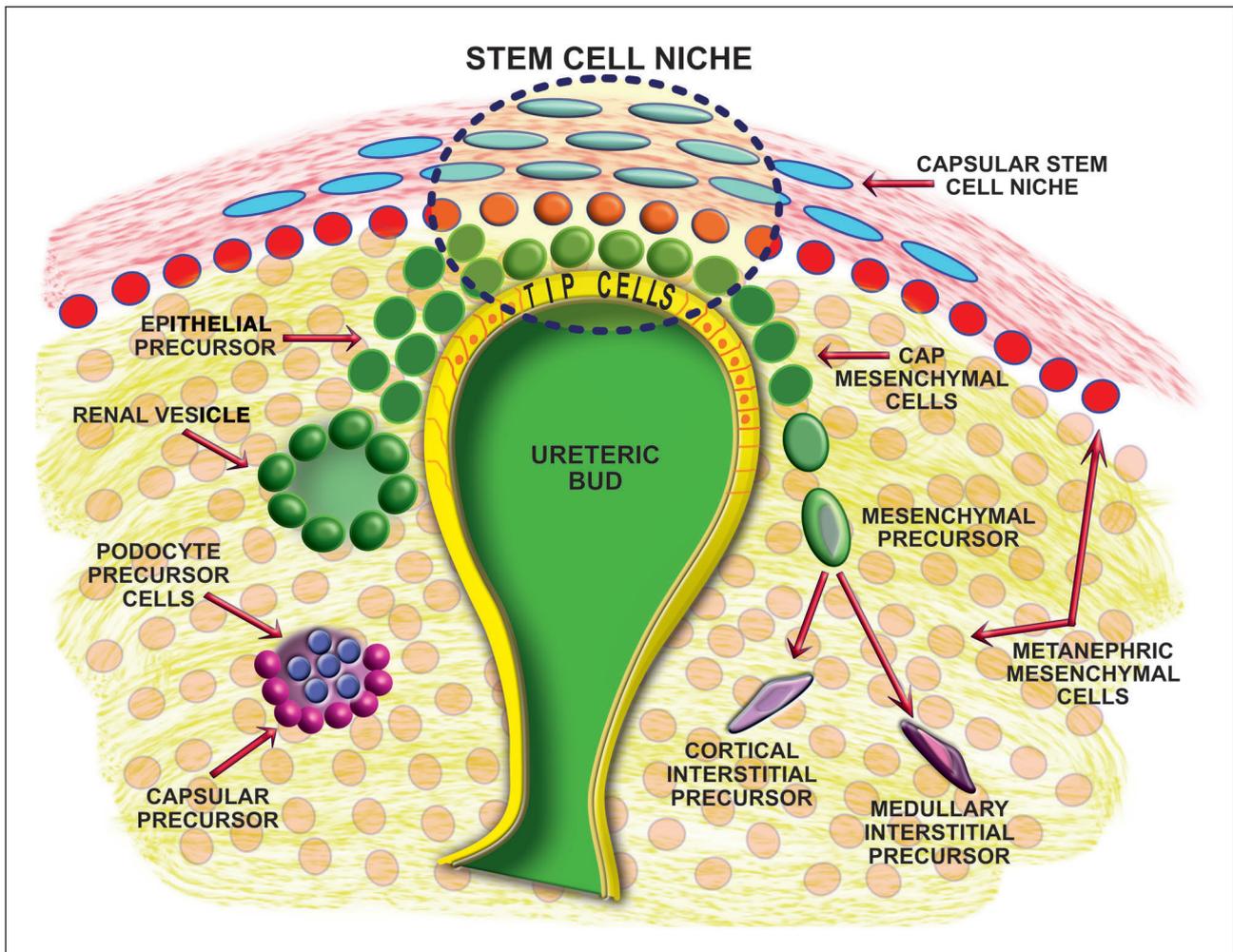


Figure 1 (LECT 39). Stem/progenitor cells operating in the preterm human kidney.

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LECT 40

PRENATAL DIAGNOSIS OF MALFORMATIVE UROPATHIES

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The sonographic prenatal screening involves performing an ultrasound scan in the first, second, third trimesters of pregnancy. By ultrasound it is possible to assess the presence of the bladder and the renal arteries as early as the first trimester. However, the morphology of the kidney is clearly visible only in the second trimester screening scan. In the first trimester (before 14 weeks of gestation) the diameter of the bladder is always below 7 mm. An increased value suggests the diagnosis of megacystis, which is always a sign of early urethral obstruction. In most cases in which the diameter of the bladder is between 8-12 mm, we witness a spontaneous resolution. In all cases in which the value exceeds 15 mm, fetuses are diagnosed with obstructive uropathy associated with renal dysplasia. Ultrasound screening in the second trimester of pregnancy has resulted in

increasing recognition of fetal hydronephrosis. The prevalence range is 2-5.5% and it depends on diagnostic criteria. The condition is bilateral in 17-54%. Hydronephrosis defined as moderate is when the antero-posterior renal pelvic diameter (APRPD) is ≥ 4 mm and it appears to be associated with an excellent prognosis. Moderate/severe hydronephrosis, defined for APRPD ≥ 7 mm (with dilation of the renal calices) is associated with a worse prognosis and it is a condition that requires closer monitoring. The most important prognostic factor is considered evolutivity; for this reason, the finding of hydronephrosis during the second trimester screening involves performing a second control scan at 28 weeks of gestation. Third trimester scanning is more specific for diagnosing of antenatal hydronephrosis than second trimester scanning. A persistent hydronephrosis is defined when the renal pelvis are > 10 mm in the third quarter. The amount of amniotic fluid and the bladder volume represent important indirect indices of renal function. The evolution of hydronephrosis over time and the association with other signs allow us to perform a differential diagnosis of transient hydronephrosis (41-88%), pelvic-ureteric junction obstruction, mega-ureter (13-30%), vesico-ureteric reflux (10-20%), vesico-ureteric junction obstruction, megaureter (5-10%), multicystic dysplastic kidney (4-6%), duplex kidneys (\pm ureterocele) (2-7%), posterior urethral valves 1-2% and other uncommon anomalies.

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LECT 41

PRENATAL AND POSTNATAL URINARY TRACT DILATION: CLINIC AND ECHOGRAPHIC FOLLOW-UP

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Urinary tract (UT) dilation is frequently sonographically identified in fetuses: it can reflect the presence of possible congenital anomalies of the kidney and urinary tract (CAKUT) but, in the majority of the cases, it is transient or physiologic and has no clinical significance. Currently there is a lack of consensus and uniformity in defining and classifying UT dilation, this is one of the main reasons of the poor correlation between prenatal and postnatal ultrasound findings and the ultimate urological diagnosis. We report the widest classification systems in use by medical specialists [1-4] and the most recent UT dilation classification system (UTD) [5] proposed by a consensus conference of eight scientific societies of radiologists, nephrologists and urologists in order to develop a unified grading system and a standardized scheme of follow-up for perinatal UT dilation.

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LECT 42

CAKUT AND THE PEDIATRIC NEPHRO-UROLOGIST TODAY

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CAKUT (Congenital Anomalies of the Kidney and Urinary Tract) include a series of morphological and/or functional renal anomalies and urinary tract diseases. They represent 20-30% of all alteration identified in the antenatal period and occur in 3-6/1,000 live births. CAKUT includes different levels of alterations in the urinary system: hydronephrosis, vesicoureteral reflux, duplex collecting system, megaureter, and kidney dysplasia. CAKUT are associated with urinary tract infection (UTI) and constitute the major cause of renal failure in the pediatric age and one of the major factors in young adults needing renal replacement therapy, but the majority of children with CAKUT is in good health, has a good prognosis and does not require surgical therapy. An important role to improve the natural history of these syndromes is the prenatal diagnosis with ultrasound. It is clear that establishing a prognosis in the prenatal period is not easy. The level of dilatation of the urinary tract is not always correlated with the prognosis. The finding of oligohydramnios in gestational age is an excellent indicator of abnormal renal function in the fetus. It is always important to evaluate the presence of a visible ureter [1] during fetal ultrasound and to confirm its presence after birth. The ultrasound data in antenatal and postnatal period do not always correlate with diagnosis and prognosis. There are no indications to anticipate the date of delivery of fetuses with CAKUT considering the risks related to premature birth. After birth, almost all children with CAKUT are in good health. The most sensitive, but fortunately rare, cases are males affected by Posterior Urethral Valves (PUV), which may present important renal failure at birth. When indicated it is suggested the introduction of antibiotic prophylaxis accompanied by instrumental investigations such as ultrasound, micturating cystourethrogram (MCUG), renal scintigraphy and magnetic resonance. Often in children with CAKUT it is necessary to extend the follow-up after adolescence. Unfortunately, however, a proper transitional care between pediatric nephrourologist and adult nephrologists/urologists is often missing. Treatment of this kind of diseases, when possible, is more conservative and in a constant effort to preserve renal function. Antibiotic prophylaxis should be considered in selected situations to prevent recurrent UTI and/or renal damage. Surgery is proposed only for cases of severe functional impairment [2] and in patients with chronic UTIs. Current criteria that bring us to decide for surgery are the following:

recurrent UTIs, especially in children under 1 year of age, children with VUR of high bilateral grade, complicated double district kidney (**Fig. 1**) with or without ureterocele, worsening hydronephrosis > 3 cm or with visible changing during the follow-up (**Fig. 2**), bilateral dilatation and pain symptoms. Surgical correction includes preferably endoscopic, laparoscopic and robotic approaches [3]. The conservative approach in CAKUT is

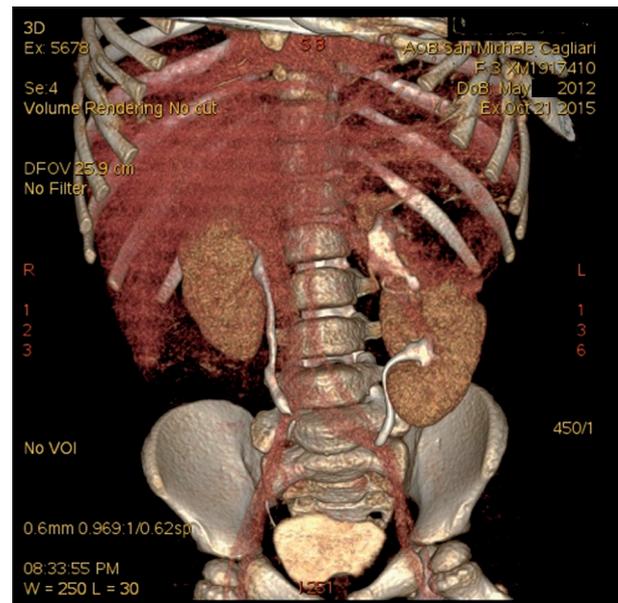


Figure 1 (LECT 42). Renal computerized tomography: upper pole double district, left kidney, complicated by an abscess in a 3-year-old female.

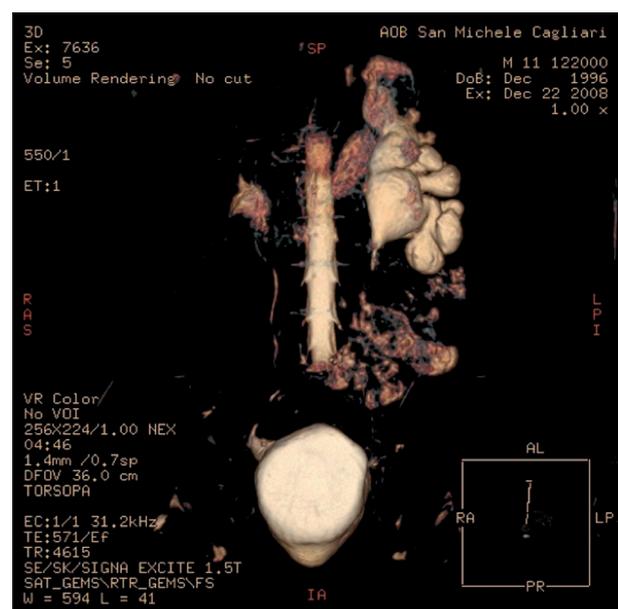


Figure 2 (LECT 42). Magnetic resonance urography: left uretero-pelvic junction obstruction in a 12-year-old male.

prevalent. The role of the pediatric nephrologist is to preserve the function of the kidney as long as possible, avoiding a worse dysplasia, which can be caused by obstruction and recurrent UTIs. It is important to identify a population at risk, differentiating between CAKUT with and without clinical connotation, applying a tailored approach to the individual patient, avoiding unnecessary investigations and treatments and finally encouraging a better interaction between gynecologists, pediatricians, nephrologists and urologists.

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LECT 43

RENAL CHANGES IN EXTRARENAL PATHOLOGIES: DOWN SYNDROME AND BETA-THALASSEMIA

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For many years, kidney disease has not been considered a frequent complication in patients affected by congenital extra renal diseases, including Down syndrome (DS) and Beta-thalassemia (BT). In recent years, developmental pathological changes have been reported in patients affected by chromosomal abnormalities. In particular, renal pathological changes have been described in subjects with DS, with emphasis on glomerular lesions [1]. Moreover, congenital podocytopathy with podocyte loss and glomerular crescents has been described by our group in a newborn, prospecting the hypothesis that glomerulopathies might originate in the perinatal period or, putatively, in the intrauterine life [2]. On the basis of these data, this study aimed to analyze the kidney of fetuses affected by DS and BT, in order to verify the presence of renal changes

originating in the intrauterine life in carriers of these two chromosomal abnormalities. 10 fetuses with a genetic diagnosis of trisomy 21, DS, and 5 carriers of BT, with a gestational age ranging from 13 up to 22 weeks, from women who underwent voluntary interruption of gestation, were analyzed in this study. At autopsy, kidneys were formalin-fixed, routinely processed and paraffin-embedded. Tissue sections were stained with H&E and Periodic Acid Schiff stain. 10 healthy fetuses were utilized as a control group. The kidneys of subjects with DS showed larger glomeruli, as compared with fetuses of the control group (**Fig. 1**). Moreover, some large glomeruli with an “unconventional” shape were detected in all DS kidneys: they appeared asymmetric (**Fig. 2**), and sometimes they appeared fused together (**Fig. 3**). Pathological changes were also detected in the kidney of BT subjects. The lesions were mainly observed at glomerular level, glomerular hypertrophy (**Fig. 4**) representing the most relevant change, with sinusoidal dilatation (**Fig. 5**). Sometimes the Bowman space appeared enlarged (**Fig. 6**). Moreover, a significant reduction of podocyte precursors was constantly detected in a percentage of glomeruli suggesting the diagnosis of congenital podocytopathy. Our preliminary data clearly show that pathological renal changes may be present before birth in carriers of congenital diseases, in which normally kidney disease is not considered a frequent complication [1]. In particular, we want to underline the presence of relevant renal changes in carriers of BT, in whom podocytes appeared to be the principal target of glomerular pathology. This finding confirms previous data from our group, regarding the possibility that podocytopathies might originate during intrauterine life, and represent a new chapter of the fetal programming of adult renal diseases. In conclusion, our findings suggest to perform regular monitoring of renal function in subjects affected by DS and in carriers of BT, in order to detect early renal injuries possibly originating by some derangement of nephrogenesis occurring before birth. Further immunohistochemical studies are needed, utilizing antibodies against WT1 (Wilms tumor 1) [3] in order to better evaluate changes in podocyte number in DS and BT patients, paralleling changes in podocyte burden previously reported to occur during gestation [4].

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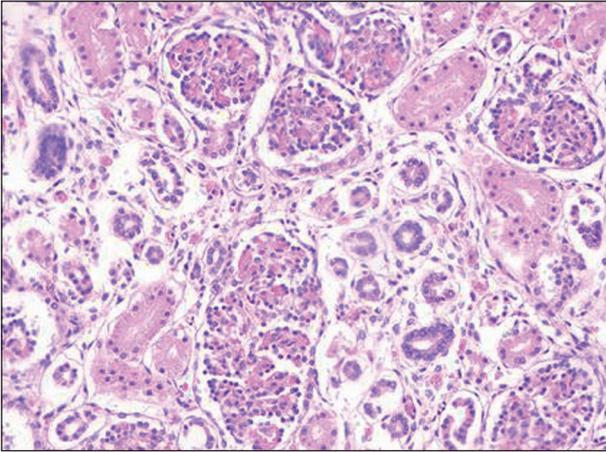


Figure 1 (LECT 43). Large glomeruli in a fetus of 19 weeks.

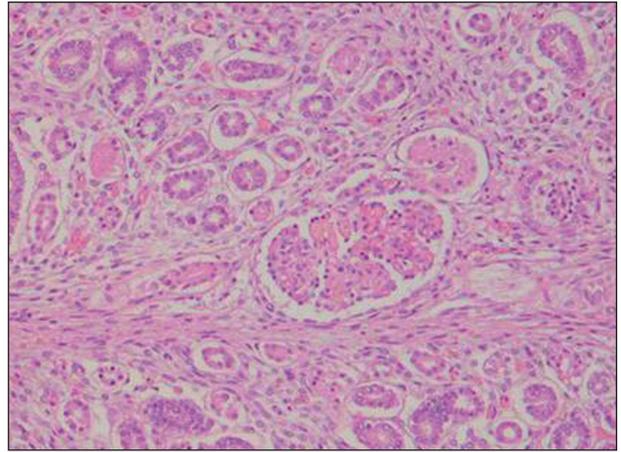


Figure 2 (LECT 43). Glomeruli appeared asymmetric.

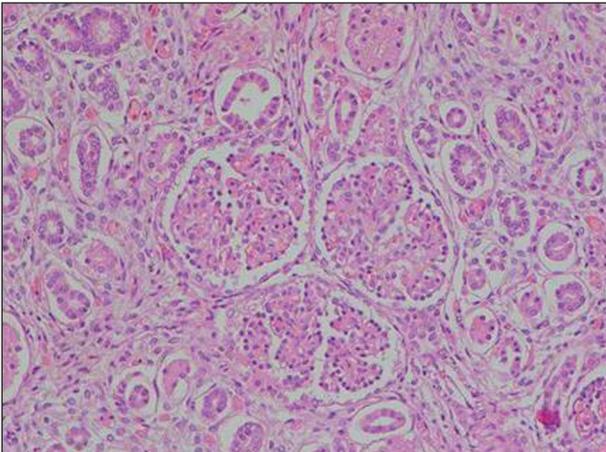


Figure 3 (LECT 43). Three glomeruli appeared fused together.

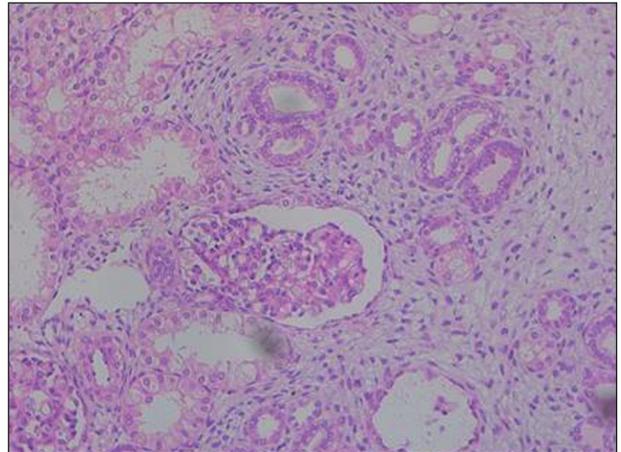


Figure 4 (LECT 43). Glomerular hypertrophy in an embryo of 14 weeks.

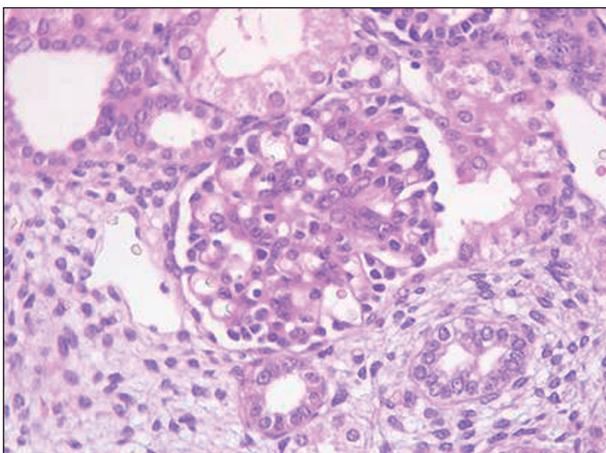


Figure 5 (LECT 43). Glomerular capillary dilatation.

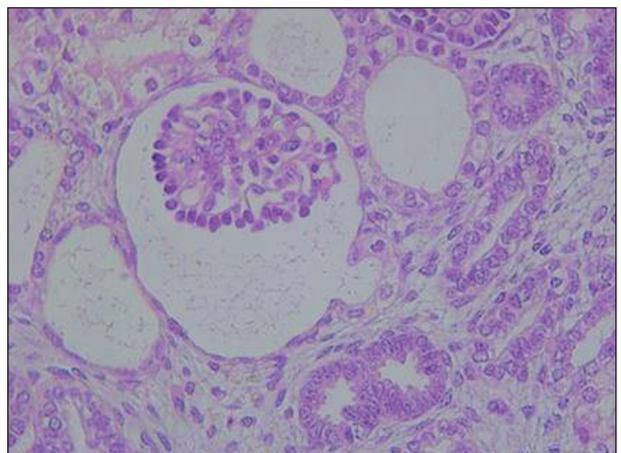


Figure 6 (LECT 43). Enlarged Bowman space.

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LECT 44

DOES THE RABBIT NEPHROGENIC ZONE IN CULTURE REFLECT CAUSES FOR IMPAIRED NEPHROGENESIS?

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Intrauterine as well as extrauterine influences are held responsible to cause prematurity of renal parenchyma and impaired nephrogenesis in preterm infants, leading to a high incidence of severe kidney diseases later in life. Although involved noxae and resulting molecular effects are quite different, all of them converge to the nephrogenic zone, that is restricted to the outer cortex of a developing kidney. Covered by the organ capsule, it consists of aligned ureteric bud-derived collecting duct (CD) ampullae, containing epithelial stem cells, nephrogenic mesenchymal stem cells, renal vesicles and S-shaped bodies. Due to the complex composition of the nephrogenic zone and the different noxae, it is appropriate to investigate impaired nephrogenesis by an adequate *in vitro* system. In this case, microsurgical isolation and culture of the nephrogenic zone from neonatal rabbit kidney is particularly well suited. In fact, it shows a microarchitecture which is largely comparable with the human specimen. Moreover, a decisive advantage is that it can be easily and quickly isolated in original composition by microsurgical techniques and pieces of the explant are consequently available for a variety of advanced culture experiments. Formation of renal spheroids can be used for drug toxicity testing. Mounting in a tissue carrier makes it possible to register functional differentiation of the CD epithelium. Perfusion culture within an artificial interstitium enables to investigate spatial development of parenchyma. On the one hand these recent findings define the route to solve future tasks, on the other hand actual pathologic data inform about intrinsic cell biological risks during generation of renal parenchyma.

LECT 45

IS THE PLACENTA AN INNOCENT BYSTANDER IN PERINATAL PROGRAMMING?

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During pregnancy, a well functioning placenta is needed to ensure appropriate growth and development of the fetus [1]. Indeed, a mal-functioning or “insufficient” placenta has been recognized as the “cause” of Intrauterine Growth Restriction (IUGR) [2], leading to decreased oxygen delivery as well as altered placental transport of nutrients, mainly amino acids and lipids, but also micronutrients such as iron and folate. A number of previous studies from our lab support this hypothesis, demonstrating a specific placental phenotype of IUGR [3], recently confirmed with decreased levels of placental Transferrin Receptor (TFRC – mediating cellular iron uptake) or of Sodium-coupled Neutral Amino acid Transporter 2 (SNAT2) in IUGR *versus* controls [4, 5] (summarized in **Tab. 1**). Maternal nutritional status, diet and exposure to environmental factors are increasingly acknowledged as potentially affecting placental gene expression, thus modifying placental function. These epigenetic associations link intrauterine environment to adverse perinatal outcomes reprogramming the fetal epigenome with several mechanisms, such as methylation or miRNA, thus affecting gene expression and activity in preeclamptic (PE) and IUGR tissues [6]. Changes in miRNA expression pattern have been observed in placental tissue and associated with several pregnancy pathologies as preeclampsia (↓ miR-21, ↑ miR-155, ↓ miR-223), GDM (↓ miR-132), IUGR (↓ miR-21, ↓ miR-210) and preterm birth (↑ miR-493, ↑ miR-338) [7]. In this context, an active placental metabolism is crucial to support both trophoblast invasion and placentation [8]. Alterations in early implantation may lead to mismatches in oxygen (O₂) delivery to different areas of the placenta, with less O₂ exchange between the uterine and the umbilical circulations [9]. Mitochondrial DNA (mtDNA) copy number is positively correlated with the number of mitochondria. We have previously demonstrated altered mitochondrial content in IUGR placentas [10], with higher mtDNA levels in IUGR maternal blood [11]. Moreover, we measured the functionality of the respiratory complexes (RCC) by high-resolution respirometry (HRR), in order to assess potential alterations in placental energy metabolism [12] (summarized

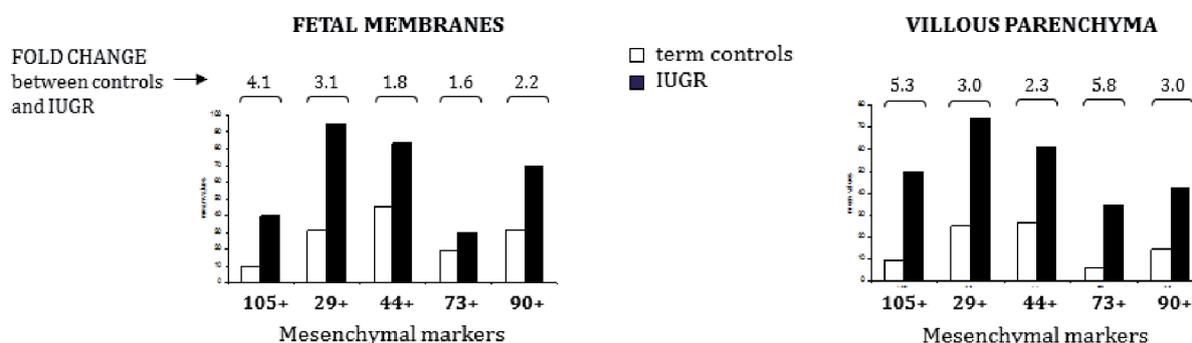
Table 1 (LECT 45). Research findings in normal and abnormal placental development.

References	Topic	Study Description	Results
Mandò et al., <i>Pediatr Res</i> , 2013 [4]	Sodium-Coupled Neutral Amino acid Transporter 2 (SNAT2)	SNAT2 gene expression and its intron-1 levels methylation	mRNA levels significantly decreased in intrauterine growth restricted (IUGR) placentas, with reduced umbilical blood flows. Methylation levels were steadily low in both IUGR and controls
Mandò et al., <i>Placenta</i> , 2011 [5]	Transferrin Receptor 1 (TFRC), cellular iron uptake on trophoblast membranes	TFRC mRNA and protein expression and localization in 50 IUGR and 56 control placentas	TFRC gene and protein expression significantly lower in IUGR placentas vs controls ($p < 0.05$), especially in the most severe IUGR group. TFRC protein was predominantly in syncytiotrophoblasts
Lattuada et al., <i>Placenta</i> , 2008 [10]	Mitochondrial DNA (mtDNA) content in control and IUGR placentas	mtDNA content (Real-Time PCR) in 26 control pregnancies (AGA) and 24 IUGR	Increased mtDNA content ($p = 0.004$), inversely related to oxygen (O_2) tension in the umbilical vein
Colleoni et al., <i>Am J Obst Gynecol</i> , 2010 [11]	mtDNA content in maternal circulation of pregnancies complicated by IUGR	MtDNA in 13 non-pregnant women; 45 control pregnancies, in 3 trimesters; and 12 complicated by IUGR	Highly significant progressive reduction in circulating mtDNA in pregnant women of I-II-III trimesters compared to non-pregnant women ($p < 0.001$). mtDNA content was significantly increased in women with IUGR fetuses compared control ($p < 0.001$)
Mandò et al., <i>Am J Phys Endocr Metab</i> , 2014 [12]	mtDNA and nuclear respiratory factor 1 (NRF1) levels in placental tissue and cytotrophoblast cells; gene and protein expression of respiratory chain complexes (RCC) and their O_2 consumption	mtDNA, NRF1, RCC expression (Real-Time, Western Blotting), O_2 consumption (HRR) in 8 IUGR, 6 PE, and 8 pregnancies	Lower mRNA levels of mt complexes II, III, and IV in IUGR cytotrophoblast cells; no differences at the protein level. mtDNA increased in IUGR placentas ($p < 0.017$). Both mtDNA and NRF1 expression lower in isolated cytotrophoblast cells ($p < 0.05$). RCC activity was increased in placentas of IUGR fetuses ($p < 0.017$)
Mandò et al., <i>Stem Cells Transl Med</i> , 2016 [17]	Characterization of cells isolated from placental membranes and basal disc (pMSCs) of IUGR and physiological placentas	Viability and proliferation of cell culture. Hematopoietic, stem, endothelial, and mesenchymal markers (flow cytometry). Multipotency of pMSCs and expression of mt genes (Real-Time PCR)	Cell viability high in all samples, proliferation rate lower in IUGR compared to controls. Multipotency of IUGR pMSCs was restricted because their capacity for adipocyte differentiation was increased, whereas their ability to differentiate toward endothelial cell lineage was decreased. Mitochondrial content and function higher in IUGR pMSCs

in **Tab. 1**). Preliminary observations suggest similar changes in placental mitochondria, DNA content and function of obese pregnant women. These pregnancies are characterized by low-grade inflammation and oxidative stress [13]. Moreover, dysregulated mt genes methylation (D-loop and CO1 hypomethylation) might expand our findings of higher mtDNA content in fetal cord blood of IUGR and PE [14]. These preliminary data may indeed suggest a compensatory attempt of fetuses to increase energy production through higher mtDNA content and RCC (CO1) expression, representing a further link between epigenetic changes and perinatal programming of diseases. Another issue is related to the placental hormonal function. The placenta as a source of a wide array of hypothalamic or pituitary hormones was a hot topic in the 60-70s, then neglected because of the radioactive techniques needed at that time. Steroid hormones, and in particular estrogens, are important for uterine/placental vascular

adaptations to pregnancy, but also essential for trophoblast cells syncytialization in placenta. During pregnancy, the feto-placental unit is a source of estrogens through its aromatase enzyme Cytochrome P450 (CYP19) involved in estradiol (E2) production [15]. Interestingly, CYP19 levels appeared significantly higher in IUGR placentas that we recently analyzed. We might speculate that the CYP19 alterations have an estrogen-related protective action in more severe IUGR placentas, which we showed to be characterized by increased mtDNA [16]. Ongoing analyses will evaluate if these placental molecular alterations result in E2 hormone altered production. Placental mesenchymal stromal cells (p-MSCs) may also represent an interesting point to evaluate in order to understand normal and abnormal placental development. In IUGR pregnancies, p-MSCs have lower proliferation rate with earlier shift towards homogeneity than in controls. *In vitro* findings also demonstrate that multipotency of IUGR-

1) EARLIER mesenchymal cell ENRICHMENT after 7 days of culture both in IUGR fetal membranes and villous parenchyma vs CONTROL



2) HIGHER ADIPOGENIC DIFFERENTIATION in IUGR mesenchymal stem cells vs term controls

3) LOWER ENDOTHELIAL DIFFERENTIATION in IUGR mesenchymal stem cells vs term controls

Figure 1 (LECT 45). Analysis of mesenchymal stem cells isolated from Intrauterine Growth Restriction (IUGR) and control human placentas.

derived p-MSCs is restricted, as their capacity for adipocyte differentiation is increased, whereas their differentiation ability towards endothelial cell lineage is decreased (**Fig. 1**) [17]. These findings are indicative of changes that may also be reflected in the developing fetus (summarized in **Tab. 1**). The potential role for p-MSCs in pregnancy pathologies, as well as the striking mitochondrial changes involved in energy production, open new perspectives for understanding the development of the diseases and potential routes of prevention and treatment.

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LECT 46

INSULIN SENSITIVITY IN IUGR: FROM PLACENTA TO ADOLESCENCE

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Subjects born with intrauterine growth restriction (IUGR) have an increased incidence of cardiovascular disease, hypertension and type 2 diabetes mellitus in adulthood compared with the general population. These features are shared with obese subjects, and derive primarily from increased insulin resistance. Fetal growth is driven mainly by the IGF system as experiments in knockout mice have shown, however, insulin is a well known determinant of fetal growth as well, and the IGF system is of importance in regulating glucose metabolism and insulin sensitivity. We reported that human IUGR subjects present increased insulin-like growth factor (IGF)-2, IGF binding protein (IGFBP-1) and IGFBP-2 content in placenta, if compared with appropriate for gestational age (AGA) newborns. The increase in IGF-2 could be a compensation for reduced insulin bioactivity in placenta. Moreover, we described increased IGFBP-2 cord serum concentrations

in IUGR compared with AGA, and a positive relationship of serum IL-6 with IGFBP-2, although IL-6 concentration did not show any changes in the two situations. We described instead increased IL-6 mRNA and protein concentrations in placental lysates from IUGR. Finally, we reported a negative effect, although not a major effect, of IL-6 placental concentration on birth size. In detail, whereas the amount of total insulin receptor (IR) was similar in both AGA and IUGR, activated IR was significantly higher in IUGR. Total IR substrate-1 (IRS1) was increased in IUGR, whereas total IRS2 and activated IRS1 were similar. AKT content was reduced and activated AKT was undetectable in IUGR placentas. C-Jun N-terminal kinase content was reduced in IUGR. Total and activated ERK1/2 were similar in IUGR and AGA groups, and total SOCS2 was increased in IUGR. IL6 lysate concentrations correlated with AKT content and activated IR. SOCS2 correlated negatively with all growth parameters at birth. Close relationships of insulin action in placenta with fetal growth were shown. Insulin resistance and type 2 diabetes are related by association with high serum concentrations of proinflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)- α , and low adiponectin concentrations, as studies in obese subjects have confirmed. As markers of insulin sensitivity in placenta we further studied adiponectin and resistin contents in placentas from IUGR and AGA newborns. Adiponectin was significantly lower in lysates from IUGR, insulin and resistin concentrations were positively correlated, and placental adiponectin concentration was positively correlated with the weight of the placenta, birth weight and head circumference. Fetal programming of the endocrine axis related to intra-uterine growth and events occurred during pregnancy contribute to the timing of puberty and to future reproductive capacity. Pubertal development disorders influence not only sexual maturity, but also adult height, bone mineral density and reproductive health. Adipokines play a significant role in the metabolic syndrome and in cardiovascular diseases, have implications in regulating insulin sensitivity and inflammation, and significant effects on growth and reproductive function. Precocious pubarche and precocious adrenarche have been shown to be more frequent in subjects with a low birth weight. Girls with previous prenatal growth restriction have been described to have more frequently than the general population idiopathic functional ovarian hyperandrogenism

Table 1 (LECT 46). Main findings concerning the relationship between adiponectin, leptin and onset of Polycystic Ovarian Syndrome (PCOS).

Adipokine	In vivo/species	Main conclusions
Adiponectin	<i>In vitro</i> /Humans and Bovine	There is strong evidence for a direct link between fat cell metabolism and ovarian steroidogenesis, suggesting that disruption of adiponectin and/or its receptors plays a key role in pathogenesis of hyperandrogenism in PCOS.
Adiponectin	<i>In vivo</i> /Humans	Adiponectin levels seem to be lower in women with PCOS compared with non-PCOS controls. Low levels of adiponectin in PCOS are probably related to insulin resistance but not to testosterone levels. Total adiponectin should not be used as a biomarker of PCOS severity. Further investigations are needed to understand the role of High Molecular Weight adiponectin levels in PCOS.
Adiponectin, Leptin	<i>In vivo</i> /Humans	PCOS does not appear to have an independent effect on the adipose expression of leptin, adiponectin, and IL-6 or the circulating adipocytokines.
Leptin	<i>In vivo</i> /Humans	A significant association was found between the Pro1019Pro in <i>LEPR</i> gene and PCOS, and a highly significant association was found between the Gln223Arg in <i>LEPR</i> gene and PCOS.

and hyperinsulinism. Adipokines represent an important link between nutritional status and pubertal physiology and several pubertal disorders, such as premature adrenarche, premature pubarche, polycystic ovarian syndrome and constitutional delay in growth and puberty. The possible causes of these associations are thought to be insulin insensitivity, increased central adiposity, increased IGF-I levels between the age of 2 and 4 years and metabolic and hormonal patterns that are common in children born small for gestational age (SGA) with excess weight gain in early childhood. High levels of IGF-I and insulin resistance stimulate adrenal androgen secretion and the development of precocious pubarche. Interestingly, one study evaluated the development of premature AGA and full-term SGA children, observing that menarche occurred 6 months earlier in the preterm group and 12 months earlier in the SGA group with respect to full-term AGA controls. The interval of time between onset of puberty and menarche was similar in all groups. Polycystic Ovarian Syndrome (PCOS) is one of the most common disorders in females, affecting approximately 15% of women during reproductive age. It is classically characterized by three distinctive features: hyperandrogenism, ovarian dysfunction and polycystic morphology pattern of ovaries on ultrasound scan. A pivotal role in the pathophysiology of this syndrome is played by visceral adiposity and insulin resistance. Findings related to PCOS and adiponectin and leptin are summarized in the table below (**Tab. 1**). In conclusion, at least in part insulin sensitivity is set *in utero* and remains tightly related to pre- and postnatal longitudinal growth, weight increase,

pubertal development, pubertal disorders and reproductive capacity.

LECT 47

MATERNAL HYPERGLYCEMIA, NEONATAL AND ADULT DISEASE

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Hyperglycemia is one of the most common medical conditions complicating pregnancy and it occurs in one in six pregnant women (16.8%). The majority of cases (84%) are due to gestational diabetes mellitus (GDM), while 16% are women with pregestational diabetes [1]. Hyperglycemia in pregnancy is associated with higher incidence of maternal complications (hypertensive disorders, caesarean section, birth trauma and subsequent development of type 2 diabetes) but also perinatal morbidity and mortality. Long-term outcome data show that prenatal exposure to maternal hyperglycemia increases the risk of metabolic complications in later life.

FETAL COMPLICATIONS

In pregestational diabetes, hyperglycemia during the critical period of organogenesis may lead to a high risk of spontaneous abortions and congenital anomalies (diabetic embryopathy). The risk of isolated and multiple congenital anomalies is highest in infants of mothers with pregestational

diabetes (adjusted odd ratios [OR] 3.17 for isolated and OR 8.62 for multiple anomalies), and congenital malformations account for approximately 50% of the perinatal deaths in infant of diabetic mothers [2]. Rates of miscarriage are two to threefold higher in women with pregestational diabetes than among non-diabetic women. Possible reasons for this increase in risk include a higher rate of congenital malformations, toxic effects of hyperglycemia and maternal vascular disease leading to utero-placental insufficiency [3]. Hyperglycemia during the second and third trimester occurs in pregestational diabetes but also in GDM and leads to “diabetic fetopathy”, resulting in fetal hyperglycemia, hyperinsulinemia, and macrosomia. The prevalence of macrosomia in developed countries is between 5% and 20%; however, an increase of 15-25% has been reported in the last decades, associated with an increase of maternal obesity and diabetes [4]. The pathophysiology of macrosomia can be explained based on Pedersen’s hypothesis of maternal hyperglycemia leading to fetal hyperinsulinemia and increased utilization of glucose and, hence, increased fetal adipose tissue. Macrosomic fetuses in diabetic pregnancies develop a unique pattern of overgrowth, involving the central deposition of subcutaneous fat in the abdominal and interscapular areas [5]. They have larger shoulder and extremity circumferences, a decreased head-to-shoulder ratio, significantly higher body fat and thicker upper-extremity skinfolds. Consequently, one of the most serious complications of vaginal delivery in macrosomic babies is shoulder dystocia, which is associated with birth trauma. Macrosomia is associated with excessive rates of neonatal morbidity. Macrosomic neonates have 5-fold higher rates of severe hypoglycemia and a doubled increase in neonatal jaundice in comparison with the infants of mothers without diabetes [5]. Stillbirth is another well-known complication of diabetic pregnancies. In GDM, chronic fetal hyperinsulinemia results in elevated metabolic rates that lead to increased oxygen consumption and fetal hypoxemia; the placenta may be unable to meet the increased metabolic demands leading to intrauterine fetal death. In poorly controlled pregestational diabetes the long standing hyperglycemia leads to a placental insult early in pregnancy during trophoblastic invasion; a high glucose concentration will also lead to excess reactive oxygen species (ROS), that can determine cell death and tissue damage and thus influence placental development [6]. This results in a smaller

than expected placenta and fetal growth restriction, eventually leading to stillbirth.

NEONATAL COMPLICATIONS

Infants of diabetic mothers are at increased risk of mortality and morbidity compared with infants born to non-diabetic mothers. Preterm delivery both spontaneous and medically indicated occurs more frequently in diabetic than non diabetic pregnancies. Newborns of diabetic mothers have a higher risk of respiratory distress syndrome (RDS) due to surfactant deficiency because these babies are more likely to be delivered prematurely and because maternal hyperglycemia appears to delay surfactant synthesis [7]. Metabolic complications (hypoglycemia, hypocalcemia and hypomagnesemia), polycythemia and hyperbilirubinemia occur frequently in newborns of diabetic pregnant women. Babies of diabetic mothers are at increased risk for transient hypertrophic cardiomyopathy that is thought to be caused by fetal hyperinsulinemia, which increases the synthesis and deposition of fat and glycogen in the myocardial cells; infants often are asymptomatic, but 5% to 10% have respiratory distress or signs of poor cardiac output or heart failure [8].

LONG TERM CONSEQUENCES

There is now robust evidence that a hyperglycemic intrauterine environment is responsible not only for significant short term morbidity in the fetus and the neonate, but also for an increased risk of developing diabetes as well as other chronic, non communicable diseases at adulthood like obesity, cardiovascular and renal diseases. The mechanisms by which excess maternal weight and/or diabetes during pregnancy may lead to disease in the offspring at childhood and adulthood are not fully understood, but epigenetic changes induced by exposure to maternal hyperglycemia during fetal life are implicated. Epigenetic modifications, such as DNA methylation, can regulate gene expression without altering the underlying DNA sequence, and determine abnormal proliferation of fetal adipocytes and muscle cells, together with hyperplasia of pancreatic beta cells and neuroendocrine cells. This causes development of obesity, hypertension, and type 2 diabetes mellitus later in life [9]. Thus, hyperglycemia in pregnancy gives rise to a vicious cycle in which diabetic mothers have babies with epigenetic changes who are prone to develop metabolic disease later in life, which will give rise to a new generation of mothers with GDM. Consequently, a tight maternal glycemetic control can prevent not only short-term fetal and neonatal

morbidity but also long term complications, breaking this vicious cycle. This highlights the importance of developing strategies for screening and managing women with GDM.

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LECT 48

PLASTICS AND THE NEWBORN

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Plasticizers, bisphenol-A (BPA) and phthalates are present in almost any material we use, especially in the food and beverage packages, from which they may leach out in small amounts into food and drinks. Besides, they are found in other countless consumer products used in our daily life, increasing the risk of background exposure in human population. Human exposure to BPA and phthalates, including that of pregnant women occurs through diet, air, dust,

water, and skin contact. Newborn infants are already exposed to these endocrine disrupting chemicals during the prenatal period via the placenta, and continue to be exposed, after delivery, through bottle feeding, breastfeeding and, if hospitalized, through medical devices in the intensive care units. BPA and phthalates may be present in various medical devices such as intravenous administration sets, infusion bags, stopcocks, angiographic injectors and syringes, pump, surgical instruments, intravascular catheters (possible), gastrointestinal tubes and accessories, percutaneous implanted long-term catheter, umbilical catheters. Very high exposures to BPA and phthalates can occur during medical treatment, especially in premature infants undergoing intensive medical treatments. Data from animal and human studies suggest that exposure to BPA and phthalates during critical periods of development may disrupt the development of the fetus and young child as a consequence of its interaction with estrogen, androgen and thyroid receptors. BPA and phthalates are also epigenetically toxic. Prenatal and neonatal exposure to low doses of BPA and/or phthalates may induce up/down regulation in gene expression that may possibly result in adverse health effects such as neural and immune disorders, infertility, and late-onset complex diseases.

LECT 49

HOW CAN THE INCREASE IN CHILDHOOD CANCER BE EXPLAINED? THE ROLE OF TRANSPLENTAL AND TRANSGENERATIONAL CARCINOGENESIS

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BACKGROUND

Cancer is generally associated with old age and the explanation most clinicians (and others) advance for its continuous increase in prevalence, observed throughout the 20th century in all the industrialized countries, is that it results from the progressive accumulation over the time of aging of genetic oxidative/stochastic damages, with an additional role for the improvement of diagnostic capacities. It is too often forgotten that this increase concerns persons of all ages and, particularly in the last decades, the youth.

METHODS

Literature review. The aim is to reconsider, in the light of the new epigenetic models of environmental carcinogenesis and of transgenerational cancer transmission, the recent epidemiological data that confirm a substantial increase in childhood cancer throughout Europe, hardly explainable by the current stochastic mutational paradigm.

RESULTS

Carcinogenesis is a long and complex process. As far as the increase in childhood cancer is concerned, the key factor for its occurrence should be looked for in the parents (exposure of the reproductive cells) or the fetus (exposure in the really first stages of the ontogenetic development).

CONCLUSIONS

The two main possibilities that have to be considered are: 1) the direct exposure of the embryo/fetus to physical agents or, through transplacental transmission, to biological (viruses) or chemical agents, capable of directly damaging the fetal DNA or of inducing epigenetic changes in the fetal tissues (fetal programming); 2) the transgenerational transmission of epigenetic “signatures” through the gametes. Admitting the importance of such mechanisms would have several implications. The first would be recognizing the highly underestimated role of the environmental pollution in the genesis and the progressive increase in prevalence of cancer. In particular, we hypothesize that the “initiating” stage of cancer might take place much earlier, in the fetus or even in the parents’ gametes, and that the increasing trends in cancer in the very early childhood should be seen as a sentinel sign of a possible transgenerational amplification of (epi)genetic/programmatic changes with their associated pathologies.

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LECT 50

NEW FORTIFIERS FOR THE NEWBORN

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Human milk (HM) has an essential role in the nutrition of preterm infants due to its various bioactive and immunomodulatory components. Evidence shows that preterm infants fed HM have lower rates of infection, necrotizing enterocolitis (NEC) and improved neurodevelopmental outcomes compared to infants fed preterm formula. The American Academy of Pediatrics strongly recommends the use of human milk also for preterm infants because of its unique advantages with respect to host protection and improved developmental outcomes. Own mother’s milk (OMM) should be the primary diet, and if OMM is not available or not in sufficient quantity, pasteurized donor HM obtained from a recognized HM bank should be used. Given its unique and numerous advantages, HM should be considered as the first choice for the nutrition of preterm infants, but, since the protein, mineral and energy content of human milk is not suitable to meet the high needs of VLBW infants, it should be fortified. Fortification of HM for tiny preterm infants is one of the most important nutritional interventions with the purpose to provide appropriate nutritional intake and appropriate growth. The rationale of the fortification is to optimize the content of human milk in a way to meet the special needs of this special group of infants.

Today, food-industry produced multicomponent fortifiers are available to supplement HM and contain varying amounts of protein, carbohydrate, calcium, phosphate, other minerals (zinc, manganese, magnesium, and copper), vitamins, and electrolytes. Fortification of HM, although crucial, has become more complex than anticipated. Current standard fortification methods have yielded inadequate protein intakes resulting in slower growth compared to preterm formulas. Improvement of outcomes depends on new models dealing with the large variability of HM composition, and solving the problem of protein undernutrition. Individualized fortification, particularly adjustable fortification has been shown to be effective and practical in attaining adequate protein intakes and growth. Beside the improvement of the strategies of fortification, also the optimal qualitative composition of fortifiers is still to be defined. Most commercially available multi-nutrient fortifiers and protein concentrate are derived from bovine milk, which has a protein composition very different from that of HM. Moreover, cow milk protein intake in the first month of life has raised concerns because of its association with allergies. The recent Consensus Statement on Human Milk in Feeding Premature Infants [1] drew its attention on routinely used bovine milk-based fortifiers, whose bovine protein might be associated with intestinal inflammation in ELBW infants, and consequently on investigation on exclusive HM diets (human milk-based fortifier and donor human milk if OMM was unavailable). Clinical trials reported that exclusive HM diet is associated with less NEC, less NEC requiring surgery, and lower mortality in ELBW infants than in those who receive OMM plus bovine milk-based products. The Panel concluded: "Studies for quality improvements of bovine milk-based fortifiers are in progress. Human milk-based fortifiers are available, probably are of better quality than cow's milk based fortifiers, but are very expensive at the moment." Our group recently observed that donkey milk is more similar to HM than bovine milk in term of quantitative and qualitative composition, such as protein, carbohydrate and lipid content. Moreover, donkey milk has a PUFA (polyunsaturated fatty acids) content equivalent to HM and is rich in lysozyme, a protein characterized by antibacterial properties able to protect the newborn from pathogens and the milk from degradation. Donkey milk has been suggested as the best substitute of HM due to its composition. Based on these considerations, donkey milk can be considered more suitable than bovine milk to constitute the base of the protein support for VLBW in-

fant and preterm newborns. The Neonatal Unit of the University of Turin is currently performing a multi-center, randomized, controlled, single-blind clinical trial to evaluate the use of a multi-component fortifier and a protein concentrate derived from donkey milk for the nutrition of VLBW or infants of gestational age < 32 weeks, comparing it with a multi-component fortifier and protein concentrate derived from bovine milk. This trial is performed in collaboration with the Italian National Research Council of Turin and the University of Cagliari. Our hypothesis is that feeding these newborns, according to adjustable fortification principles, with HM fortified with a multi-component supplement and a protein supplement both derived from donkey milk will improve the feeding tolerance and the clinical, metabolic, neurological and auxologic outcome at short- and long-term.

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LECT 51

OLIVE OIL AND MATERNAL AND NEONATAL HEALTH

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The new design of the Mediterranean diet (MD) pyramid includes as a base of the pyramid a balanced lifestyle, cooking methods, traditional, local and eco-friendly products, conviviality, physical activity with adequate rest, a particular caloric restriction and frugality. MD pyramid confirms the importance of daily food intake of vegetables, cereals, fruits, legumes, as well as nuts and seeds, rich in micronutrients, considering the consumption of water and herbal infusions fundamental too. Moreover, it confirms that the principal source of fat must be Extra Virgin Olive Oil High Standard (EVO HS) [1]. Therefore, EVO HS is considered a key feature of the healthy properties of MD for its composition of fatty acids, vitamins and polyphenols. However, these must be bioavailable to allow EVO HS to have its nutraceutical properties, which include: antioxidant, anti-inflammatory, anti-cancer, antimicrobial and antiviral, hypoglycemic properties, and its protective effects for heart,

brain, during pregnancy and breastfeeding [2]. The main phenolic components for its nutraceutical properties in EVO HS are hydroxytyrosol, tyrosol, and oleuropein. Oil production and extraction technologies, (essential is the extraction at low oxidative stress) determine the final content of polyphenols in virgin olive oil. Despite few information on the epigenetic effects of olive polyphenols being presently available, the similarities between many effects of different plant polyphenols at the molecular level suggest that the well documented epigenetic effects observed for many other plant polyphenols could also be largely documented in olive polyphenols [3]. It has also been found that if mothers have consumed an adequate amount of olive oil during pregnancy, their kids are exposed to a lower risk of wheezing in their first period of life [4]. Dietary supplements with n-3 long chain polyunsaturated fatty acids (LCPUFA), also present in EVO HS, may change the developing immune system of the newborn before allergic responses are established, particularly for those with a genetic predisposition to the production of immunoglobulin E (IgE) antibodies [5]. EVO HS, for its content of oleocanthal, a natural anti-inflammatory substance, may have an effect on many inflammatory diseases [2] also in the early period of life.

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LECT 52

OBESITY IN CHILDREN: ANTE-, PERI- AND POST-NATAL DETERMINISM

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Child obesity has recently become an important public health problem due to its increasing incidence, with impact on the morbidity and mortality rates. The incidence of overweight and obesity is increasing in both developed and developing countries at a rate of 2% per year. It is a chronic disorder of the nutritional status with multifactorial determinism as a result of genetic, environmental, lifestyle, psychological factors and the interactions between them. The impact of obesity in pediatric pathology is due to cardiovascular, renal, metabolic complications, as well as the psychological and social inclusion problems of the child. Previous studies identified some critical periods in the determination of obesity, namely pregnancy, period of childhood 'adiposity rebound' development (age 3-6 years) and puberty. Prevention and nutritional measures are not adequate, beginning from the moment of birth, and even before this moment, during the intrauterine life, and then during childhood and adolescence, clearly showing a need of major improvements. Therefore, the proper management of obesity should involve adequate nutritional measurements even since the moment of conception in order to influence ante-, peri- and post-natal factors that determine obesity. The alarming increase of obesity determined researchers to identify risk factors in order to prevent this disorder. Identifying risk factors of ante-, peri- and post-natal obesity and reducing the incidence of obesity in children has a particular impact on adult health, preventing the complications of the disease (heart disease, diabetes, metabolic syndrome and cancer). Antenatal factors that lead to obesity involve maternal factors. These factors are related to the diet of pregnant women's in order to obtain an adequate gestational weight gain, according to the recommendations of the Institute of Medicine (IOM). Perinatal factors include newborn nutrition, in relation to the proper content of proteins in formulas, with a preference for those with a low content, but also genetic factors. Regarding this fact, we performed a study and we pointed out that the risk of obesity in newborns was greater for the variant allele of the *TGF-β1* 869 T>C polymorphism and for C allele of the *PPARγ2* 34 C>G gene polymorphism. We also observed that gestational weight gain is a very good predictor of birth weight, being positively correlated with bioimpedance parameters. The postnatal period is influenced by all the factors (genetic, nutritional, lifestyle) that present an impact on childhood period. Our studies show that GG genotype of *LEPR* 223 gene and AA genotype

of *LEPR* 1019 gene, *IL-6* 572 C allele carriers and *IL-6* 190 C allele carriers are higher in children with obesity and are correlated with anthropometric parameters and serum leptin and adiponectin. The assessment of the nutritional status in the child includes anthropometric and bioimpedance measurements, genetic and laboratory tests. The diagnosis is established on the corroboration of the clinical, genetic and anthropometric data. The treatment of child obesity imposes an adequate prophylaxis by providing a proper diet intake and the increase of physical activity, and the avoidance of junk food intake, with high caloric value. The curative treatment involves diets with a more reduced caloric intake, but not very restrictive because of developmental processes of growing organisms. This study pointed out 2 important aspects: 1) the important role of alimentation in the critical periods of pregnancy (adequate gestational weight gain) and in the neonatal period (reduced protein intake during infancy) and 2) the role of gene polymorphisms in the determinism of child obesity. Therefore, it is very important to elucidate the role of all these factors in the obesity development mechanism in the newborn and the child, in order to prevent and to diagnose this disease early by taking under consideration the environmental and genetic factors, but also food habits. In conclusion obesity is a chronic, multifactorial, nutritional disorders determined by ante-, peri- and post-natal factors, and by the interaction between them.

LECT 53

NUTRITION AND CYSTIC FIBROSIS

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Cystic fibrosis is a progressive genetic disease with poor prognosis, characterized by an exocrine system dysfunction, caused by CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation. Currently, the life expectancy of an infant with cystic fibrosis is between 30 and 40 years, and it mainly depends on the severity of lung involvement; the type of genetic mutation obviously has great importance too. Over 80% of cystic fibrosis patients have pancreatic insufficiency with malabsorption syndrome, particularly fat malabsorption, and consequent repercussions on growth and nutritional status.

The auxological indices used to assess a state of malnutrition are mainly the length/height percentile < 5th in all patients, weight/length ratio percentile < 10th in children under two years and BMI (Body Mass Index) percentile in patients over the age of two years. Taking into account pancreatic insufficiency and increased caloric consumption linked to the greater respiratory effort, the daily energy intake should be around 120-150% compared to a healthy child of the same age. About 40% of total calories should be provided by lipids, in particular 2-5% of essential fatty acids; protein intake should be high (2-3 g/kg/day); carbohydrates should preferably be complex sugars with a percentage of simple sugars not higher than 10%. The administration of pancreatic enzymes at very high doses (to be taken at each meal) allows to obtain adequate nutritional absorption and a satisfactory nutritional state. Current guidelines recommend a daily intake of pancreatic enzymes between 2,000 and 10,000 lipase units per kg of body weight. Supplement of vitamins, especially fat-soluble vitamins (A, D, E, K), and mineral salts are essential as well. Breast milk is the ideal food in the infant with cystic fibrosis as for the healthy child, while the use of hydrolyzed should be restricted to patients with severe malnutrition. Adequate nutritional status has a positive effect on lung function and, ultimately, on the course of the disease.

LECT 54

DIAGNOSTIC AND MANAGEMENT IN DIGESTIVE HEMORRHAGES IN CHILDREN

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Digestive hemorrhage in children may evolve from being occult to major events including vital risk. The etiology includes extra digestive diseases (acute pathology of the nervous system, coagulopathies, portal hypertension) or digestive diseases (infections, malformations, inflammations) with a variable frequency in relation to different ages. The history, encumbered by numerous opportunities of error, needs a systematic exploration: the severity of blood loss, its reflection on vital organ function, detection of underlying (malformative) or acquired medical or surgical causes. Treatment is guided

by the severity of blood loss, by the necessary medicines with etiopathogenetic impact. Digestive endoscopy, in addition to its important role as a method of diagnosis, is also sometimes a therapeutic method. Acute gastrointestinal hemorrhage is an indication for a therapeutic endoscopic intervention. Bleeding from esophageal varices can also be treated by endoscopic sclerotherapy, band ligation or a combination of these techniques. Diffuse mucosal bleeding from duodenitis or gastritis is usually not responsive to endoscopic intervention. Lesions that may be treatable with this therapy include bleeding lesions, angiomata and polyps. There are five well-established types of therapeutic intervention for acute gastrointestinal bleeding: injection, coagulation or thermal therapy including argon plasma coagulator, laser therapy, endoscopic hemostatic devices and ligation therapy.

LECT 55

CESAREAN SECTION: PAST, PRESENT AND FUTURE

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Caesarean section is a surgical procedure of ancient origins: it was performed to extract the baby after the woman had died. Today, cesarean section is necessary when vaginal birth is not possible or poses the mother or the child at high risk. In the last thirty years, this procedure has been increasingly used, particularly in Western countries, with values ranging from 14% in the Scandinavian countries to 30-35% in the USA and approximately 38% of deliveries in Italy. The increase in delivery by caesarean section is due to several factors: the progress of anesthetic techniques and surgical procedures, advanced maternal age of first delivery (32 years in the third millennium), growing medical litigation and the consequent use of defensive medicine [1-3]. Furthermore, other causes include the organization of hospitals, with the Healthcare system paying higher refunds for caesarean sections compared to natural deliveries, and the right of women to avoid pain and give birth “on request” by caesarean

section. In this study we analyzed the characteristics of all patients who underwent caesarean section at the Maternal University Hospital in Sassari in 2014. This hospital is a third-level of high specialization, attracting pregnant patients from central and northern areas of Sardinia. Mothers who had a caesarean section were older and had on average a higher education level (over 78% had a high school degree, a bachelor's or master's degree) compared to women who gave birth by vaginal delivery. Moreover, they often lived in towns of Central and Northern Sardinia referring to hospitals without a neonatal intensive care unit, and they were then admitted to our Clinic for severe diseases of prematurity needing a fast and safe delivery, namely caesarean section. In the future, particularly in industrialized countries, it will be possible to contain the prevalence of cesarean section within the current percentage (38%), but it will probably not be reduced. In fact, several factors related to global social and cultural changes will be likely to play a role, such as a very low birth rate (around 1 child per couple), an average older age of first pregnancy (related to high levels of education and career), and a greater degree of self-determination in choosing the mode of delivery compared to the past. In the present circumstances, caesarean section should not be demonized, as with its low anesthetic and surgical risk it guarantees a safe childbirth for both mother and child.

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LECT 56

NEONATAL BACTERIAL AND FUNGAL INFECTIONS: WHAT'S NEW?

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Neonatal infections are the leading cause of neonatal mortality and morbidity, with estimated 1.6 million neonatal deaths due to infection occurring each year worldwide. Extremely preterm infants in neonatal intensive care units (NICUs) are at higher risk of infection, with increasing risk with decreasing gestational age. A recent report by Stoll et al. for the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network (NRN) shows the 20-year (1993-2012) trends in neonatal infections for infants born at 22-28 weeks gestation and with birth weight 401-1,500 g [1]. Overall, 2% of infants had early-onset sepsis, and among infants surviving more than 3 days, 32% were diagnosed with late-onset sepsis. The percentage of infants with early-onset sepsis remained stable over the study period. The percentage of infants with late-onset sepsis remained substantially stable during the years 1993-2004 (only a slight increase was reported for some gestational ages), while in the years 2005-2012 the rate of late-onset sepsis was reported to decrease for infants of each gestational age. A relevant problem in recent years has been the emergence of multi-drug resistant bacterial strains. Previous reports showed the transmission of microorganisms in neonatal units and the emergence of multi-drug resistant bacterial strains in late-onset infections in newborn infants admitted to NICUs. In a recent work conducted for the NRN, Weissman et al. studied the resistance patterns of *E. coli* isolates from neonatal early-onset sepsis or meningitis [2]. Importantly, the authors found a high rate of ampicillin resistance, no aminoglycoside resistance, and resistance to third-generation cephalosporins in only a single strain. These results are of extreme importance given that ampicillin is a recommended antibiotic for early-onset sepsis, and the authors highlight the importance of continuing surveillance of antibiotic resistance to ensure that standard empiric regimens remain effective. In face of the emergence of resistant microorganisms, a number of experts and institutional guidelines stress the importance of the development of an antibiotic stewardship program in each hospital, which should be specific for NICUs [3]. Specific challenges in building a neonatal antibiotic stewardship program include the difficulty in diagnosing infection in newborn infants, the poor standardization of the duration of antibiotic treatment among NICUs and even inside single NICUs, the management of chorioamnionitis, and the management of perioperative prophylaxis.

On the other hand, several laboratories in the world are actively working for the discovery of new antibiotic and antimicrobial drugs. It is worth citing here the discovery of antibiotic teixobactin from soil bacteria [4], antibiotic lugdunin from human nasal commensal bacteria [5], novel approaches to deliver antibiotics in the intracellular environment to reduce the microbial reservoirs [6, 7], and novel synthetic chemical approaches to antibiotic synthesis [8]. Infection caused by *Candida spp.* still represents a burden in NICUs, especially for extremely low birth weight infants (< 1,000 g), and it is associated with high mortality and poor neurodevelopmental outcome. Beyond drugs traditionally used in neonatal medicine, a new class of antifungals, the Echinocandins, appears suitable for neonatal use, in particular micafungin, for which pharmacokinetic studies on neonates are available [9]. While Echinocandins penetrate the brain tissue, they do not penetrate the CSF and the vitreous; a dilated retinal exam is required to exclude endophthalmitis before micafungin is used as first-choice treatment [9]. High-risk infants with suspected or possible *Candida spp.* infection could benefit from empirical antifungal therapy, that has been associated with increased survival without neurodevelopmental impairment [9]. High-risk populations include ELBW neonates with sepsis and risk factors for fungal infections (presence of a central catheter, endotracheal tube, thrombocytopenia, prolonged treatment with broad-spectrum antibiotics, gestational age < 28 weeks). Altogether, the field is moving towards a slight but progressive reduction in the incidence of infection in NICUs, as demonstrated by the trend in the years 2005-2012 reported by the NRN. The reduction of infection and the related mortality and morbidity is a primary goal in the NICU. Despite the important armamentarium available to fight infection, one must not forget that simple measures like hand hygiene, judicious antibiotic use and antibiotic stewardship are fundamental to limit the emergence of resistant strains.

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LECT 57

THALASSEMIA: THE STATE OF THE ART

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Beta-thalassemias are a group of hereditary hemoglobinopathies that cause worldwide severe anemias and are particularly frequent in the temperate regions of the world. Lifelong blood transfusions and iron chelation therapies are the mainstay of the treatment. Hematopoietic bone marrow transplantation is possible for a minority of patients and carries a risk of death ranging from 5% for siblings donor to 30% for unrelated matched or haploidentical siblings donors. More general and safer definitive treatments are urgently needed. To this aim, two main approaches

are generally sought with equal intensity: one aimed at correcting the disease by gene transfer and the other by reactivating the synthesis of the postnatally suppressed fetal globin genes. Although hemoglobinopathies were the first genetic disease to be discovered, the possibility of a genetic treatment by gene transfer of a normal beta-globin gene has lagged behind for many years due to the difficulties in correcting structural as opposed to enzymatic gene defects. In the year 2000, the first clinical trial of gene therapy has provided the proof of concept that thalassemia was amenable to a cure, and several others studies have later flourished, promising to bring to reality what was until recently thought of as a dream. Clinical trials in larger samples of patients with β^0 , β^+ -thalassemia, β -thal/HbE and sickle cell diseases with diverse genotypes have been successful in reaching transfusion independence in up to 85% of the patients and also in the more difficult to treat β^0 -type of thalassemia. Beside traditional gene therapy, the new technology of genome editing by CRISPR CAS9 technology promises to achieve gene correction by homologous recombination at unprecedented high efficiency and it is expected to enter into the clinical stage soon. The technology exploits an adaptive immune system that relies on base pairing of nucleic acids to recognize self from non-self. The most useful CRISPR system is derived from *S. pyogenes* and it has been optimized for gene therapy purposes. It is based on single guide RNAs that directs the endonuclease CAS9 protein toward DNA targets to be modified *in vivo*. Recognition of the nucleic acid follows the simple Watson and Clegg base pairing, editing is achieved through enzymatic cleavage of the DNA strands by CAS9 endonucleases, and gene correction by the use of the endogenous mechanism of DNA repair: non homologous end joining or homology directed DNA repair. Recent preclinical studies of its application to the cure of thalassemia will be presented and discussed. Induction of fetal or adult globin synthesis with novel approaches is also in the clinical phase and important advances are predicted to sprout from the great improvements made in the knowledge of the regulation of the fetal globin genes. Different biological products directed to stimulate activin II receptor and increase hemoglobin production are in phase 3 clinical studies. We will present examples of some of the later achievements in the search for a definitive cure for beta-thalassemia and hemoglobinopathies in general.

LECT 58

VITAMIN D DEFICIENCY: WHAT'S NEW?

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Vitamin D (VD) is a prohormone that is crucial in the control of calcium and phosphorus metabolism and is an essential determinant of bone health in childhood and adolescence.

Two forms of VD are of practical importance: VD₂ (ergocalciferol – plant-derived), and VD₃ (cholecalciferol – animal-derived). They can be ingested from different dietary sources, but the main source of VD (cholecalciferol) is exposure to ultraviolet B (UV-B) component of sunlight, as skin synthesis contributes 80% to 90% of an individual's VD levels. Both VD₂ and VD₃ are biologically inactive; their activation requires sequential hydroxylation reactions in the liver (25-hydroxylation) and kidney (1-hydroxylation). 1,25-dihydroxyvitamin D₃ (1,25[OH]₂-D₃) or calcitriol, the biologically active form of VD, circulates in the blood functioning as a hormone, and exerts its action through the cytosolic vitamin D receptor (VDR), that is nearly ubiquitously expressed. Calcitriol has various biological activities aimed at maintaining bone health: increased intestinal absorption of calcium and phosphorus, bone resorption, and decreased renal excretion of calcium and phosphorus. Together with parathyroid hormone (PTH) and calcitonin, calcitriol acts to maintain plasma calcium levels within the normal range. Moreover, VD can influence the host's immune system through the modulation of both innate and adaptive immunity and the regulation of the inflammatory cascade.

BODY VITAMIN D STATUS

It is measured by serum concentration of 25-hydroxyvitamin D₃ (25[OH]-D₃), or calcidiol. Both the American Academy of Pediatrics (AAP) [1] and the Institute of Medicine (IOM) [2] use the following calcidiol concentrations to classify VD status in the pediatric population: severe deficiency for values < 5 ng/mL; deficiency for values between 5 and 15 ng/mL; insufficiency for values between 16 and 20 ng/mL; sufficiency for values between 21 and 100 ng/mL; excess for values between 101 and 150 ng/mL; intoxication for values > 150 ng/mL. In contrast, other scientific societies establish the cutoff for VD sufficiency at ≥ 30 ng/mL.

HYPOVITAMINOSIS D

It is widespread and represents a re-emerging global health problem. Pediatric groups at risk for deficiency or insufficiency of VD have been identified (**Tab. 1**). Hypovitaminosis D has skeletal and extraskeletal consequences.

Skeletal consequences

VD sufficiency and adequate intake of calcium are both required for bone health. VD deficiency leads to a decreased intestinal absorption of calcium and phosphorus. Consequent hypocalcemia determines release of PTH, which exerts its actions on specific target organs. In particular, PTH increases renal calcium reabsorption and phosphorus excretion, stimulates renal conversion of 25(OH)-D to 1,25(OH)₂-D, and promotes calcium absorption from the bone, thus decreasing bone mineralization. When VD deficiency continues for weeks to months, stunted growth and florid rickets may develop. Rickets is primarily caused by a nutritional deficiency of VD, but other rare forms exist. Nutritional rickets has a variable clinical presentation. Children with rickets commonly present with symptoms of bone deformity and muscle weakness, but irritability and impaired growth may also occur. Severe VD deficiency may give rise to hypocalcemic seizures or tetany, especially in newborns and adolescents. Cardiomyopathy and even heart failure may rarely develop.

Extraskeletal consequences

Epidemiologic studies have suggested that VD deficiency may play a role in various chronic diseases.

Table 1 (LECT 58). Pediatric groups at risk for vitamin D deficiency.

Newborns born to mothers with vitamin D deficiency
Preterm infants
Exclusively breastfed Infants
Infants without vitamin D supplementation
Children with darker skin
Children living at higher latitudes during winter and spring season
Children with restricted sunlight exposure
Obese children
Children with severe liver or renal failure
Children with chronic diseases reducing fat absorption
Children with skin diseases
Children under chronic treatment with anticonvulsants or systemic glucocorticoids
Migrant children and adopted children

VD deficiency has been found to be associated with autoimmune diseases including type 1 diabetes and multiple sclerosis. The association between VD intake during early life and a reduced risk of developing type 1 diabetes has been recently confirmed. Deficiency or insufficiency of VD has been shown to increase the risks of elevated blood glucose levels, hypertension and metabolic syndrome. VD deficiency has also been associated with increased risk of cardiovascular disease and cancer. Finally, the deficiency of VD has been linked with pediatric disorders such as acute respiratory infection, asthma, food allergy and atopic dermatitis. Adequate levels of VD in pregnancy have been associated with a reduced risk of schizophrenia.

VITAMIN D SUPPLEMENTATION

In efforts to prevent rickets, and to achieve and maintain the target VD concentrations (> 20 ng/mL), recommendations have been made. AAP guidelines published in 2008 stated that “breastfed and partially breastfed infants should be supplemented with 400 IU/d of vitamin D beginning in the first few days of life”, and that “supplementation should be continued unless the infant is weaned to at least 1 L/day or 1 qt/day of vitamin D-fortified formula or whole milk”. It was also stated that “All non-breastfed infants, as well as older children who are ingesting less than 1,000 mL/day of vitamin D-fortified formula or milk, should receive a vitamin D supplement of 400 IU/day” [1]. In 2011, the IOM [2] proposed a Recommended Dietary Allowance of 400 and 600 IU/day of VD for healthy infants younger than 1 year and for children from 1-18 years, respectively. Preterm infants are at risk of developing hypovitaminosis D. A clinical report by the AAP has recommended a VD intake of 200 to 400 IU/day in enterally fed preterm infants [3]. In contrast, a VD intake of 400-800 IU/day in preterm infants is supported by a recent position statement [4]. Currently, universal screening of 25(OH)-D levels is not recommended, while pediatric groups at higher risk for VD insufficiency or deficiency should be screened, and supplemented where appropriate [4].

TREATMENT

The treatment of VD deficiency rickets is aimed at replenishing the stores of 25(OH)-D, and should be restricted to children with symptomatic hypovitaminosis D. US guidelines recommend a 2- to 3-month dosing regimen of VD therapy of 1,000 IU/day in neonates, 1,000 to 5,000 IU/day in infants 1 to 12 months old, and 5,000 IU/day in children over 12 months old [5]. This course of high-dose therapy should be followed by a maintenance dose (400 IU/day in all age groups, or 800 IU/

day in at-risk populations). Oral calcium (500 mg/day) should also be administered as supplements or by increasing dietary intake [6]. The patient’s response to treatment should be monitored closely. Future research should focus on establishing the optimal 25(OH)-D concentrations for children and on determining the health consequences of hypovitaminosis D.

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LECT 59

METABOLOMICS AND AGGRESSION (ACTION PROJECT)

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Human aggression is a complex and widespread social behavior that is overrepresented in individuals affected by severe mental illness (SMI). A substantial proportion of the liability threshold

for aggressive behavior is determined by genetic factors, as demonstrated by large longitudinal twin studies showing a heritability of 50% to 80% [1]. Environmental moderators might modulate this genetic liability to aggressive behavior through modification of gene expression via the epigenetic machinery. Indeed, epidemiological studies have shown that the longitudinal developmental trajectory of aggression is significantly influenced by environmental determinants, even in the presence of high genetic risk [2]. For instance, factors such as low income, presence of mothers who smoked during pregnancy, mothers' coercive parenting behavior, and family dysfunction predict the manifestation of aggressive behavior in children [2]. These specific alterations in the genetic and epigenetic make-up of aggressive individuals might determine changes in the physiological pathways of the Peripheral and Central Nervous System (PNS and CNS) regulating behavioral control. Signatures of these alterations might be detected through the analysis of peripheral set of metabolites (metabolome). In fact, a recent data synthesis has shown that biochemical markers, such as inflammation markers, neurotransmitters, lipoproteins, and hormones of various classes, are significantly altered in individuals with aggressive behavior [3]. As there is no evidence directly linking aggressive behavior with alterations in the metabolome, we selectively reviewed recent metabolomics studies in autism spectrum disorder (ASD), and bipolar disorder (BD), two psychiatric conditions where aggressive behavior is often a prominent clinical manifestation. Concerning ASD, we found consistent evidence, mainly in urine specimens, for perturbation of several biochemical pathways. Among these, the tryptophan-nicotinic acid metabolic pathway, the amino acid metabolism pathway, the oxidative stress pathway, as well as the mammalian microbial co-metabolism pathway were significantly perturbed in affected individuals compared to healthy controls. Other studies found higher urinary levels of succinate and glycolate, of 3-(3-hydroxyphenyl)-3-hydroxypropanoic acid, of 3,4-dihydroxybutyric acid, glycolic acid and glycine, of cis-aconitic acid, of phenylalanine, of tyrosine, p-hydroxyphenylacetic acid, and homovanillic acid, but lower levels of hippurate, 3-hydroxyphenylacetate, vanillylhydracrylate, 3-hydroxyhippurate, 4-hydroxyphenyl-2-hydroxyacetate, 1H-indole-3-acetate, phosphate, palmitate, stearate, and 3-methyladipate in ASD individuals compared to healthy controls. Studies in BD individuals found that metabolomics profile in

cerebrospinal fluid (CSF) [4] and serum [5] had specific biochemical signatures different from those of healthy controls. Specifically, isocitric acid (isocitrate) levels resulted significantly higher in the CSF from BD patients compared to healthy controls, independent of medication [4]. Interestingly, gene expression analysis of two subtypes of isocitrate dehydrogenase (IDH), a key element of the metabolic pathway of citrate and isocitrate, namely *IDH3A* and *IDH3B* genes, showed a significant down-regulation in the dorsolateral prefrontal cortex of BD patients compared to healthy controls [4]. In the second study, the same group identified higher serum levels of pyruvate, N-acetylglutamic acid, α -ketoglutarate, and arginine in 54 BD patients than in 39 healthy controls [5]. Conversely, serum levels of β -alanine, and serine were significantly lower in BD patients than in healthy controls [5]. Taken together, these findings show specific metabolomics signatures of aggression in psychiatric conditions. Although it remains to be established whether these metabolomics signatures are specific to aggression, or are rather the result of other factors, such as the comorbidity with mental illness and/or the presence of a pharmacological treatment, these results indicate the potential of this approach in exploring the pathophysiology of this behavioral phenotype. It is conceivable that the joint analysis of genomics, epigenomics, and metabolomics data might help refining the prediction of aggressive behavior particularly, but not only, in at risk populations such as those affected by SMI.

ACKNOWLEDGEMENTS

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LECT 60

DOCEMUS FOUNDATION: A CONTRIBUTION FOR FINANCING NEW CLINICAL LABORATORIES AND SCHOOL TRAINING PROGRAMS IN LOW-INCOME COUNTRIES

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Docemus is an independent, non-profit Italian organization working around the world with the support and patronage of both the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC) and the School of Medicine of the University G. D'Annunzio, Chieti, Italy. Equity, social justice, self-sufficiency, and sustainability are the key values of Docemus; they are pursued by providing several investments and free high quality medical support to clinical laboratories mainly in Africa and in other low-income countries. All the members of the board are laboratory medicine professionals with a long time experience in clinical chemistry and biochemistry. They are involved in planning and realizing specific projects and missions in various low-income countries. The foundation promotes the fundraising for financing several activities such as: (a) new construction and general restoration of clinical laboratories; (b) upgrade of laboratory equipment and analytical methods; (c) personnel training by organizing either periods of attendance at Italian public clinical laboratories or local training sessions with the contribution of Italian physicians and technicians. Long-term goals are the following: to build laboratories fulfilling all the requirements (professional skill and technological innovation) for the early and accurate laboratory diagnosis and monitoring of most of the diseases affecting population in those countries; to improve the quality in laboratory medicine; to increase the availability of new laboratories; to implement new analytical methods; and ultimately to make native clinical pathologists completely

independent in managing the organization and the clinical activities of their laboratories. Since in clinical laboratories a huge technological gap exists between current tools (analytical methods and equipment) commonly used in high-income countries and those in low-income countries, Docemus is primarily involved in reducing this gap, with an active contribution of the *in vitro* diagnostic (IVD) industries. In particular, the effort is focused to reduce manual, low-standardized, and in-home methods widely used in African laboratories by automated, standardized methods, reducing the analytical error as well as increasing the productivity. The challenge is to maintain a low-cost per test. Therefore, IVD companies are called to offer analytical platforms, not necessarily new or next generation, but fully functional, easy and friendly to use in the routine, utilizing commercial kits easy adaptable and optimized on these instruments, and supported by technical assistance with the availability of spare parts. Over the past years, several analytical instruments have been placed in various African laboratories with the sponsorship of SIBioC and IVD industries. Docemus is also involved in introducing the culture of the analytical quality control and in promoting procedure standardization, hygiene and efficiency of the total testing process. Docemus has launched a partnership with other medical specialties and international organizations, like UNICEF, EMERGENCY and others. Currently, Docemus has entered into an agreement with general surgery, ophthalmology, nutrition, clinical pharmacology, and environmental laboratory with the aim to develop common projects for improving patient care and the quality of health care systems in several African countries (**Tab. 1**).

MISSIONS IN AFRICA

UGANDA: project for a laboratory and clinic in Uganda

Upon invitation and request from the local community, Docemus is exploring the possibility to build a laboratory for analysis with a clinic in Uganda, Kagadi Town, in the North West of the country, 5 hours-drive from the capital Kampala, 2 on a dirt road. Kagadi Town is a rural country of 30,000 inhabitants and it is expanding. In July 2012, Kagadi and its district, Kibalee, dominated front-page news all over the world because of the last outbreak of Ebola virus. Two Docemus operators went on an exploratory mission in the second half of June 2013, with the economical support of SIBioC. Docemus came back there

Table 1 (LECT 60). Main activities.

No.	Project	Country
1.	Teaching sessions for native physicians and technicians	Democratic Republic of the Congo
2.	Comparison between popular traditions and conventional medicine	Cameroun
3.	Preparation of a new clinical laboratory in the Emergency hospital of Port Sudan	Sudan
4.	Teaching transfusion medicine in Bangui	Central Africa Republic
5.	Cross-match learning in the laboratory of the Emergency clinic	Sierra Leone
6.	Building a new laboratory in Kagadi Town	Republic of Uganda
7.	Building a new laboratory for the Hargeisa hospital, with a special area for malnutrition	Somaliland ^a
8.	Building a new laboratory for the diagnosis of anemia in pediatric age	Kenya

^aSomaliland is not internationally recognised. Source: BBC Somaliland profile, available at: <http://www.bbc.com/news/world-africa-14115069>, last access: September 2016.

once more in August 2014 with the economical support of SIBioC. The aim of the mission was to verify, after having received a formal invitation from the competent local authorities of Kagadi Town and from the competent district authorities of Kibalee, the possibility to use some rooms in a wing of the Kagadi Town Hospital to activate a transfusion center, which is lacking in the hospital at the moment. There have been several meetings with the competent local authorities in Kagadi and district authorities in Kibalee, up to Kampala where we met the director of Uganda Blood Transfusion Services, Dr. Dorothy Kyeyune and the Health Minister of Uganda Dr. Ruhakana Rugunda. The meetings had positive results and we are now waiting to be formally entrusted to proceed. Several rooms were assigned to Docemus: some of them will be used as transfusion center, while the remaining spaces, in a next step, will be restructured to transfer and reorganize the main laboratory in order to have the laboratory and the transfusion center in the same facility.

CAMEROUN

The main idea of the mission in Cameroun was to investigate the existence of traditional medicine systems, which are deeply rooted, diffused and alternative to the science-based medicine. By extension the work tried to highlight the importance of diagnosis and the choice of treatment offered by

the laboratory for analysis, in a context of scarcity of resources. According to WHO, 80% of the population in Africa chooses traditional medicine rather than science-based medicine. In Cameroun, the health system – public, private or traditional – is a fee-for-service system. Another issue is the lack of an effective codified pharmacopoeia of the products used in traditional medicine and an evident sanitary anarchy, despite the decennial trials of systemization promoted by the most important national and international organizations of sanitary reference. Docemus produced 2 documentaries: one includes 35 video-interviews to medical personnel, researchers, shamans, and patients, while the other one is photographic. Mario Trave produced them and this was made possible thanks to the support of Dr. Rachel Kamgaing, CIRCB Lab Manager (Centre International de Référence “Chantal Biya” pour la recherche sur la prévention et la prise en charge du VIH/SIDA).

CENTRAFRICAN REPUBLIC

The mission of June-July 2014 consisted in restarting the National Center of Blood Transfusion in Bangui, closed due to the Country instability, with a project in collaboration with the WHO and EMERGENCY, and to support and control the laboratory of analyses in the EMERGENCY’s Pediatric Clinic in Bangui. Docemus collaborated in the blood collection in the local villages, in teaching the proper procedures and the validation process of blood bags, in the organization, in the control of blood supplies and in devices monitoring.

SIERRA LEONE

Docemus in April 2012 carried out a mission in the EMERGENCY’s Surgical Center in Goderich, a village in the periphery of Freetown. The main aims of this mission were: a) the training of the local technicians in the routine use and routine and extraordinary maintenance of the analyzer for the hematology (Medonic, Menarini, Italy), previously bought by EMERGENCY; b) the training to use a water bath to perform the cross-match and a mini rocker shaker for blood bags, with a scale for bloodletting given by Docemus. The successful learning was verified with particular care and attention.

SOMALILAND: Hargeisa – Mohamed Aden Sheik Children Teaching Hospital

In 2012, Docemus received the request of cooperation from MASCHT (Mohamed Aden Sheik Children Teaching Hospital) Non-profit Organization to set up the laboratory for analyses and the acquisition of knowledge and skills by the Somali

technical staff. This is a pediatric hospital and in particular 2 master programs in pediatrics and laboratory medicine will be established, recognized by the local government. In January 2013, our experienced laboratory technicians went to Hargeisa to set up the laboratory, to make it operative and to train the properly selected local technicians. The laboratory is currently operational and performs all the analyses necessary for the efficient functioning of the hospital.

SUDAN: The EMERGENCY's Clinic Laboratory

In 2010, Docemus presented a project for: the full furniture of the laboratory for analysis of the EMERGENCY's Pediatric Clinic in Port Sudan; the sending of Docemus personnel necessary for setting up the laboratory and make it operative; the training of local technicians. This project was presented to the Fondazione CariChieti (Italian bank) that funded the whole project with 25,000.00 €. In September 2011, 2 experienced Docemus technicians went to Port Sudan where they stayed for about 2 months, to set up the laboratory, made it operative and train the local technicians. The laboratory is currently operative and performs all the analyses necessary for the proper functioning of the EMERGENCY's Clinic.

LECT 61

NEW NMR BASED TOOLS FOR CLINICAL AND TRANSLATIONAL RESEARCH WITH FOCUS ON NEWBORNS AND CHILDREN

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In vitro Nuclear Magnetic Resonance (NMR) has found its way into large scale screening of body fluids in clinical/translational research and epidemiology. Based on its high reproducibility and transferability, it is a tool to integrate spectra measured under identical standard operation procedures (SOP) of multiple research groups into common analysis, be it targeted or un-targeted. Modern NMR spectrometers based on their digital receiver characteristics have very high dynamic range ($2 \cdot 10^5$) and therefore can detect and quantify a multitude of metabolites simultaneously. For quantification only 1 reference standard is needed for all metabolites. Routinely urine, plasma, serum

and cerebrospinal fluid (CSF) are investigated under screening conditions.

QUANTITATIVE TOOLS BY NMR

Plasma, serum

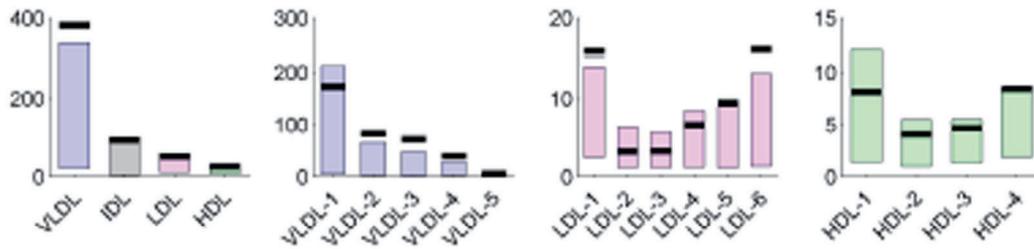
NMR has unique capabilities in terms of the analysis of lipoproteins in plasma and serum, where in a few minutes total cholesterol, free cholesterol, triglycerides, phospholipids, APO-A1, APO-A2, APO-B as well as particle numbers and particle sizes can be analyzed of total plasma or serum, the main fractions VLDL, LDL, IDL and HDL (VLDL: very low density lipoprotein, LDL: low density lipoprotein, IDL: intermediate density lipoprotein, HDL: high density lipoprotein) as well as their subclasses are calculated (VLDL 6, LDL 6, HDL 4). It is known that some sub-fractions of LDL and HDL depending on their concentrations are a measure for the risk for cardiovascular diseases [1, 2]. Well established for adults, the technology has not been much used for newborns or children. Lipoproteins play a role not only in cardiovascular diseases, but also in many other diseases like obesity, diabetes, metabolic syndrome, high blood pressure, cerebrovascular diseases, atherosclerosis and various types of cancer, just to mention a few. **Fig. 1** shows an extract of an automated lipoprotein main and subclass analysis report [3].

Urine

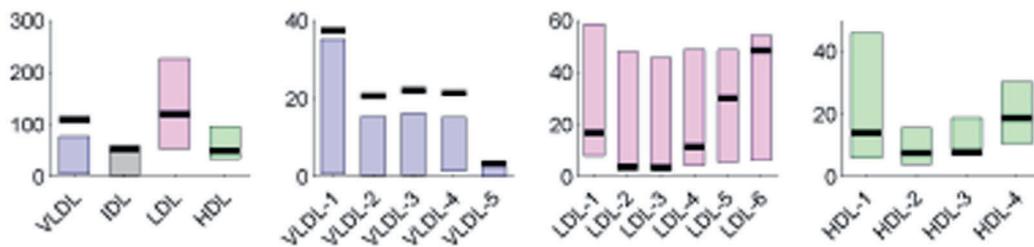
Another tool NMR can provide is automatic and precise metabolite quantification in urine. Fully validated quantification (based on DIN-spiking and numerical spiking) can deliver more than 130 metabolites completely unattended. Using spectral databases of pure compounds in body fluids acquired under identical SOPs, it is also possible to identify a much larger number of compounds under full automation (> 500). Quantification can be relative to creatinine and/or absolute. Due to the fact that only 1 quantification reference is needed for all metabolites, concentration values for all metabolites can be generated for every sample measured and analyzed. In doing so, a highly populated concentration distribution can be rapidly generated at least for every endogenous metabolite. Such a concentration distribution can indicate health issues, if a sample shows concentrations outside the range identified as standard (e.g. outside 99% of all values). Such ranges can be made specific, if the metadata for the samples measured are available as well. In this case it is for example possible to calculate the distribution for male children in the age of 1 to 5 years only and do the same for female children. Many other questions can be investigated with regard to the concentration

Lipid Distribution Overview

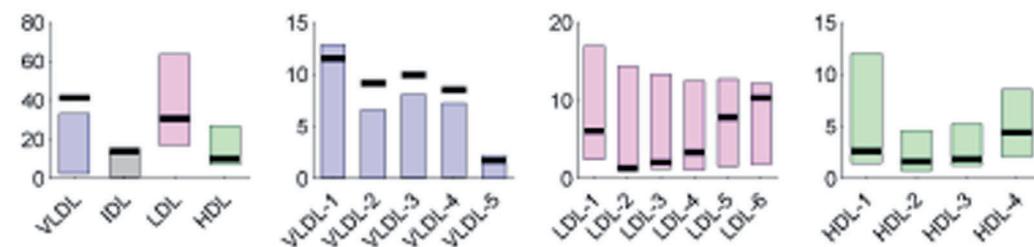
Triglycerides distribution (concentrations in mg/dL together with 95% range of model)



Cholesterol distribution (concentrations in mg/dL together with 95% range of model)



Free Cholesterol distribution (concentrations in mg/dL together with 95% range of model)



Phospholipids distribution (concentrations in mg/dL together with 95% range of model)

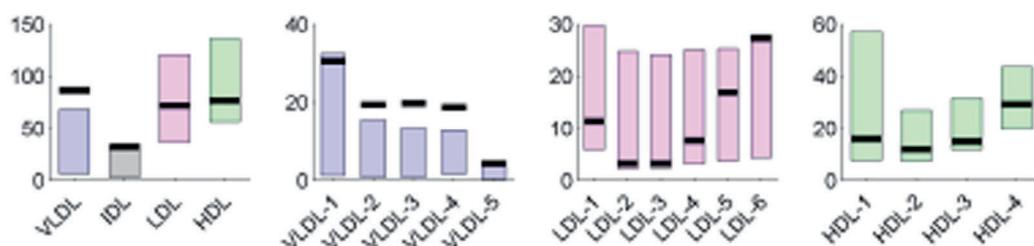


Figure 1 (LECT 61). Extract of a lipoprotein subclass report of a donor having a cardiovascular event 3 days after sample collection.

	mg/l	mmol/mol Crea	LOD	Distribution
Succinic Acid	0.20	62	20	
Citric Acid	≤ LOD	≤ LOD	490	
Dimethylamine	0.36	112	20	

Figure 2 (LECT 61). Extract of automated urine quantification, demonstrating the concentration profile of some endogenous metabolites.

distributions per compound, e.g. using geographical origin and diet. **Fig. 2** shows an extract of the quantification of endogenous metabolites in children urine. As it can be seen, such distributions are by no means Gaussian. This also indicates that a large number of samples is needed to create a meaningful concentration distribution, in order to define a normal range. The black bar in the distribution curve represents a new sample, which is checked against the concentration distribution. Besides endogenous metabolites, markers of inborn errors of metabolism can also be identified and quantified. In this case concentration distributions are difficult to obtain, as these diseases are fortunately very rare. Based on the above-mentioned spectral database of reference compounds it is currently possible to identify more than 200 IEM marker compounds and to precisely quantify about 80 of them. It is also shown how NMR compares to GC-MS, which is a standard in the pediatric lab. Simple straightforward preparation and interrupt free operation under full automation demonstrate the strength of NMR (**Fig. 2**).

Statistical analysis on all body fluids mentioned

While normal NMR spectral profiles of adults can be generated from a large number of data available, the numbers are small for children. A normal model for healthy children would allow checking new samples against such a model as it was published for newborns in Turkey [4]. Therefore, the collection of normal children urine has been started to generate such a well-populated healthy NMR profile. New samples can be tested against such a model in search for potential diseases in an untargeted mode. Metabolite signals leading to deviations from normality can be identified using spectral databases or 2-dimensional NMR technology in combination with high-resolution mass spectroscopy. Such healthy models can also be used to judge the treatment efficiency for example for dedicated diet for inborn errors of metabolism. An example is shown for PKU (phenylketonuria).

SUMMARY

High resolution NMR can be used efficiently in body fluid analysis, delivering a multitude of relevant parameters as well as statistical results with a single measurement. This contribution also stresses the strength of NMR in this complex mixture analysis.

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LECT 62

METABOLOMICS AND AUTISM

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Metabolomics is the study of the small molecules, namely metabolites, contained in body fluids as well as in human cells, tissues or organs. Metabolites are involved in primary and intermediary metabolism. The Metabolomics Society defined metabolomics as “the study of metabolic changes”. The term “metabolomics” is equivalent to metabolite target

analysis, metabolite profiling, metabolic fingerprinting, metabolic profiling. Metabolomics provides a functional readout of changes determined by genetic blueprint regulation, protein abundance and modification, and environmental influence. The importance of metabolomics is closely related to the importance of discovering the gap between genotype and phenotype, the latter being not necessarily predicted (in whole or in part) by the genotype. In fact, the phenotype is the result of the dynamic interplay between genome and environment; this complex, individual interplay originates many biochemical reactions, each with individual dependencies to various environmental influences (drugs, nutrition, gut microbiota, etc.). As a consequence, the diagnosis and the management of most human diseases cannot be merely based on the genomics and on the assessment of genetic risk factors; in the era of a multi-“omics” system biology approach, the metabolome is the closest biological representation of a clinical trait [1]. Therefore, the success of the precision medicine strategy, namely the tailored medical approach to each individual with chronic or acute diseases, lies on the identification of the molecular phenotype for adopting the most appropriate medical intervention and pharmacological treatment. Autism Spectrum Disorders (ASD), a diagnostic category assembling a group of complex neurodevelopmental disorders characterized by mental retardation and associated with life-long disabilities, is a perfect example highlighting the importance to assess and to monitor over time the patient’s molecular phenotype. About 15% of ASD are associated with known genetic mutation [2]. ASD is highly heritable; a recent Sweden study including more than 2 millions families demonstrated that heritability of ASD is around 50% of the population [3]. However, despite the relevant role of genetic risk factors (monozygotic and dizygotic twins, parental age and difference in age between parents, etc.), environmental risk factors can significantly influence the development and the severity of the disorder. This means that in absence of any genetic risk factor for ASD, the genomic approach is negligible; more important, non-genetically determined ASD can be recognized and diagnosed later (mainly between 12-24 months of life) on the basis of mental retardation and child behavior. On the other hand, the metabolomics approach at birth, consisting in the identification of the urinary or blood metabolome and its changes over time, allows to establish earlier whether or not a baby can be considered at risk to develop ASD due to environmental factors. In a recent review

of the literature on the role of metabolomics in autism, we analyzed experimental results published in the literature [4-6]. The comparative analysis of these studies showed that the main metabolic perturbations consist of: high concentrations of mammalian-microbial co-metabolites; nicotinic-acid metabolism; production of cellular energy due to mitochondrial dysfunction; antioxidant status; amino acid metabolism. One of the most relevant factors modulating gene expression by epigenetic mechanisms is fetal/neonatal gut colonization and dysbiosis. There is a large worldwide consensus on the role of an intact gut microbiota in shaping brain neurochemistry and emotional behavior. Gastrointestinal flora can be considerably altered by several environmental factors, such as: maternal bacterial flora and diet; perinatal antimicrobial use; mode of birth (spontaneous delivery or caesarian section); type of feeding; dietary intake. Notably, psychological stress during pregnancy and at birth can induce changes in the composition of gastrointestinal microbial flora. Gut dysbiosis raises abnormal metabolites and their escape into the bloodstream; most of them are neurotropic. This means that they rapidly pass the blood brain barrier and then could act as neurotransmitters or could modify biochemical pathways within the central nervous system, altering neurotransmitters synthesis and release. In conclusion, alterations in the composition and metabolic products of the gut microbiome have been implicated in the complex pathophysiology of ASD and these alterations can be easily revealed by changes in urine metabolome of newborns. The early identification of risk factors for ASD can improve children outcome by early therapeutic interventions such as gut microbiota transplantation. This implies a drastic reduction in the severity of ASD symptoms and, in turn, a better socio-relational outcome and a considerable money saving.

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LECT 63

BIG DATA, METABOLOMICS AND MICROBIOMICS FROM THE FETUS TO THE ADULT: TOWARDS 10 P PEDIATRICS

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Big data are going to make a real revolution in Medicine. With their characteristics (velocity, variety and volumes), they are actually penetrating medical systems and will become “our world”. Physicians of the immediate future will become coaches of big data. 80% of human diagnostics could be substituted by computers to prevent human errors, to anticipate not only the diagnosis of patients but also their outcome and, for example, to compare radiological images with millions of images and not only with the limited experience of the radiologist. In a few words: Big data + Machine intelligence = Artificial intuition. Big data is a big deal. New languages of medicine have emerged in recent years, they are presented in **Tab. 1** and **Tab. 2**. Metabolomics enables us to identify and quantify thousands of metabolites at the same time in the different liquids of the organism, thus it represents a new holistic (all in one tool), sensitive, and predictive approach to diagnosis. Some of its most important applications are represented by pharmacometabolomics and nutrimental metabolomics. The challenge of systems medicine is to interpret the body structure as a whole and not as a sum of single parts. Metabolomics represents the unique metabolic fingerprint of an individual. From small molecules to big ideas. The human gut harbors a diverse and complex community of microbes, termed gut microbiota, that normally assemble during the early phases of life. We have made

Table 1 (LECT 63). The new languages of medicine: metabolomics.

Systems Biology
Systems Medicine
Networks Medicine
Omics Technologies
Data trained Medicine
Big data in Medicine
Resilience, antifragility, fragility
Holistic Medicine
Individualized Medicine

Table 2 (LECT 63). The new languages of medicine: microbiomics.

Microbiota
Holobiont
Bacterial Networking
Bacterial Sociomicrobiology
Body Ecosystem
Co-evolution
Symbiotics
NEI (Neuro-endocrin-immunological) System
Bacteria and brain evolution
Microbiota transplant

substantial progresses in identifying the bacteria that live in our intestine and their hierarchical relationships (sociomicrobiology) and our three major enterotypes have been identified. Thus the microbiome is a cutting-edge topic in medicine. It is considered the conductor in the orchestra of immune-neuroendocrine communication. There are “good guys” and “bad guys” and we must start to analyze them and understand their action. Drugs can also influence the health of the gut microbiota, which is also able to modify the pharmacokinetics and pharmacodynamics of drugs, thus creating vicious circles. We have a genetic predisposition but the environment is much more important: epigenetics comes from the Greek word (*epi*), which means above. It means that for multigenic and multifactorial diseases (> 98% of diseases) the environment is more important than our genes. Our genes are not our destiny, or at least they are only in part. Diet is the most important epigenetic factor and our bacterial cloud is of the utmost importance. We are not individuals, but rather are walking ecosystems. From a practical point of view, only the interaction of a certain kind of diet with a certain type of intestinal microbiota produces metabolites that are able to pass into the bloodstream and modulate the structure and function of our organs, including the brain. How does the gut

microbiota talk to the brain? The language is an enigma. Many diseases derive from an unbalance of the organism induced by an inappropriate diet that leads to dysbiosis, that is, the abnormal colonization by an intestinal bacterial species that outweighs the others. Generally, a healthy gut microbiota is characterized by a high number and variety of microbial species: they protect us against the deleterious effects of inflammation connected to diseases such as diabetes and obesity. Again, an increased Firmicutes-to-Bacteroidetes ratio seems to be a signature of metabolic diseases influencing processes related to energy harvesting, intestinal permeability, bile acid metabolism, brain functions related to metabolism and immunomodulation. In fact, the intestinal microbiota interacts with the immune system and orients it throughout our lives, but especially during any time windows when our development, both fetal and in the first years of our lives, is at risk. Perinatal programming of organs (mainly related to endocrine and mitochondrial programming) explains the consequences of early events during fetal development even in the medium or long term. All these things taken together explain why the improvement and optimization of nutrition play the leading role in maintaining a person's state of health, in preventing diseases and their complications, and in expressing the individual response to the different pathologies. Furthermore, the positive message is that the rebalancing of the intestinal microbiota with prebiotics, probiotics, and symbiotics (prebiotics + probiotics), as well as with the transplant of intestinal microbiota with bacteria from a healthy subject, can lead to an improvement in our state of health and, even better, to its consolidation. We should know our gut microbiota and what kind of enterotype we are, we should protect it and respect it. A first key concept is that a person's life must be seen as a continuum from prenatal life (even before conception!) to that of the adult, and the most important period in this journey is the time spent in the womb. Another key concept is our extraordinary basal interindividual variability. This wide variability that swings from fragility to resilience to antifragility is enormously accentuated after acute stimuli such as neonatal asphyxia, sepsis, or prolonged abstinence from food: only the application of holistic technologies such as metabolomics and microbiomics is able to provide an image of the incredible complexity of every individual and capture his or her distinct and unique metabolic, microbiomic fingerprint and the pathways from a state of health to one of

illness and from the latter to recovery. This heritage will take us from pure research to the patient's bedside, thus providing an example of translational research, together with a universe of computer clouds composed of an enormous amount of data. The future has been traced and goes in the direction of the 10 P Medicine, namely the 10 P Pediatrics, as they have been recently defined: personalized, perspective, predictive, preventive, precise, participatory, patient-centric, psycho-cognitive, postgenomic and public. In short: individualized medicine.

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LECT 64

MICROBIOTA AND ASTHMA: "HOT TOPIC" VS. IMPLICATIONS FOR THE PRACTITIONERS

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By using culture-independent microbial identification and new sequencing techniques, researchers have recently postulated the role of a newly identified organ, the microbiota. The microbiota is currently considered a part of the human organism with profound implications on growth and development. Recent data collected on the microbiota impact on "educating" the immune system in infancy/childhood suggest the need to shift the paradigm from the "hygiene" to the

“microbiota” hypothesis, with regard to individual risk of subsequent developing of asthma and allergic disease. This presentation starts by presenting key epidemiologic studies supporting the existence of an intricate relationship between early development of microbiota in children and specific risk factors and exposures and their impact on development of allergic diseases. Further on, some studies identifying mechanisms by which microbiota can specifically alter the immune development and function and evaluating the prevalence of asthma, corroborated with the microbiome in certain population of children, are presented. Lessons learned from real life and their impacts on the activity of the practitioners are illustrated, suggesting effective methods for reducing the burden of asthma in children.

LECT 65

ADDING VALUE TO OBSTETRICS, FROM GENOMICS TO EPIGENETICS: AN ECOLOGICAL PERSPECTIVE

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The aim of the Genome Project was to understand how genes work and the causal link between their alterations and diseases. The Project started in 1990, it involved a number of international research institutes, and was coordinated by National Institutes of Health and by the US Department of Energy. The Project was terminated in advance, in 2003. The dream was: “*nosce te ipsum*”. The hope was that knowing about genes would have led to the complete understanding of human nature, but this did not happen. We understood, on the other hand, that we are not predetermined, at least not entirely, but we are also determined by our interaction with the environment, which starts right from the germinal cells of our parents. In fact, not only the composition and expression of their DNA play a role, but also the epigenetic modifications that they were subject to during conception, then continuing during intrauterine life, concretized at birth and, afterwards, during breastfeeding, weaning and following nutrition, together with our style of life. This way we can continuously change our destiny, the path of our life on the Earth. We understood that the environment could change the expression of DNA. Epigenetics can be defined as the study of the

changes in genetics that do not cause changes to the inherited sequences of DNA. Epigenetic mechanisms can ensure that the characteristic phenotypic traits of parents are transmitted both directly, via microRNA, as well as indirectly, via other mechanisms (i.e. DNA methylation) to the offspring. An example of this can be seen in rats, where loving mothers give birth to very affectionate offspring. On the other hand, less loving mothers will have less loving offspring. This is known as the transmission of behavior, and transmission can be completely changed if the baby rat born of a less loving mother is entrusted to a very loving mother. The baby rat will “inherit” the behavior of the mother it has been entrusted to, showing that the acquisition of this behavioral trait is acquired and not genetic. In fact, if baby rats are exposed “to love” they will reduce DNA methylation processes in a particular position of their genetic code. This modification is activated immediately after birth, and the consequences remain for the entire lifetime. This example shows the responsibility that obstetricians have during birth, which goes far beyond this single moment. In fact, during the very first moments of life, numerous nucleotide sequences are activated and disabled, and the ways in which this process takes place cause long term consequences. An example of this can be seen in the administration of medication during labor in humans. A strong inverse relationship has been found between the amount and duration of exposure to fentanyl, used for epidural anesthesia, together with the amount of synthetic oxytocin, on the suckling ability and on the ability of reaching the mother’s breast the first hour after a vaginal birth. The more exposure newborns have to synthetic oxytocin and fentanyl, the more difficulty they will have to reach the mother’s breast. Another paradigm, which we are slowly starting to refute, is considering microorganisms exclusively as enemies of eukaryotic organisms. We now know that we live side by side with about 10,000 billion microorganisms, which live inside our bodies – above all in our intestines – but also in our mouth and genitals. How can we positively interfere during pregnancy and childbirth with these processes that we are starting to find out about? An example could be to carry out fewer cesarean sections. Cesarean sections can actually reduce mortality and maternal and perinatal morbidity if carried out following precise medical instructions and indications. There is not, however, any medical proof to show the benefits of caesarean sections for mothers and newborns when there is no clinical justification. On the contrary, women who undergo a cesarean

section are subject to a higher rate of the following: maternal death, maternal morbidity, peripartum hysterectomy, subsequent periods of hospitalization during the postnatal period, amniotic fluid embolism, placental accretism, displaced attachment of the placenta in subsequent pregnancies. Babies born via cesarean section are subject to a higher rate of the following: iatrogenic lacerations during cesarean delivery, respiratory morbidity, poor cardiovascular adaptation, reduced ability to be breastfed, anemia. Babies born via cesarean section have a higher risk of asthma, laryngitis, gastroenteritis, ulcerative colitis, celiac disease, infections of the lower respiratory tract, juvenile idiopathic arthritis (JIA), leukemia, death, obesity, type 1 diabetes, metabolic syndrome, cancer. In the pathogenesis of this “bad adaptation to birth”, one of the most important factors is probably the failure to acquire maternal microbiota, which takes place as a consequence of a caesarean section, thus resulting in the altered maturation of the neonatal immune system, which can lead to changes both in our “internal environment” as well as towards the outside environment.

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LECT 66

IMPLICATION OF METABOLOMICS FOR MATERNAL-FETAL MEDICINE

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Systems biology has been widely adapted and applied to characterize a wide range of disease states, but so far it has been limitedly utilized in reproductive biology and pregnancy. High-throughput technologies and computational biology afford and provide a unique opportunity to advance knowledge and in-depth study across a continuum of both healthy and high risk pregnancies, the latter resulting in adverse outcomes and related maternal-fetal complications. Prediction and prevention of the major diseases in pregnancy such as gestational diabetes mellitus, chorioamnionitis or preterm birth is nowadays a major global concern. Due to the concept of fetal programming, development of embryo and fetus is a complex process that can be altered even by subtle changes in the intrauterine environment and can endure into adulthood. This influences the long-term outcomes and prospects of the baby not only in the perinatal period but also over the entire length of its life. Metabolomics, especially global untargeted approach called as fingerprinting, has a great impact in gaining new insights of molecular changes underlying disease onset, monitoring disease progression and identifying novel biomarkers of clinical relevance. Fingerprinting aims to measure the complete set of metabolites (the intermediates and products of metabolism) and obtain unique, disease-specific metabolite patterns that allow to map the blood metabolome and to decipher biological processes to create dynamic picture of the phenotype. Recently, we have applied untargeted metabolomics approach to the study of gestational diabetes mellitus (GDM) and chorioamnionitis. Multiplatform (LC-QTOF/MS, GC-Q/MS, CE-TOF/MS) analysis of plasma allowed to clearly discriminate GDM pregnant women from controls in the 2nd trimester of gestation. Specific metabolic pattern that are indicative of low-grade inflammation and altered redox-balance, which may reflect on the pathophysiological context of GDM, was observed. Meaningful information of altered metabolic pathways was also achieved in the study of amniotic fluid in case of chorioamnionitis associated with perinatal brain damage. These discovery-based studies and many others with promising biomarkers candidates have to be validated in further, independent research with larger sample size involved. This is the main bottleneck and a key challenge of most metabolomics-based studies that could be changed by improving sample collection guidelines, standardizing methods and establishing of scientific collaboration.

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LECT 67

AN ETHICS FOR RELATIONSHIPS IN NEONATOLOGY

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WHY ETHICS FOR RELATIONSHIPS IN NEONATOLOGY? HOW IS IT TO BE PRACTICALLY OPERATED?

We know that handling by staff and parents entirely affects the baby immediate environment. That clearly shows that the functioning and maintenance of this environment requires a particular know-how from the health professional in neonatology. This know-how cannot be generated at random: it must be precise and, paradoxically, both singular and reproducible. Quite naturally, in a first spontaneity, the gestures of the care-giver are often, and in spite of himself, shaped by everyday social ways of “being in relation”. These modes are themselves conditioned by dominant cultural codes that infiltrate this intersubjective space and which, too often, come on the way of the quality of clinical practice. This context opens the field for clumsiness. Pity, good intentions, or blinding rigidity in the application of protocols are part of it as well. This mode of interacting is also a form of self-subjection that stifles creativity. For instance, the practical gestures of the “gentle handling” run the risk of becoming fake and bringing about a restrained form of acting and being. This mode made of precautions driven by the fear of disturbing an equilibrium actually disturbs the equilibrium. Assuredly, we handle the baby slowly and carefully; however, the baby has become an object. The caregiver is separated from his “power of acting”. This brings about the risk of making mistakes in his judgments: his capacity for a sharp observation is limited. It is a common attitude for beginners and one that haunts as well the everyday practice of experienced caregivers. This mode of being has become natural for them: a ‘being-with’ that only goes through the cognitive

level, making inaccessible the felt resonance of the presence in its density – the unique experiential mode of the newborn. The bionomic mode takes the caregiver out of his everyday interhuman experience. As Bachelard puts it in the context of scientific observation: “It is only through a derealization of regular experience that we can reach a realism of the scientific technique”. It is not a mode of being that is directly reacting to these everyday attitudes, but one that implicitly invalidates their production. It is as if the caregiver, through this way of interacting, could “trick his own culture”.

HOW TO MOVE FROM DAILY INTERSUBJECTIVE EXPERIENCE TO THE BIONOMIC MODE?

Through the experiential knotting, a radical move happens both for the carer and the newborn. Production of an interruption with the flow of what was before. “Rupture, fissure, break {...} that produces the plane of immanence” – immanence in the sense of a sharp presence to the actual data. Taking the kilos into consideration, taking into account this actual mass in our hands, even if it is only 950 grams, brings this reversal about that makes it possible for us to arrive, almost in spite of ourselves, right in the heart of this singular experience. This can happen on a daily basis, enabling the capacity of capturing details, the relations between them, and a countless number of emerging events. The baby and the care-giver land in the actual data of their environment: the baby is no longer a generic term; it is this baby, this existential singularity here and now that is in front of me and in my hands. The caregiver is for the baby this reachable bodily mass. This effective interaction felt by both protagonists is a mutual exchange of experiences; a genuine communication is established. In these conditions, we observe a stabilization of the newborn’s vital parameters, and he becomes less irritable. The entire context sharpens and we note that parents become more aware of the importance of their presence for the well being of the child. The everyday routine gestures of the caregiver are disarmed by this flow of realism: his “power of acting” is liberated. The mode of being socially conditioned and full of precautions “in the fear of” is replaced by a free, frank and unequivocal mode of acting and infra-verbal communication abilities that are so precious in NICU. Through this practical methodology that is precise, singular and reproductive, each moment of the encounter is the occasion for an ethical caring gesture: the baby, caregivers, parents, and also everyone in the NICU team are brought in contact with their own “power of acting”. Beyond the fact

that it brings back the good functioning of the baby immediate environment and physiology, this gesture participates implicitly in the production of an effective culture of care – a culture close to the ancient wisdom's concern to inhabit the world.

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LECT 68

DO THE NEW EUROPEAN REGULATIONS HELP NURSES?

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Since 25th November 2015, a EU regulation about fair working hours in public health has entered into force. It establishes the respect of the eleven consecutive hours of rest, every 24 hours and not exceeding the 48-hour working week. This adjustment regulation refers to the Law no. 66/2003 which represents Italy's acquisition of the European legislation created to protect the physical and psychological integrity of workers. In the following report we will try to discuss such regulation and how it has affected on the assistance of nurses working in the neonatal field. I performed an analysis of the European directives referring to the implementation of the directives 93/104/EC and 2000/34/EC, concerning some aspects of the organization of working hours. The previously mentioned regulation defines and clarifies what is meant by working hours and rest, and it identifies in how many hours they have to be organized. As for the formulation of the shifts and the hourly division, the distribution in 7 hours in the morning, 7 hours in the afternoon and 10 hours in the night appeared to be the most balanced. In particular, the night shift, although onerous, has always remained contained within the 10 hours of service, which is the maximum limit to ensure safety of patients and staff. Currently, researches in Europe and in the USA are highlighting a strong trend to limit the working hours of the staff. Several studies point out that many working hours have an impact on chronic fatigue. This impact is greater when referring to

the field of intensive care. The night shifts generate sleep deprivation; tiredness affects patient's safety and fatigue can cause errors.

A study carried out in a French intensive care unit concluded that 46% of the staff of intensive assistance was suffering from exhaustion and 24% from depression. The number of working hours was associated to a high level of burnout. *Intensive Care Medicine* published an article showing how the night shift decreases cognitive skills of the ICU staff (research published by *Editorial Daily Network*). Another emerging concern is related to injuries. Field studies highlight that in health care, unlike in other critical fields (construction, agriculture, metal processing), they are steadily increasing. In the health field, the number of nurses has decreased over time, while the index rate of accidents tends to increase, as well as their severity. A clear relationship emerged between the accidents and reduced attention level, especially close to the weekly rest period, when the tiredness accumulated by several days of continuous shifts is dominant. During the calendar year, the peak of accidents occurs during the summer months. In July, in particular, it is double compared to the other months and in this case it definitely affects on the ability to focus and on the well being in the workplace. It is a time of the year when the working pace in hospitals is still very strong, but the staff decreases due to the holidays.

LECT 69

BIRTH AND DEVELOPMENT OF A NEONATAL RESPIRATORY ECMO CENTER: NURSING ASPECTS

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In September 2015, the Neonatal Intensive Care Unit in Milan launched the Neonatal Respiratory Extra-Corporeal Membrane Oxygenation (ECMO) Center. The nurses and physicians of the ECMO team received a specific training on extracorporeal techniques in order to master the procedure. The ECMO group is continuously active and updated in order to improve the ECMO program and ensure the best assistance for the neonates supported by ECMO procedure. Extracorporeal support is recommended in case of severe respiratory diseases – such as MAS (Meconium Aspiration Syndrome),

pulmonary hypertension, pulmonitis, CDH (Congenital Diaphragmatic Hernia) – or cardiac diseases – such as myocarditis or congenital heart defects – which are reversible but non-responsive to conventional intensive care and standard therapy. ECMO artificially supports cardiorespiratory functions. ECMO gives the neonate a chance of survival during the acute disease phase, allowing rest and recovery for the organs involved. Connected to the patient's vascular bed, the ECMO machine performs pulmonary and cardiac functions replacing native heart and lungs. Adopting extracorporeal membrane oxygenation for severe respiratory failure aims to maintain adequate O₂ and CO₂ exchanges and minimize ventilation-induced lung injury (VILI). The ECMO circuit has 4 major components: 2 cannulas (venous cannula and arterial cannula) that connect the circuit to the vascular bed of the neonate; the pump that establishes blood flow replacing the heart pump; the membrane oxygenator that performs pulmonary functions; the heat exchanger that keeps blood normal temperature (37°C) through the circuit. Even if the circuit material is biocompatible, blood contact with non-biological material activates the inflammatory response and coagulation cascade. In order to prevent these complications, anticoagulant therapy is necessary, increasing the risk for severe bleeding. There are two types of ECMO: veno-venous ECMO (V-V ECMO), in which oxygenated blood is returned to a vein, and veno-arterial ECMO (V-A ECMO), in which blood is returned to an artery. Due to Poiseuille law ($R = 8\eta L/\pi r^4$), cannula dimensions need to be as big as possible in order to permit an adequate and efficient blood flow for the procedure (120-150 ml/kg/min). For this reason, V-A ECMO is recommended in neonates. Therefore, the venous cannula is positioned in the right internal jugular vein and the arterial cannula is positioned in the right carotid artery. Creating a new ECMO program requires a scrupulous preparation of the staff, which needs to master the procedure in the best way to guarantee the quality of care. Neonates supported by ECMO require a well-trained multidisciplinary team with specific skills in preventing, restricting and solving complications due to the procedure. Nursing care must include: constant extracorporeal circuit monitoring and managing, which requires basics of hemodynamics and of machine mechanisms; continuous intensive care of the neonate, exposed both to disease and procedure risks. Due to these aspects, ECMO nurses' training was based on frontal theoretical

lessons and practice exercises with medical staff. The purpose was to reach the same skills for each team component. The training program is characterized by continuous training once a month and by practical simulations, as to both apply skills achieved by theoretical lessons and coordinate members in the best way possible. Comparison with high experience in pediatric ECMO centers allowed answering nursing care important questions, for example: airways managing considering high blood risk; timing for positioning invasive devices; cannula insertion point dressing; cannulas and circuit fixing; neonate correct position; passive movement therapy; emergency events managing. Nurse staff draws up an ECMO protocol based on these features, constantly reviewed as to guarantee the best quality of nursing care. In March 2016, we had the first neonate supported by extracorporeal circulation, due to H1N1 influenza with severe respiratory failure. The procedure was successfully completed. For the first time the ECMO program has been tested: satisfying results did not stop staff training and project improvement, as we believe that constant review is necessary to reach maximum validity and quality of care.

LECT 70

LATCH SCORE, MATERNAL PERCEPTION AND SUCCESS OF EXCLUSIVE BREASTFEEDING: THE NURSERY EXPERIENCE IN CAGLIARI

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INTRODUCTION

Breastfeeding is a very important practice in the public health system and in the last few years it has become even more important, enough to be acknowledged by UNICEF as a right in the article 24 of the UN *Convention on the Rights of the Child*. It has been reported that every year in the world, 10.9 millions children die under the age of 5. In two third of the cases, death occurs under the first year of life and it's associated with malnutrition. Nowadays mothers are supported in breastfeeding by the availability of classes for expectant mothers and by the accessibility of information thanks to the mass media. This has led an increasing number of women to choose breastfeeding in the last years, creating

the need to train professionals and to develop research in this field. The UNICEF-WHO document *Global strategy for infant and young child feeding* recommends exclusive breastfeeding for the first 6 months of life, followed by continued breastfeeding with the introduction of complementary food and up to 2 years, or until the mother and the child wish. The advantages of maternal milk for both the infant and for the mother are well known. In fact, maternal milk is species-specific and contains all the fundamental elements for the growth of the infant. Furthermore, several studies demonstrated its protective effects against several pathologies and its role in promoting bonding between mother and child. In fact, in the correct position for the breastfeeding, it is possible for the neonate to see the mother's face. Furthermore, in the reproductive life of a woman, the act of feeding her child is a very significant moment from a psychological point of view. Therefore, besides the clinical benefits, breastfeeding makes her feel new emotions and improves her self-esteem. The consciousness of the presence of the milk, the possibility to see it and a less painful breast are all factors that persuade mothers to have faith in their own abilities to produce it and feed their newborn. Nevertheless, difficulties are frequent in the beginning, making mothers frustrated towards healthcare professionals or even their neonates. Mothers often think that infants dislike being breastfed because they interpret some normal behavior of the neonates such as moving the head or the legs and the arms or fighting with the whole body as a rejection of breastfeeding. Some women who have recently given birth can experience several psychological problems, including anxiety and depression, and they can feel inadequate to fulfill the needs of a neonate. Thus, healthcare professionals have a role of primary importance in supporting mothers during breastfeeding and they should be trained to establish a helpful relationship with the mothers. Healthcare personnel uses different scales to evaluate breastfeeding, one of the most common tools being the LATCH score. This is an assessment tool elaborated by Jensen, Wallace and Kelsey in 1994 and it provides a systematic method to collect different information concerning breastfeeding. It is important to highlight that besides the collection of information and evaluation, healthcare professionals should offer psychological and practical support to the new mothers.

METHODS

This study investigated the attitude of the mothers towards breastfeeding and the correlation between

LATCH score and the perception that mothers had towards breastfeeding, the eventual need of further consultations, and the success of exclusive breastfeeding one month after the delivery. The study was performed between September 2014 and July 2016 in the Neonatal Section of the AOU Cagliari and the sample included healthy women, mothers of healthy neonates born at term. A pediatrician counseled each mother about breastfeeding on the second day after the birth. After each counseling, the LATCH tool was filled out and mothers were asked if they need further consultancies concerning the breastfeeding. Subsequently, a survey of 9 questions was proposed to the new mothers, in which 6 questions aimed to investigate the need for further support. A month after the delivery, a group of mothers was called to find out which type of feeding they were performing.

RESULTS

Our data show that the majority of women were primiparae. 76% of them had previously received information about breastfeeding during pregnancy and were positive about breastfeeding. Concerning LATCH scores, several mothers obtained a score of ≥ 7 (61%), but more than a half of them requested another consultancy concerning breastfeeding, while some of them did not perform exclusive breastfeeding at the first month of life of the neonates.

CONCLUSIONS

The study results showed that the majority of the women who according to the LATCH score were not at risk of non-exclusive breastfeeding needed further support for breastfeeding. LATCH score is a good evaluation scale for breastfeeding and it is particularly useful for the first approach of the dyad mother-child, but the insecurities and the needs of the mothers should be taken into account as well.

LECT 71

PRESSURE ULCERS IN THE HOSPITALIZED PRETERM INFANT

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Skin lesions are a widely recognized complication in adult hospitalized patients. Although they represent a similarly significant problem in infants, studies in the pediatric and particularly in the neonatal

population are very limited. Indeed, the prevention and management of skin lesions is very important, especially in a busy Neonatal Intensive Care Unit, where several important risk factors are present, such as the immaturity of the integumentary system, a spontaneous or induced reduced mobility, and the widespread use of devices and their related fixing systems. The most common skin injuries in this area are stripping lesions, dermatitis associated with incontinence, lesions caused by chemical and thermal damage, pressure ulcers, surgical wounds and lesions caused by congenital abnormalities. In this report, however, we will mostly deal with pressure lesions in babies with limited mobility; in addition, we will also discuss about skin tears. Pressure ulcers are skin lesions with necrotic evolution, affecting the epidermis, the dermis and the subcutaneous layers and, in severe cases, also the muscles and bones. This kind of lesions is due to a prolonged compression between a bony prominence (skull, sacrum) and a rigid outer surface (bed, pillows, devices), resulting in insufficient tissue perfusion, and the related skin complications. Lesions may be single, or multiple. In the neonatal field, infants at highest risk are those with reduced mobility due to forced postures (for example surgical and/or curarized infants), or extreme prematurity, or undergoing therapeutic procedures (for example, induced hypothermia, cast, ankle leather cuff traction), and infants suffering from neuromotor problems. An additional risk factor is represented by the presence of more devices in different locations and for a long period of time, such as an endotracheal tube, electrodes, the cuff for blood pressure monitoring, vascular catheters, bladder catheters, drains, etc. Other risk factors are gestational age less than 32 weeks, weight lower than 1,000-1,500 g, dehydration, malnutrition, edema, and the presence of factors that negatively affect oxygenation and tissue perfusion (blood glucose alterations, cardio-vascular instability, use of vasopressors). Skin tears, on the other hand, are wounds produced by the action of shear forces, friction and/or blunt devices, resulting in separation of the skin layers. A skin tear can be partial thickness (epidermal separation from the dermis) or full thickness (epidermis and dermis separation from the underlying structures). Both intrinsic and extrinsic factors can contribute to the development of skin tears, some of which have yet to be determined. Factors that may increase the risk of skin tears are the presence of bruising, a long-term corticosteroid use, skin cleansers, cardio-vascular and/or lung disorders, dehydration and malnutrition, previous

skin tears (because the area, although healed, has a reduced tensile strength), and preterm infants, in whom the dermal-epidermal junction and the stratum corneum are poorly developed. In a newborn of gestational age around 24 weeks the subcutaneous tissue is scarce and the dermis is placed over the muscle, so even a simple maneuver of removal of a patch or an adhesive dressing can result in loss of full-thickness tissue. The preservation of the skin integrity is one of the indicators of neonatal welfare and, as pointed out by the Code of Nursing Practice in 2009, nurses as professionals are responsible of nursing assistance, and are committed to protect it and prevent any lesions. More attention should be given to the prevention of pressure lesions, in order to avoid preventable injuries that affect the newborn quality of life, and may increase the risk of infections, which leads to longer hospital stay and higher costs.

LECT 72

STABILIZATION AND TRANSPORT OF ASPHYXIATED NEWBORNS TO THE REFERENCE CENTER

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Perinatal asphyxia, more appropriately known as hypoxic-ischemic encephalopathy (HIE), is the medical condition resulting from deprivation of oxygen during the birth process for duration sufficient to cause neurologic injury. Laboratory studies have demonstrated the complex relationship between fetal and newborn asphyxia and brain damage. Most cases of perinatal asphyxia are not only caused by intrapartum events, but are often associated with underlying chronic maternal or fetal conditions. Among the intrapartum causes, obstetric emergencies are the most common ones and are not always preventable. Screening of high-risk pregnancies with ultrasound, Doppler velocimetry, and antenatal testing can aid in the identification of situations at risk. During asphyxia, some portions of central nervous system (CNS) are deprived of oxygen supply, which may lead to permanent brain injury manifested as cerebral palsy as well as cognitive defects. Until recently, no treatment has proved to be effective for preventing brain damage, even though it has been demonstrated that the damage is progressive and that there is a window

of opportunity to arrest some of the evolving brain injury. New guidelines for the resuscitation of newborns recommend that therapeutic hypothermia (HT) should be the standard method of treatment offered to neonates with acute perinatal asphyxia. The quality of care during and after resuscitation and the mode of transport can have a relevant impact on the outcome, either improving the newborn's condition or resulting in the deterioration of the neonate's brain. The therapeutic effect depends on the starting time of the cooling procedure and delayed treatment must be avoided. For this purpose, neonatologists or pediatricians from referring hospitals who do not have the equipment for hypothermia, should begin the cooling process while waiting for the arrival of a neonatal transport team (within 6 hours of birth). In that short time it is necessary to identify infants at risk for HIE, following guidelines for HT and initiating cooling using simple methods, the so-called low-tech cooling methods. In conclusion, a good resuscitation, a correct stabilization with early cooling, and HT have proved to be successful treatments for the reduction of brain injury in asphyxiated newborns.

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LECT 73

PERINATAL ASPHYXIA: LESSONS FROM METABOLOMICS

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Despite the advent of a more intense monitoring of the fetus well-being during either the gestational and the perinatal period, perinatal asphyxia (PA) with its most feared consequence – cerebral palsy (CP) – is still one the leading causes of permanent impairment in infants and of neonatal mortality [1]. With increased longevity in this group of patients, it has been estimated that there are nowadays nearly 3 times as many adults as children with cerebral palsy.

This figure is a heavy challenge for social services and health systems [2] and an increasing medical liability issue. PA, neonatal encephalopathy (NE), hypoxic-ischemic encephalopathy (HIE) and CP have been extensively studied both in animal models and in their clinical scenarios, looking for insights into the etiopathological mechanisms underlying these polymorphic pathological entities. An in-depth understanding of the injury cascade leading to cerebral palsy through different pathological steps might help in creating a “general clinical approach” to reduce prenatal, perinatal and postnatal risks and a “personalized therapeutical” one to ameliorate the outcomes. In this view, metabolomics represents an intriguing tool to better understand the inner modifications occurring at the different metabolic pathways as a consequence of the severe disturbance of cells and tissues homeostasis. Metabolomics is an approach to identify, in a qualitative, semi-quantitative or quantitative way, a huge number of small metabolites (molecular weight below 1,500 Da). After a perinatal prolonged partial asphyxia or a sudden sub-total asphyxia, the metabolic profile from either urine or plasma/serum, of the newborn – if the hypoxic insult is of a sufficient degree or prolonged beyond the ability of the fetus to compensate – shows a clear and peculiar modification. This may be helpful, together with clinical signs and instrumental identification of cerebral and cardiac variations, to the diagnosis and, to some extent, to the prognosis of the hypoxic insult. From a medico-legal point of view the metabolomics snapshot at birth may be very useful to discriminate among prenatal and perinatal causes and the ability to following the clinical-biochemical evolution in the first days of life may help to better evaluate the clinical management of the fetus/newborn. Several blood biomarkers have so far been investigated and proposed as diagnostic tools to monitoring the clinical evolution and to predict final outcome, but they failed in their task. Given the clinical complexity underpinning perinatal asphyxia, it seems difficult to rely on a single biomarker to fully predict outcomes and even a panel of biomarkers may underrepresent the biological phenomenon. Therefore, following the clinical evolution of all the infants born with clinical signs of perinatal asphyxia and submitted to therapeutic hypothermia by an intensive monitoring of urine metabolomics profile may represent an efficient alternative. A real option is represented by the metabolomics profile as a by-

product of the hypoxic-ischemic insult in the first moments after delivery and its attitude to follow the metabolic modifications over-time, compared with a control population of at term newborns without any medical issues, in a non-invasive way during and after the therapeutic hypothermia. Animal studies (either on mice, rats, piglets, and non-human primates) on perinatal asphyxia are consistent with the identification of a distinct profile in these newborns, mainly characterized by an increase in intermediates of Krebs cycle (such as succinic acid, fumarate, malate and alpha ketoglutarate) together with an increase in lactate and glycerol, with an altered amino acid profiles (such as alanine, glycine and branched-chain amino acids – BCAA) and with release in blood and in urine of components of cell membranes. Authors explained this metabolomics modification as an extreme attempt to compensate the energy failure by shifting the entire energetic production pathway from an aerobic to an anaerobic one and as a consequence of the mitochondrial dysfunction. However, it is well known that translation of animal research results to human context may be not a simple task. An effort to create a national/international network to collect urine samples and – whenever possible – blood specimens of newborns diagnosed with severe and mild HIE, together with a larger dataset of biological samples coming from healthy at term newborns, seems at the moment a compelling need. This would allow to create a robust metabolomics model to help clinicians to better manage the hypoxic-ischemic insult, as well as forensic expert to discriminate beyond any reasonable doubts the newborn in whom the damage has to be attributed to a professional conduct under the clinical standard from the one in which the damage recognizes causes other than the medical liability.

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LECT 74

NEWS ON THE TREATMENT OF GENETIC DISEASES

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Until few years ago genetic diseases and syndromes had no hope of real treatment. After clinical and/or molecular diagnosis, the only possibility of treatment was related to a symptomatic approach to the different medical problems and, when indicated, to a psychomotor and cognitive rehabilitation. These approaches are still absolutely valid and are the core messages of “pediatrics disability”. More recently, however, innovative therapeutic indications are in progress. From a medical point of view, we are now aware that early growth hormone treatment has completely modified the natural history of Prader Willi syndrome, particularly in terms of stature and prevention of the classical obesity. In patients with tuberous sclerosis, everolimus, an mTor inhibitor, succeeded in achieving an impressive reduction of cerebral multiple or infiltrating subependymal giant cell astrocytoma (SEGA). To date however this reduction seems to be strictly related to the assumption of the drug, with new growth of the lesions when the drug is interrupted. An interesting trial is ongoing regarding pharmacological treatment of patients affected with achondroplasia. The subcutaneous injection of natriuretic peptide C analog, BMN111, seems to promote long bone growth and to improve other skeletal anomalies in the mouse model and in a little cohort of patients. Further research is in progress on the same diseases regarding the use of statins and meclizine. Pharmacological studies are also ongoing in the treatment of hypo/anhidrotic ectodermal dysplasia. Study on dogs showed a clear improvement in sweating glands and teeth after neonatal administration of an analog peptide of EDA-A1 protein. The “correct peptide” is able to normally stimulate the specific receptor, obtaining a regular formation of tooth and sweating glands. The first affected neonates have been treated with this new molecule and we are waiting for clinical results after a proper clinical follow-up. Other examples are available on new researches related to treatment of genetic disease starting from induced pluripotent stem (iPS) cell approach. An example of this strategy could be considered the very brilliant data related to Down syndrome published by Jiang et al. in 2013. The authors in fact succeeded in inserting the *Xist* gene on one of the three 21 chromosomes in iPS cells of affected patients, and this insertion was able to inactivate the “treated chromosome”. In conclusion, even if most of the data currently available are preliminary and related to animal or iPS models, the treatment of genetic syndromes seems to be a new, more concrete perspective.

LECT 75**EXPANDED NEWBORN SCREENING FOR INBORN ERRORS OF METABOLISM: THE LYSOSOMAL DISEASES**

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BACKGROUND

The inclusion of Lysosomal Storage Diseases (LSD) on the newborn screening panel has been the subject of considerable debate. LSDs screening presents several challenges due to the nature of the conditions and lack of information regarding phenotypic spectrum, timing of treatment interventions, as well as a lack of professionally approved guidelines. We report our experience of LSDs screening for Fabry, Pompe, Gaucher and Mucopolysaccharidosis Type I (MPS I) diseases in North East Italy.

METHODS

The enzyme activities of α -galactosidase A (GLA), α -glucosidase (GAA), β -glucocerebrosidase (ABG) and α -L-iduronidase (IDUA) were analyzed on dried blood spot (DBS) by stable isotope dilution flow injection analysis MS/MS (FIA-MS/MS) using the NeoLSD kit (PerkinElmer®). Infants with low activity (< 0.25 ile) were recalled for a second DBS. If low activity were confirmed, neonates were referred to our Unit for clinical assessment and further investigations.

RESULTS

Of 17,365 newborns screened, 53 (0.31%) were recalled for a second DBS, and 13/53 (25%) underwent confirmatory testing including clinical evaluation, enzyme assay in leucocytes/lymphocytes and mutational analysis. The diagnosis of an LSD genotype was confirmed in 4 newborns, with a total incidence of 1 in 4,341 births. The number of confirmed positive cases corresponded to detection rates of 1:8,682 for Pompe disease and 1:17,365 for Gaucher and Fabry disease. LSD genotype was classified as a genotype of unknown significance/onset and pseudodeficiency in 5 newborns (3 with Fabry disease and 2 with MPS I). Among the newborns who screened positive for GAA deficiency, 1 was diagnosed with the infantile form and 1 with the late-onset disease.

DISCUSSION

Our experience highlights some difficulties with results interpretation due to a lack of published information on novel mutations, genotype-phenotype cor-

relations, and pseudodeficiency alleles. Clinicians experienced challenges in determining proper surveillance of individuals with non-classic forms of diseases and presumed pseudodeficiencies due to the absence of professionally agreed guidelines. Determining appropriate timing of treatment is also difficult. In conclusion, high costs and great risk of complications associated with treatment were unique to LSD and required a supportive approach to counseling.

LECT 76**GAUCHER DISEASE**

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Gaucher disease (GD) is the most common lysosomal storage disease. GD is caused by the deficiency of the enzyme glucocerebrosidase (GBA), required for the degradation of glycosphingolipids. Mutations in the *GBA1* (acid- β -glucosidase) gene, an 11 exons gene located on chr. 1q21-22 and coding for a 497 amino acid protein, are responsible for the disease. Nearly 300 mutations are known (frame-shift mutations, point mutations, deletions, insertions, splice site mutations, recombinant alleles) and they are classified based on the phenotypic correlation as:

- “null”, 84dupG (84 GG), no enzyme production, the phenotype is related to the second allele,
- “severe”, 1448T>C (L444P), enzymes are produced but, when inherited with a null or another severe mutation, are usually associated with GD type 2 or 3, and
- “mild”, 1226A>G (N370S), only associated with GD type 1.

GD is an autosomal recessive disorder with a most likely underestimated prevalence of 1/40,000 births, in the general population (vs Ashkenazi Jewish of 1/850, carrier rate 1/17). GBA deficiency results in the accumulation of its immediate substrates, glucosylceramide and its deacetylated form, glucosylsphingosine, predominately in lysosomes of the reticular endothelial system cells. In fact, Gaucher cells are characterized by striated “wrinkled tissue paper” cytoplasm. GD is a multi-system disorder characterized by (hepato)-splenomegaly, peripheral blood cytopenias (anemia, thrombopenia), bone disease, gammopathies with or without malignancies and, in some patients, neurological manifestations. GD has a continuous spectrum of severity and it is sub-classified into

three sub-types based on the following neurological features:

- Type 1 (OMIM #230800), 95% – chronic non-neurological, characterized by the lack of CNS involvement; 1/40,000-60,000 non Jewish, 1/600-850 Ashkenazi Jewish.
- Type 2 (OMIM #230900), 1% – severe neuropathic, with infantile onset and a life expectancy < 2 years.
- Type 3 (OMIM #231000), 5% – attenuated chronic neurological symptoms, with a pathognomic supranuclear horizontal gaze palsy, accompanied by visceral involvement and survival into adulthood.

Since the availability of effective therapies, expanded newborn screening programs on dried blood spots (DBS) for the detection of GD and other lysosomal storage diseases have been advocated for many years. The expanded newborn screening program is currently accessible in Italy, even though it is not available in all areas of the country. Treatment modalities include various supportive therapies (pain reduction, blood transfusions, orthopedic surgery) combined with two major therapeutic approaches for GD type 1:

Enzyme Replacement Therapy (ERT):

- Alglucerase (*Ceredase, Genzyme Corp*),
 - Imiglucerase (*Cerezyme, Genzyme Corp*),
 - Velaglucerase alfa (*VPRIV, Shire Human Genetic Therapies, Inc*),
 - Taliglucerase alfa (*ELELYSO, Pfizer Inc*),
- and Substrate Reduction Therapy (SRT):

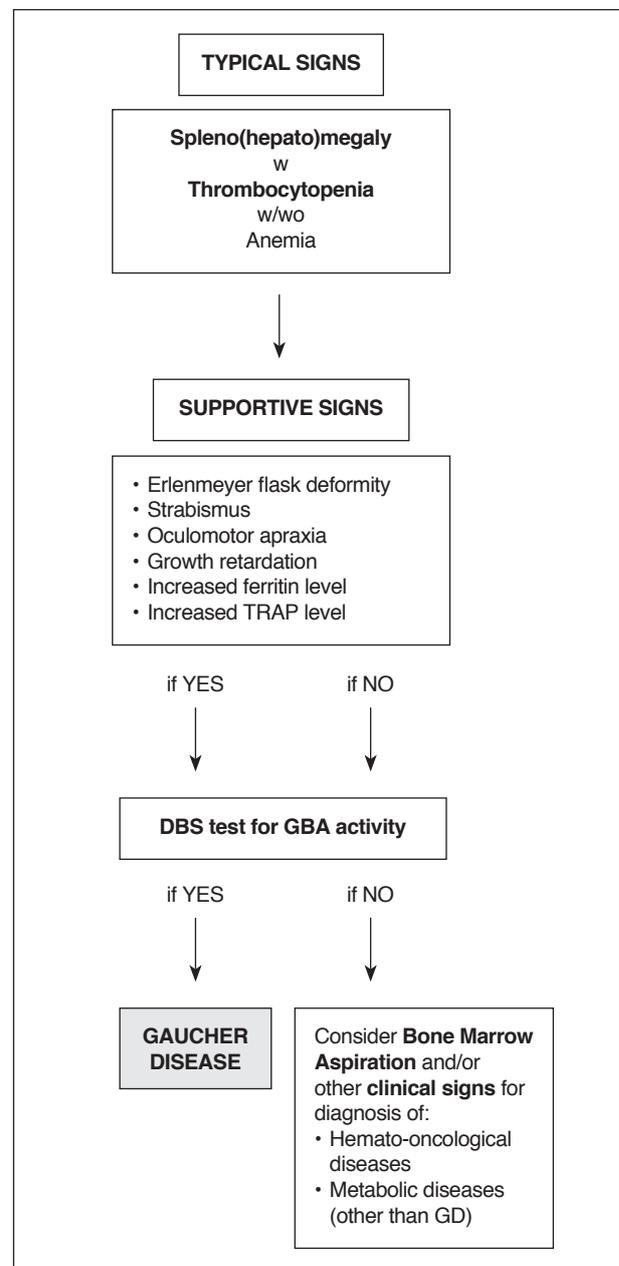
- Miglustat (*Zavesca, Actelion Pharma., Switzerland*),
- Eliglustat tartrate (*Genz-112638, Genzyme Corp*).

The aim of ERT is to provide the appropriate amount of the enzyme to permit excess material degradation. Because ERT does not cross the blood brain barrier, it is not indicated for GD type 3 and GD type 2.

The aim of SRT is to minimize the amount of production and the intracellular accumulation of excess glucosylceramide. According to the International Collaborative Gaucher Group Registry data, the diagnosis of GD type 1 is normally reached only 10-15 years after the onset of symptoms. GD patients are most likely to be referred to hematologists, because the most common disease presentation is splenomegaly with cytopenia. Only a minority of hematologists-oncologists considers GD in the differential diagnosis, even in the presence of all its typical signs and symptoms. Missing a diagnosis

of GD in a patient presenting with splenomegaly may lead to a delayed treatment initiation and an increased disease-related morbidity and mortality, while a well-established therapy for this condition is available. Moreover, a missed diagnosis could mean that a patient may have to undergo invasive procedures, such as bone marrow aspiration. Since clinical manifestations of GD are evident since childhood in two thirds of patients, it was considered appropriate to develop an algorithm (**Fig. 1**)

Figure 1 (LECT 76). Diagnostic approach for Gaucher disease in children.



W/wo: with/without; TRAP: tartrate-resistant acid phosphatase; DBS: dried blood spot; GBA: glucocerebrosidase. Modified from: Di Rocco M et al., 2014 [1].

to support clinicians in promoting a timely diagnosis and early access to therapy for pediatric (≤ 18 years) patients with GD type 1 [1]. After an appropriate waiting period, an observational multicenter cross-sectional study (GAU-PED study) has been set up to evaluate the prevalence of GD patients among children referred to the hematology pediatric units for splenomegaly and selected on the basis of the indications contained in the diagnostic algorithm. Patients will be screened for the GBA activity, by

means of a DBS sample. GBA deficiency will be confirmed using the gold standard GBA analysis. The results of the GAU-PED study will validate the diagnostic algorithm and will help to identify signs and symptoms useful for a prompt diagnosis of GD.

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