# Selected Lectures of the 14ᵗʰ International Workshop on Neonatology

## THE REVOLUTION OF MICROBIOMICS
### NUTRITION, BACTERIA AND PROBIOTICS IN PERINATAL AND PEDIATRIC HEALTH

## CAGLIARI (ITALY) · OCTOBER 24ᵀᴴ-27ᵀᴴ, 2018

The Workshop has been organized with the patronage of the Italian Society of Neonatology (SIN), the Italian DOHaD (Developmental Origins of Health and Disease) Society (SI-DOHaD), the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC), the Union of European Neonatal and Perinatal Societies (UENPS), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the Union of Middle-Eastern and Mediterranean Pediatric Societies (UMEMPS), the European Association of Perinatal Medicine (EAPM), the Italian-Romanian Pediatric Society (IRPS) and the Italian-Arabian Pediatric Society (IAPS).

## WORKSHOP'S PRESIDENTS

Vassilios Fanos (Cagliari, Italy), Egbert Herting (Lübeck, Germany), Moshe Hod (Tel Aviv, Israel), Manuel Sánchez-Luna (Madrid, Spain), Michele Mussap (Cagliari, Italy), Sergio Bernardini (Rome, Italy), Antonio Ragusa (Massa Carrara, Italy), Ana Maria Ciubara (Galati, Romania), Gavino Faa (Cagliari, Italy), Giuseppe Floris (Leuven, Belgium)

## WORKSHOP'S HONORARY COMMITTEE

S. Bernardini (Rome, Italy), P. Biban (Verona, Italy), M. Bruscagnin (Turin, Italy), G. Buonocore (Siena, Italy), P. Calò (Cagliari, Italy), A.M. Ciubara (Galati, Romania), G. Corsello (Palermo, Italy), G.C. Di Renzo (Perugia, Italy), C. Fabris (Turin, Italy), V. Fanos (Cagliari, Italy), G. Finco (Cagliari, Italy), M. Hod (Tel Aviv, Israel), V. Modica (Byblos, Lebanon), G. Monni (Cagliari, Italy), F. Mosca (Milan, Italy), R. Mura (Cagliari, Italy), G. Perricone (Palermo, Italy), D. Pisano (Cagliari, Italy), C. Romagnoli (Rome, Italy), M. Sánchez-Luna (Madrid, Spain), S. Vendemmia (Aversa, Italy), F. Vicariotto (Milan, Italy), P. Vezzoli (Cagliari, Italy)

## WORKSHOP'S INTERNATIONAL FACULTY

E. Bertino (Turin, Italy), G. Buonocore (Siena, Italy), L. Cataldi (Rome, Italy), G. Corsello (Palermo, Italy), C. Dani (Florence, Italy), G.C. Di Renzo (Perugia, Italy), G. Faa (Cagliari, Italy), V. Fanos (Cagliari, Italy), F. Ferrari (Modena, Italy), E. Herting (Lübeck, Germany), S. Iacobelli (Saint Pierre, France), P. Paolillo (Rome, Italy), A. Ragusa (Massa Carrara, Italy), F. Velardi (Rome, Italy)

## OTHER PRESIDENTS

Giovanni Ottonello (Cagliari, Italy), Denis Pisano (Cagliari, Italy), Melania Puddu (Cagliari, Italy)

**LECT 1**

# PERINATAL PROGRAMMING: FROM THE WOMB TO THE ADULT. THE FIGO LIFE CYCLE APPROACH

M. Hod

*Department of Obstetrics and Gynecology, Rabin Medical Center, Tel Aviv, Israel*

*Chairman, FIGO Pregnancy and NCD Committee*

The first nine months of life shapes the offspring's adulthood, while simultaneously impacting maternal life after pregnancy. Such long-term effects on the health of mothers and their children are mediated through epigenetic, physiological, endocrine, and biochemical pathways, and by imprinting of responses to stress. They contribute to the increased post pregnancy risk of developing non-com-municable diseases (NCDs) that are passed on from one generation to the next through the critical period of pregnancy. A need for an all-encompassing approach for improving maternal and fetal medicine (MFM) is thus required to interrupt the vicious cycle that starts at pregnancy disorder to improve maternal life in this generation and fetal life of the future generations. The holistic approach is needed to merge the importance of maternal and fetal health in the MFM sub-specialty. We, at the RMC, present a new three-floor holistic and multidisciplinary model for maternal and fetal medicine. The model's first floor is pre-pregnancy care and involves family planning and assessment of the prior risks for NCDs and their pre-pregnancy control and prevention. It continues through the introduction of the inverted pyramid of antenatal care that shifts the emphasis from the third to the first trimester of pregnancy, offering a multi-disciplinary screening and risk assessment followed by individually tailored prevention and management pathways. The third floor is the post pregnancy health management to minimize long-term damage. This model of MFM care is proposed for improving maternal outcome and preventing short and long-term complications not only to the mothers but also to their children and the next coming generations.

## FETAL ORIGINS OF ADULT DISEASES – PROGRAMMING AND IMPRINTING IN UTERO
### Low birth weight – IUGR (increased risk for lifelong cardiovascular diseases and diabetes)

Barker was the first to demonstrate how low birth weight is associated with elevated risk for cardiovascular diseases (CVDs) in adulthood. He postulated that fetal shortage of nutrients and oxygen due to placental insufficiency is associated with the fetal development of physiological pathways for stress adjustment underlying recruitment of the same pathways in adulthood leading to the development of higher adulthood susceptibility to obesity, diabetes mellitus, hypertension, and CVDs. According to Barker these "programmed changes" are metabolic adaptations to fetal under-nutrition expressed in enhanced catabolism, and self-consuming of substrates for energy supplies. A prolonged fetal adjustment period to under-nutrition also reduces the endocrine concentration of fetal growth hormones (FGH), via the reduced transfer of amino acids and glucose across the placenta, due to decreased maternal Insulin-like growth factor (IGF). These changes are followed by reduced rates of fetal growth also creating a process response to stress that is then recruited in adulthood causing metabolic disorders and CVDs. Low birth weight was shown to be associated with an increased rate of ischemic heart diseases in adulthood. Studies with 3 large cohorts (> 16,000 individuals) in the UK have shown that mortality from ischemic heart disease later in life were 2 fold higher in those born < 2.5 kg at birth compared to the ones born > 4.3 kg. Thin or stunted and small trunk babies who were born due to *in utero* undernutrition, hypoxia, and other changes, are pre-disposed to significant diseases in the long term. Furthermore, increased mortality rates from coronary heart diseases (CHDs) are found among men born with a low birth-weight, low placental weight or narrow head circumference. The prevalence of Type 2 diabetes mellitus and impaired glucose tolerance later in life are 3 fold higher in people who were born with the smallest (< 2.5 kg) birth weight compared to the largest who weighed > 4.3 kg at birth. There is evidence that deficiency in insulin production and insulin resistance are both determined *in utero*, and that low birth-weight babies develop *in utero* the 'insulin resistance syndrome' which is prevailed in their adulthood, causing an impaired glucose tolerance, hypertension and high concentrations of triacylglycerol. The extreme example of the long-term impact of nutrient shortage during pregnancy was discovered with the

Dutch study of individuals who were *in utero* during the Dutch famine of 1944-1945. This study provides evidence linking fetal undernutrition to programmed insulin resistance and Type 2 diabetes. Their glucose tolerance tests at age 50 years were all higher than in those conceived before or after the famine. This study has also provided evidence for long-lasting epigenetic effects transferred from the newborn to their progenies not through the mother but the father, indicating the profound impact of undernutrition on the DNA methylation of germ cells associated with facilitated aging-related diseases for the generations to come. Another example is the Chinese famine during 1954-1964, which was identified to be associated with a higher likelihood to develop metabolic syndrome in adulthood. Based on all these changes tomes magazine published its series of articles on the way the first 9 months shape the person health throughout life.

*Blood pressure and hypertension – preeclampsia*

A multitude of studies has found a trend in which each 1 kg increase in birth-weight is associated with a fall of around 3.5 mmHg in blood pressure in adult life. There is a strong association between hypertension disorder in adulthood to low birth-weight, thinness, stunting and below average head circumference.

All the examples above have demonstrated how birth weight, *in-utero* conditions, and epigenetic changes are associated with the increased adulthood morbidity from NCDs leading to a vicious cycle for generations to come.

*The maternal aspects of placenta insufficiency – increased maternal cardiovascular diseases and decreased life expectancy due to preeclampsia*

Another form of placental insufficiency is preeclampsia, particular the early form of the disorders. McDonald et al., in their meta-analysis of 35,000 women have shown that hypertension disorders in pregnancy are associated with increased maternal morbidity from CVDs and diabetes mellitus ten years later. Furthermore, Irgens et al. using the Medical Birth Registry of Norway (MBRN) have shown in > 600,000 women and their spouses, a ten years shortening of maternal longevity following early preeclampsia and IUGR. Thus, placental insufficiency is not only high programming susceptibility to CVDs and diabetes among babies born with the lower birth weight but also among their mothers.

*Non-communicable diseases and maternal morbidity*

Pre-pregnancy conditions of maternal health (obesity, diabetes, anemia and undernutrition, kidney,

blood and heart diseases), all impact maternal health in pregnancy. Pre-pregnancy diabetes and gestational diabetes can cause macrosomia, obstructed labor, postpartum hemorrhage as well as neonatal mortality due to prematurity, respiratory distress syndrome, hypoglycemia, etc. Maternal under-nutrition can lead to fetal metabolic and hormonal alterations causing lifelong susceptibility to certain diseases. At the same time, low birth-weight and accelerated growth during childhood have been demonstrated as risk factors for cardiovascular disease and diabetes mellitus. This vicious cycle starting from pre-pregnancy health, influencing the outcome, which in turn causes adulthood diseases associated with pregnancy disorders in the next generation and continuing, is now recognized as the link between the origin of NCD'S in neonatal life and adulthood diseases. It requires implementation of healthcare assessment and preventive interventions before pregnancy to reduce infant and maternal morbidity and mortality and preventing developing NCD'S later in life.

*The vicious cycle of the non-communicable diseases epidemic; obesity, diabetes, hypertension, metabolic syndrome – fetal programming*

According to the World Health Organization (WHO) of the 57 million who died in 2008, 36 million died from NCD's, and the WHO has stated that non-communicable diseases represent a "slow-motion disaster." The four main chronic diseases responsible for most NCDs deaths are CVDs, including heart attacks and stroke (17.3 million annually); cancer (7.6 million), respiratory diseases, such as chronic obstructive pulmonary disease; asthma (4.2 million) and diabetes (1.3 million). Intermediate risk factors predisposing to NCD's include high hypertension, elevated blood glucose, hyperlipidemia, overweight and obesity, which all can lead to the development of CVDs. The hypothesis about the Developmental Origin of Disease (DOHaD) put forward the concept that internal and external environmental conditions during pregnancy cause critical biochemical, endocrinology and epigenetic modifications in the DNA, cell differentiation and formation of specific tissues in both the mother and her fetus/newborn. 31. While at birth these functional changes are currently not detected by conventional tests and are likely to be initially masked by systemic effects. However, the slow process of their development into disorders may impact the health of the mothers and their children later in life. Epigenetic changes in DNA methylation and CpG islands cause the silencing or activation of specific genes that are essential for the

physiological function in early childhood and adult life and could lead to an accelerated DNA clocking and aging. Thus epigenetic methods could shed light on *in utero* processes that predispose individuals to diseases in adult life. The programmed *in utero* changes of the metabolism and physiology could lead to dysfunction and disease in adulthood. As such, the related pregnancy disorders such as preterm delivery, IUGR and preeclampsia can be considered as markers of increased risk of CVDs, obesity and metabolic disorders and gestational diabetes (GDM) could be the source for obesity and Diabetes Mellitus (DM), or overall – the origin of NCDs. In fact, the American Heart Association has identified the women who develop hypertension disorder in pregnancy and gestational diabetes as the two new high-risk groups for developing CVDs, which requires individual management and monitoring. In order to reduce the influence of epigenetic, biochemical, endocrine and physiological pre-conditioning of NCDs in the perinatal period, preventative measures should be introduced, including the provision of sufficient prenatal care, prevention or optimal treatment of conditions such as obesity, diabetes and chronic hypertension, and also direct the attention at pre-pregnancy assessment of their prior risks and family planning to assure women begin their pregnancy period with rich metabolic reservoirs and with a pre-planned program for their pregnancy management based on their prior risks. In this way, it may be possible to inhibit negative epigenetic, biochemical, physiological and endocrine programming. The importance of good maternal care beginning before conception and continuing during pregnancy and after delivery is therefore crucial to shape the health of mothers and their babies for life and to prevent the impact of internal and external effects of long-lasting changes thus reducing the likelihood of NCD's development in adulthood.

## LECT 2

### SINS IN PREGNANCY, SINS, AND PREGNANCY

G.C. Di Renzo

*Department of Obstetrics and Gynecology and Centre of Reproductive and Perinatal Medicine, University of Perugia, Perugia, Italy*

Sin is an offense against reason, truth, and right conscience; it is a failure in genuine love for God and neighbor, because of a perverse attachment to certain goods. It wounds the nature of man and injures human solidarity. It has been called "an utterance, a deed, or a desire contrary to the eternal law." The "sin" is also understood by a broader sense and general; even in pregnancy, we can speak of "sins", referring to behaviors and habits variously judged and/or reprehensible ... and most potentially dangerous for the maternal-fetal-neonatal health. The seven "good sins" in pregnancy: Physical activity; Cocoa/chocolate assumption; Music; Travels; Sex; Sunbathing; Environment and climate. Seven bad "sins" in pregnancy: Overnutrition and obesity; Unhealthy eating trends; Alcohol; Caffeine; Smoke; Illegal drugs; Abuse of antibiotics. All these aspects should be considered for their impact on pregnancy and infant taking into consideration that the health of the mother (and father) affects pregnancy outcomes and the health of the fetus, neonate, infant, child, adolescent, and subsequent generations. Besides these, other negative aspects can affect pregnancy: "the sins of the physicians" in the management of pregnancy! What are the desirable medical behaviors? Every obstetrician should think of the pregnant woman and the fetus first, in respect of ethical and deontological principles. Unfortunately, the supportive pastoral aspects of medical care tend to be squeezed out by one of our human sins, pride. The Oxford English Dictionary defines pride as "high or overweening opinion of one's qualities, attainments or state, which gives rise to a feeling and attitude of superiority over and contempt for others". Our pride in the application of scientific knowledge and biomedical technology is now creating an "emotional" gap in the care of patients. Machines now stand between our patients and us. A pregnancy system too proud to "care". However, also pregnancy care might be harassed by greed: the medical professional liability crisis. There is among doctors the lust of technology; Envy among specialties favors fragmentary responses to a totality of health needs. Medical over-consumption is a symbol of gluttony and inequity. A health care system that reacts to disease in anger gives the only overemphasis of curative clinical medicine. The aggressive therapy consists in the execution of documented ineffective treatments concerning the objective, to which is added the presence of high risk and/or a particularly burdensome treatment for the patient. As doctors should always react to illness with rationality and objectivity, always give priority to the patient as a person! Finally sloth as the indolent attitude toward social problems: sloth as a sin is widely prevalent in all walks of life, and the health care system can commit it with deadly consequences.

The failure of a physician and surgeon to maintain adequate and accurate good medical practice constitutes unprofessional conduct. Pregnancy and infancy are a concern for all of us, whether we are services providers, educators, and trainers, or scientific researchers, the health care system filters our inputs and modifies the output. There are issues which, admittedly, we cannot change as individuals. There are issues, however, which we are responsible and which we must change. The pregnant woman is master not only of his destiny but in part also of that of her child (the DOHaD concept). So she must favor responsible behavior and avoid bad temptations-sins! On the other hand, the pregnant woman should not be following behaviors which are not substantiated by scientific evidence just because of tradition or beliefs: sometimes these may cause more harm than good!

**LECT 3**

**THE HUMAN MILK AS A MODEL OF PREVENTION**

G. Corsello, M. Carta

*Operative Unit of Pediatrics and Neonatal Intensive Therapy, Mother and Child Department, University of Palermo, Palermo, Italy*

The term "milk" does not give the real idea of the multipotential biological effects of breastfeeding in human beings. Breastmilk is a biofluid highly flexible regarding contents during lactation both for nutrients and other functional components. Its multiple protective effects act on mothers as well as infants in different ways. During phylogenesis, the evolution marked itself also remodeling the breastmilk contents according to the features and needs of different species. In this sense, the only milk-specific for humans is breastmilk and mainly the milk of own mother according to individualized and personalized nutritional and biological needs. Adequate nutrition may be retained as a real priority in preterm and very low birth weight newborns. The nutritional strategies in such categories of high-risk infants must guarantee a correct short-term linear growth and long-term neurological and cognitive performances avoiding negative consequences for long-term metabolic and cardiovascular health. Human milk provides many and unique benefits to preterm infants: protection against infections, reduction of necrotizing enterocolitis and retinopathy of premature, improvement of neurodevelopment outcomes.

A complex neuroendocrine and immunological network moves on around human lactation. Many epigenetic effects of breastmilk components promote the gut and immune system development. Proteins, oligosaccharides, staminal cells, lymphocytes, immunoglobulins, microbiome in breast milk play a role in these processes. Several studies showed that among the clinical advantages of breastfeeding may be included in the protection against infections and the reduction of chronic diseases, such as diabetes and allergies. One of the main studies is the HMOs (human oligosaccharides). Their activity is partly direct and partly mediated by the microflora [1]. They prevent adhesion of pathogens, promote the gut maturation and cell surface glycosylation, guarantee a healthy microbial composition of the intestinal microflora [2]. Human milk oligosaccharides are represented mainly in the pool of functional components, being the third solid component after lactose and lipids. HMOs are involved in this effect also acting on the development of intestinal microbiota through its well-known prebiotic effect. No differences have been noted among term and preterm newborns concerning HMOs activity and amount in breastmilk, neither in colostrum.

Another mechanism involved in the protective effect against the infections is the protein hydrolysis within the neonatal gut [3]. Hydrolysis of proteins amplifies the number of peptides and metabolites with immunomodulatory properties. Some of these metabolites may be originated by the fermentation of proteins operated by the microbiome, with a documented postbiotic effect. Both gastrointestinal and upper respiratory tract infections may be reduced in infants fed with fermented milk, in relations to this well-documented effect [4, 5].

Prevention of infections both during neonatal and first years of age may be considered one of the main biological effects of human milk. The multifactorial action of its functional components may be considered useful because of a reduction of gastroenteritis and respiratory infections. Further studies are necessary to document the different mechanisms of such protective effects, also because of clinical use to improve the quality of nutrition in those babies, particularly the preterm newborns, when they lack maternal milk.

REFERENCES

[1] Hao Q, Dong BR, Wu T. Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst Rev. 2011;3(2):CD006895.

[2] Puccio G, Alliet P, Cajozzo C, Janssens E, Corsello G, Sprenger N, Wernimont S, Egli D, Gosoniu L, Steenhout P. Effects of Infant Formula With Human Milk

Oligosaccharides on Growth and Morbidity: A Randomized Multicenter Trial. J Pediatr Gastroenterol Nutr. 2017;64(4):624-31.

[3] Wang G, Mishra B, Lau K, Lushnikova T, Golla R, Wang X. Antimicrobial peptides in 2014. Pharmaceuticals (Basel). 2015;8(1):123-50.

[4] Prodeus A, Niborski V, Schrezenmeir J, Gorelov A, Shcherbina A, Rumyantsev A. Fermented Milk Consumption and Common Infections in Children Attending Day-Care Centers: A Randomized Trial. J Pediatr Gastroenterol Nutr. 2016;63(5):534-43.

[5] Corsello G, Carta M, Marinello R, Picca M, De Marco G, Micillo M, Ferrara D, Vigneri P, Cecere G, Ferri P, Roggero P, Bedogni G, Mosca F, Paparo L, Nocerino R, Berni Canani R. Preventive Effect of Cow's Milk Fermented with Lactobacillus paracasei CBA L74 on Common Infectious Diseases in Children: A Multicenter Randomized Controlled Trial. Nutrients. 2017;9(7):E669.

## LECT 4

## LESS INVASIVE SURFACTANT ADMINISTRATION (LISA): WHERE ARE WE NOW?

E. Herting

*Department of Pediatrics, University of Lübeck, Lübeck, Germany*

Non-invasive ventilation with the use of continuous positive airway pressure (CPAP) instead of mechanical ventilation is increasingly used in modern neonatal care. However, some infants will still develop respiratory distress syndrome (RDS) and need surfactant replacement therapy as well. At the moment, Less Invasive Surfactant Administration (LISA) is the method of choice for the combination of spontaneous breathing with surfactant replacement via a small diameter tube that is placed in the upper trachea for a short while [1]. In contrast to the INSURE (Intubate SURfactant Extubate) procedure, no positive pressure ventilation is applied. LISA was reported to be associated with the lowest likelihood of death or bronchopulmonary dysplasia (BPD) in a recent meta-analysis of 30 randomized controlled trials using different noninvasive ventilation strategies in preterm infants with a gestational age below 33 weeks [2]. A small comparative study [3] and the meta-analysis [2] seem to indicate that LISA rather than INSURE seems to be the method of choice when combining spontaneous breathing with the surfactant, but there is only limited evidence due to the size and quality of the studies performed on this topic. In Germany, LISA accounts for more than 50% of all surfactant treatments by now (see **Fig. 1**). The data were generated in the German Neonatal Network (GNN) which is a prospective cohort study
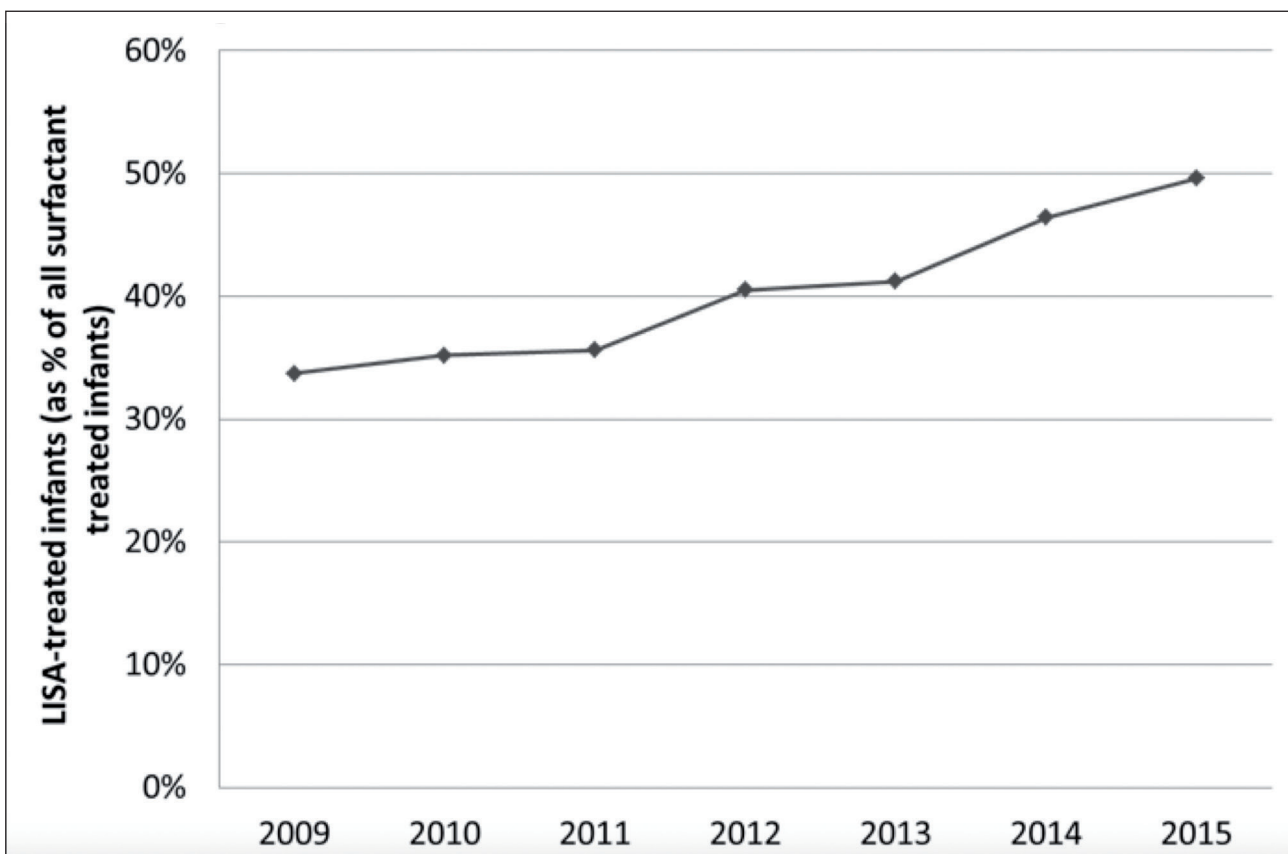


**Figure 1 (LECT 4).** Increase of LISA treatment from 2009 to 2015.

enrolling preterm infants with a birth weight below 1,500 grams. There is growing evidence on LISA now outside of Germany as well [4, 5]. LISA can prevent mechanical ventilation as demonstrated in the AMV-study [6]. When used in more than 1,000 infants outside of randomized controlled studies similar results were obtained [7, 8]. LISA needs experience but then seems to be a safe procedure. In a recent trial, the incidence of severe complications like intraventricular hemorrhage was reduced as well [9]. During application, short episodes of desaturations or bradycardia are often observed. There may be some reflux of surfactant, especially towards the end of the procedure. When LISA is used in extremely premature infants, abdominal distension should be observed since there may be an increased risk of focal intestinal perforation (FIP) especially in infants at 23 and 24 weeks of gestation [10]. Most centers use soft catheters that are introduced with the help of the Magill forceps, but the use of straight catheters for oral intubation [11] has been described as well (MIST – Minimal Invasive Surfactant Treatment). New catheters and devices, uniquely designed for LISA treatment, have been recently introduced on the market. Many centers will prefer early treatment in the first hour of life and doses between 100 [6] and 200 [12] mg/kg body weight of surfactant have been reported. A common approach also used in the NINSAPP trial [9] is to use a full vial of surfactant often resulting in doses of about 150 mg/kg. The role of analgesia and sedation in LISA is still unclear, as the success of LISA also depends on the maintenance of spontaneous breathing. Therefore, studies with nebulized surfactant, studies with pharyngeal deposition of surfactant and studies using the laryngeal mask as a tool for surfactant application are ongoing. LISA has become the standard for surfactant treatment in many NICUs across Europe. However, the number of LISA treated infants who are enrolled in randomized controlled trials is still limited, and few long-term outcome data are reported yet so that further studies are needed.

REFERENCES

[1] Herting E. Less invasive surfactant administration (LISA) – ways to deliver surfactant in spontaneously breathing infants. Early Hum Dev. 2013;89(11):875-80.

[2] Isayama T, Chai-Adisaksopha C, McDonald SD. Noninvasive Ventilation With vs Without Early Surfactant to Prevent Chronic Lung Disease in Preterm Infants: A Systematic Review and Meta-analysis. JAMA Pediatr. 2015;169(8):731-9.

[3] Kanmaz HG, Erdeve O, Canpolat FE, Mutlu B, Dilmen U. Surfactant administration via thin catheter during spontaneous breathing: randomized controlled trial. Pediatrics. 2013;131(2):e502-9.

[4] Heiring C, Jonsson B, Andersson S, Björklund LJ. Survey shows large differences between the Nordic countries in the use of less invasive surfactant administration. Acta Paediatr. 2017;106(3):382-6.

[5] Klotz D, Porcaro U, Fleck T, Fuchs H. European perspective on less invasive surfactant administration-a survey. Eur J Pediatr. 2017;176(2):147-54.

[6] Göpel W, Kribs A, Ziegler A, Laux R, Hoehn T, Wieg C, Siegel J, Avenarius S, von der Wense A, Vochem M, Groneck P, Weller U, Möller J, Härtel C, Haller S, Roth B, Herting E. Avoidance of mechanical ventilation by surfactant treatment of spontaneously breathing preterm infants (AMV): an open-label, randomised, controlled trial. German Neonatal Network. Lancet. 2011;378:1627-34.

[7] Göpel W, Kribs A, Härtel C, Avenarius S, Teig N, Groneck P, Olbertz D, Roll C, Vochem M, Weller U, von der Wense A, Wieg C, Wintgens J, Preuss M, Ziegler A, Roth B, Herting E. Less invasive surfactant administration is associated with improved pulmonary outcomes in spontaneously breathing preterm infants. German Neonatal Network (GNN). Acta Paediatr. 2015;104(3):241-6.

[8] Langhammer K, Roth B, Kribs A, Göpel W, Kuntz L, Miedaner F. Treatment and outcome data of very low birth weight infants treated with less invasive surfactant administration in comparison to intubation and mechanical ventilation in the clinical setting of a cross-sectional observational multicenter study. Eur J Pediatr. 2018;177(8):1207-17.

[9] Kribs A, Roll C, Göpel W, Wieg C, Groneck P, Laux R, Teig N, Hoehn T, Böhm W, Welzing L, Vochem M, Hoppenz M, Bührer C, Mehler K, Stützer H, Franklin J, Stöhr A, Herting E, Roth B. Nonintubated Surfactant Application vs Conventional Therapy in Extremely Preterm Infants: A Randomized Clinical Trial. NINSAPP Trial Investigators. JAMA Pediatr. 2015;169(8):723-30.

[10] Härtel C, Paul P, Hanke K, Humberg A, Kribs A, Mehler K, Vochem M, Wieg C, Roll C, Herting E, Göpel W. Less invasive surfactant administration and complications of preterm birth. Sci Rep. 2018;8(1):8333.

[11] Dargaville PA, Aiyappan A, De Paoli AG, Kuschel CA, Kamlin CO, Carlin JB, Davis PG. Minimally-invasive surfactant therapy in preterm infants on continuous positive airway pressure. Arch Dis Child Fetal Neonatal Ed. 2013;98(2):F122-6.

[12] Klebermass-Schrehof K, Wald M, Schwindt J, Grill A, Prusa AR, Haiden N, Hayde M, Waldhoer T, Fuiko R, Berger A. Less invasive surfactant administration in extremely preterm infants: impact on mortality and morbidity. Neonatology. 2013;103(4):252-8.

## LECT 5

## DRUGS AND NEUROPROTECTION

S. Perrone, E. Laschi, G. Nanni, G. Buonocore

*Pediatric and Neonatal Unit, Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy*

Perinatal brain damage in preterm infants, stroke and hypoxic-ischemic encephalopathy (HIE) in term newborns are leading causes of mortality and disability, so treatments for neurocognitive morbidity are an important health care issue.

Pathogenesis of neonatal brain injury is complex and multifactorial; it depends on the different vulnerability of brain regions, and it involves a cascade of molecular reactions that lead to cells damage and death. Pathophysiological mechanisms include the energy failure due to oxygen deprivation, glutamate release and excitotoxicity, oxidative stress (increase of free radicals, FRs) and mitochondrial impairment, microglia activation and inflammation, increased permeability of the brain-blood barrier (BBB) and neuroplasticity phenomena. All these events represent targets for promising neuroprotective therapies. The inhibition of sensitizing factors, preconditioning against damage, pharmacological blockage of mechanisms involved in primary or secondary damage, and treatment that increases endogenous reparation processes or cause reparation by themselves may be effective for neuroprotection [1]. Hypothermia is the best known neuroprotective treatment in moderate HIE: it reduces secondary energy breakdown, oxidative stress, BBB rupture extent and apoptotic cell death if started within 6 h of life, but its benefits remain partial. The efficacy of potential neuroprotective drugs has been demonstrated in animal models, especially those who target multiple pathophysiological pathways. Anti-inflammatory agents include minocycline, a semisynthetic tetracycline reducing pro-inflammatory responses via effects on microglia, and non-steroidal anti-inflammatory drugs (NSAIDs), especially COX-2 selective such as nimesulide, that act by inhibition of proinflammatory cytokine TNF-$\alpha$, prevention of BBB dysfunction and a reduction of oxidative stress. Excitotoxicity reducing agents block overstimulated postsynaptic glutamate receptors without inhibiting their physiologic functions for brain development. Magnesium sulfate inhibits NMDA receptors and has shown benefits about the incidence of cerebral palsy and motor dysfunction in preterms when administrated to pregnant women; it has shown to be also able to modulate inflammation. Topiramate is an antagonist of AMPA and kainate receptors, whose inhibition has not shown effects as deleterious on neuronal survival as those acknowledged for NMDA receptor antagonists in the developing brain; a protective effect of topiramate on preoligodendrocytes, severely affected after excitotoxic or hypoxic-ischemic stress in newborn, has been demonstrated. Antioxidant substances act at different phases of the brain injury, by reducing FRs production (through xanthine oxidase inhibition), lipid peroxidation and the activation of inducible Nitric Oxide Synthases (iNOS) and by increasing antioxidant defense mechanisms through the increase of reactive species scavengers [2]. Allopurinol is an inhibitor of xanthine oxidase, the enzyme involved in superoxide (an FR) production especially during the reperfusion phase; it has additional effects scavenging toxic FR and chelating the nonbound protein iron, a potent pro-oxidant. It has been employed in therapeutic essays in infants with HIE, showing a reduction of NO concentrations at 72-96 h of life associated with an improvement of the neurological outcome at 12 months of life. N-acetylcysteine (NAC) is an FR scavenger and restores intracellular glutathione levels; in animal studies its neuroprotective effect has been shown through a reduction of oxidative stress, proinflammatory cytokine production, and apoptosis. Recently, in a rabbit model of perinatal white matter damage, it has been shown that low doses of NAC bound to nanoparticle dendrimers specifically target activated microglia leading to significant neuroprotection. Melatonin, the main product of the pineal gland, has antioxidant, antiapoptotic and anti-inflammatory properties; it is able to cross the BBB with a safety profile. Melatonin inhibits the NOS and lipid peroxidation and favors the transcription of antioxidant enzymes; besides acute lesion enhances axonal outgrowth. Strategies against apoptotic cell death and those that favor reparation and modulation of plasticity are interesting for their therapeutic window. Erythropoietin (EPO) is a hematopoietic growth factor for which receptors exist in the central nervous system: it has an antiapoptotic effect on neurons and oligodendrocytes by decreasing proinflammatory cytokines cascade, oxidative stress, restore the energetic level and increases trophic factors involved in reparative processes. Xenon is a noble gas that blocks the NMDA but also AMPA and kainate receptor and inhibits neurotoxic cascade; Argon is another cheaper noble gas that in models of cerebral ischemia demonstrated robust neuroprotection [3].

## REFERENCES

[1] Buonocore G, Perrone S, Turrisi G, Kramer BW, Balduini W. New Pharmacological Approaches in Infants with Hypoxic-Ischemic Encephalopathy. Curr Pharm Des. 2012;18(21):3086-100.

[2] Tataranno ML, Perrone S, Longini M, Buonocore G. New antioxidant drugs for neonatal brain injury. Oxid Med Cell Longev. 2015;2015:108251.

[3] Nance E, Porambo M, Zhang F, Mishra MK, Buelow M, Getzenberg R, Johnston M, Kannan RM, Fatemi A, Kannan S. Systemic dendrimer-drug treatment of ischemia-induced neonatal white matter injury. J Control Release. 2015;214:112-20.

**LECT 6**

## CRANIOFACIAL DEFORMATIONS BETWEEN ART AND SCIENCE

F. Velardi

*Pediatric Neurosurgery and Craniofacial Surgery, Rome, Italy*

Since the beginning of its history, the western figurative art has privileged the representation of beauty, perfection, and strength, together with the supremacy of deified heroes over the rest of humanity as well as overall living entities. The depiction of all human emotions, love, passion, compassion, modesty, anger, along with the cruelty of terror, violence, and death completed the array of the ambiguous alliance between humankind and divinity. Based on the classic Egyptian, Greek, Roman and Byzantine contributions, during the Italian Renaissance, the expressive approaches and pictorial techniques addressed to the representation of human physiognomy were dominated by the canons of beauty. Proportions, symmetry, and harmony ruled its value. The golden ratio, the Fibonacci sequence, the numerical rules given by Piero Della Francesca in "*De Prospectiva Pigendi*" were the formulas to express the beauty of a divinized humanity (as well as of a humanized divinity).

During the late XV Century, new groups of contractors flowered, mainly in the Northern European countries. With the complicity of the reformist sentiment harboring in those wealthy bourgeoises and merchants middle European classes, a search for a less idealistic naturalism was favored. The innovative and revolutionary Flemish style replaced the emphatic Neoplatonic style of the old commitments ordered by the Roman Church and the old noble Italian families. Therefore, the objective representation of "imperfect" humanity and flawed settings gained dignity. Hence, portraying imperfect bodies or human deformities was no longer considered an oxymoron. The harmony of beauty was manifestly perceived even within imperfections and asymmetry. As for the major artists of the Italian Renaissance, celebrated for their heavenly technical skill and their extensive expertise on geometry and perspective, instances of pictorial "imperfections" where extremely rare (Domenico Ghirlandaio, 1490: *Portrait of an Old Man and his Grandson*, Musée du Louvre, Paris; Raffaello Sanzio, 1514: *Portrait of Fedra Inghirami*, Galleria Palatina, Firenze). Even Antonello da Messina, prominent ambassador of the Flemish style in Italy, never completely abandoned idealistic naturalism, which he, instead, masterly melted with aspects of the European descriptive realism (*Portrait of a man* [*Condottiere*], 1475, Louvre Museum; *Portrait of a man* [*Trivulzio*], 1476, Museo Civico d'Arte Antica Torino). On this regard, we consider excitingly relevant to report on the craniofacial anomalies we spotted in the tempera on panel "*Portrait of a Young Man*" that Sandro di Mariano Filipepi (1445-1510), known as Botticelli, painted in 1483 and is presently housed in London, at the National Gallery (NG626) (**Fig. 1**). Botticelli may have realized this portrait of a young man while in Florence (around 1483/1484), just coming back from his experience in Rome. It was possibly part of his private commitments from wealthy merchant families in Florence. Unfortunately, we do not know anything about the identity of the sitter, not to mention the original context of the painting. Undoubtedly, we need to consider the possibility that Botticelli did not produce a wholly accurate portrait, and his portrait cannot claim photographic accuracy. Obviously, there may be a risk of misinterpreting artistic choices as facial deformities. Yet, the young man



**Figure 1 (LECT 6).** Sandro Botticelli, *Portrait of a Young Man*.
© The National Gallery, London.

from London is not the unique occasion in which Botticelli depicted the evidence of a disease in his sitter (see the artists of the right hand spotted in the his "*Portrait of a Youth*" presently housed within the Andrew W. Mellon Collection at the National Gallery of Art Washington DC [USA] 1937.1.19). The Londoner by Botticelli shows, at inspection, some typical features of facial asymmetry:

1. Frontal bulging, more prominent on the right side;
2. Facial scoliosis, with the right convexity of the facial midline ;
3. Asymmetry of the mandible, with midline mental protuberance, deviated toward left.

By considering all these features, together with the age of the model and his overall healthy complexion, we would suppose that the man portrayed by Botticelli shows the signs of a craniofacial deformity. Concerning the differential diagnosis, we discuss and straightforwardly rule out any genetic disorder (craniofacial synostosis) as well as any consequence of a traumatic facial lesion. The assumption that the young man of Botticelli may exhibit the evidence of the long-term outcome of an untreated mild to moderate non-synostotic craniofacial deformation appears more stimulating. This condition is known, from a clinical point of view, as "positional (or deformational) plagiocephaly". In our time, it would have been classified as "Type IV", according to the classification proposed by Louis Argenta in 2004 [1]. The clinical and functional features of non-synostotic craniofacial deformations will be discussed, together with the diagnostic criteria, the natural history and the acknowledged treatment protocols [2] to be applied as best practice on real patients.

## REFERENCES

[1] Argenta L, David L, Thompson J. Clinical classification of positional plagiocephaly. J Craniofac Surg. 2004;15(3):368-72.

[2] Flannery AM, Tamber MS, Mazzola C, Klimo P Jr, Baird LC, Tyagi R, Bauer DF, Beier A, Durham S, Lin AY, McClung-Smith C, Mitchell L, Nikas D. Congress of Neurological Surgeons Systematic Review and Evidence-Based Guidelines for the Management of Patients With Positional Plagiocephaly: Executive Summary. Neurosurgery. 2016;79(5):623-4.

**LECT 7**

**NON-NUTRITIVE SUCTION AND BREATHING IN PRETERM INFANTS**

S. Ossola[1], V. Trivellin[1], M. Ragazzo[1], F. Riccaboni[2], L. Montericcio[3], L. Nosetti[2], S. Salvatore[2], L. Levrini[3], M. Agosti[1]

[1]Neonatal Intensive Care Unit and Neonatology, "F. Del Ponte" Hospital, University of Insubria, Varese, Italy

[2]Pediatric Unit, "F. Del Ponte" Hospital, University of Insubria, Varese, Italy

[3]Oro Cranio Facial Disease, University of Insubria, Varese, Italy

Oral feeding in the neonatal period requires precise coordination between sucking, swallowing and breathing. If healthy full-term infants demonstrate such skills at birth, preterm infants are generally known to have difficulty in the transition from oral tube to oral feeding, because of the lack of this coordination. Primitive sucking and swallowing movements start early at 11-13 weeks of gestation, and they are governed by highly complex neurologic pathways, throughout the pontine and medullary structures. However, if the neurogenesis of these critical structures is completed in early fetal life, the coordination of suck-swallow and swallow-respiration is an ability that infants achieve later. In preterm infants immature sucking, delayed swallow and uncoordinated suck and respiration are often potential causes of oral feeding issues and delayed hospital discharge. Swallows need to occur during a "safe phase" of the respiratory cycle, in order to avoid the risk of aspiration and desaturation. Thus coordination of suck-swallow-respiration and rhythmicity during the oral feeding remains essential. Moreover, some studies demonstrate a tightly linked, anti-phase relationship between sucking and swallowing, where the generation and the release of positive pharyngeal pressure are tightly coupled with the generation and release of negative intraoral suction [1]. Non-nutritive sucking has a significant effect on the transition from gavage to full oral feeding. Pacifiers may be used as training to prepare prematurely born infants to natural feeding, but have also been shown to have a positive effect on the development both stimulating the sucking reflex and enhancing the motor development of the articulatory organs [2]. Some studies have also demonstrated that babies who use pacifiers produce better physiological parameters, i.e., better oxygenation and lower heart rate. The most significant benefit of pacifier use in neonates is that it protects them from SIDS, in preterm infants especially, through increasing blood pressure and improving autonomic control of heart rate [3]. Considering the demonstrated advantages of a pacifier on the breath, we wanted to know if it could be helpful even in infants affected by previous Apparent Life-Threatening Event (ALTE) or Brief Resolved Unexplained Events (BRUE). In our Center For Respiratory Disorders In

Sleep of the Università dell'Insubria (Varese), we studied 10 infants aged 1 month-2 year affected by ALTE/BRUE. They underwent cardio-respiratory monitoring at home, with and without a pacifier. In the nights in which the infant used the pacifier a reduction in the number of pathological apneas (1.5 vs. 3.2 events/night) and an increase in the mean and minimum $SpO_2$ (mean $SpO_2$ 97.7% vs. 96.9%, minimum $SpO_2$ 89.7% vs. 87.7%) were observed in each case. In a recent study in 9 babies affected by mild-to-moderate Obstructive Sleep Apnea Syndrome (OSAS) and a diagnosed mandibular retrusion, we demonstrated that the moving forward of the jaw and the improved activity of facial and chewing muscles obtained through a mouth device significantly lowers the Apnea/Hypopnea Index (AHI, number of apnea or hypopnea events per hour of sleep) and determine a trend to higher $SpO_2$ levels. By complete polysomnography on the nap, we analyzed respiratory patterns of admitted babies because of ALTE or BRUE, comparing patterns recorded in sleep without and with a pacifier. 20 infants (mean age 2 months) were studied. The AHI resulted significantly lower in sleep with a pacifier (median AHI with pacifier 10 vs. median AHI without pacifier 20, p < 0.05). A trend to higher mean and minimum $SpO_2$ was observed, even if not statistically significant, probably because of the brief length of the apneas registered. The few cases in which more apneas with dummy was observed were due to gastro-esophageal reflux or to diagnosed laryngomalacia. Recently we analyzed 50 infants admitted to our Pediatric Department because of any disease, recording in each patient mean $SpO_2$ in wake or sleep, with and without dummy, in supine and 30° lifted supine position: preliminary data suggest in the whole population studied a trend to higher $SpO_2$ with dummy in supine (97.42 vs. 96.25) and 30° supine position (97.32 vs. 96.27). Preterm babies have more difficult coordination between sucking and breathing and delayed development of oropharyngeal musculature and could benefit from the use of an adequate pacifier, so we decided to investigate preterm newborns that reached term or near-term age and are closed to discharge in order to value if the positive effect of pacifier use could be confirmed on the preterm, we would have at our disposal an economical and simple tool to favor the cardiorespiratory stability of preterm infants. Preliminary data collected will be presented and discussed.

## REFERENCES

[1] McGrattan KE, Sivalingam M, Hasenstab KA, Wei L, Jadcherla SR. The physiologic coupling of sucking and swallowing coordination provides a unique process for neonatal survival. Acta Paediatr. 2016;105(7): 790-7.

[2] Fucile S, McFarland DH, Gisel EG, Lau C. Oral and nonoral sensorimotor interventions facilitate suck-swallow-respiration functions and their coordination in preterm infants. Early Hum Dev. 2012;88(6):345-50.

[3] Horne RS, Fyfe KL, Odoi A, Athukoralage A, Yiallourou SR, Wong FY. Dummy/pacifier use in preterm infants increases blood pressure and improves heart rate control. Pediatr Res. 2016;79(2):325-32.

## LECT 8

## PARENTERAL NUTRITION IN VERY LOW BIRTH WEIGHT INFANTS: TO STANDARDIZE OR NOT TO STANDARDIZE?

S. Iacobelli, F. Bonsante

*Neonatal and Pediatric Intensive Care Unit – Neonatology, La Réunion University Hospital, Saint Pierre, France*

Parenteral nutrition (PN) support is proposed from the first hours of life for preterm infants, in order to promote positive nitrogen balance and to avoid postnatal growth retardation. Today, these patients represent one of the largest clinical groups at all ages to receive PN. Traditionally, PN has been administered as a patient-tailored compound, individualized parenteral nutrition (IPN), prescribed according to the infant's needs and prepared in the neonatal yard or by the hospital pharmacy, usually on a daily basis. Over the last years, there has been a growing use of commercially batch-produced bags, delivered by manufacturers or industrially prepared, that is standardized parenteral nutrition (SPN). For preterm infants, especially of very low birth weight (VLBW), the transition to extrauterine life is associated with significant body modifications water composition, changing nutritional needs and clinical conditions, so the current challenges associated with PN are many and complex. They include i) to provide optimal nutrition intakes; ii) to reduce the risk of metabolic disturbances and infectious complications; iii) to avoid prescription and administration errors; iv) to respect some major pharmacological issues (stability, compatibility); v) to optimize workload and costs. Concerning these issues, both types of PN have advantages and disadvantages, as reported by several studies (**Tab. 1**). Alongside the above outcomes, stability of the final product, pharmacy workload and quality control considerations are often reported in favor of SPN bags, whereas the more attractive advantage of IPN prescriptions is that they can be changed

**Table 1 (LECT 8).** Clinical studies comparing individualized parenteral nutrition (IPN) versus standardized parenteral nutrition (SPN) in newborn babies indicating the main findings for several outcomes.

| Author (year) | Infants<br>· Number of SPN/IPN<br>· GA (weeks) or<br>· BW (grams, mean) | Measured outcome | | | |
|---|---|---|---|---|---|
| | | Better growth | Better nutritional intakes | Less biochemical abnormalities | Lower costs |
| Mutchie (1979) | N = 6/6<br>Preterm, term infants | IPN > SPN | NR | NR | IPN > SPN |
| Dice (1981) | N = 14/14<br>GA 31 weeks (mean) | NR | IPN > SPN | NR | SPN > IPN |
| Cade (1997) | N = 25/27<br>GA 29 weeks (mean) | No difference | NR | No difference | No difference |
| Yeung (2003) | N = 70/70<br>GA < 32 weeks | IPN > SPN | IPN > SPN | IPN > SPN | NR |
| Skouroliakou (2009) | N = 30/30<br>GA 33.9 weeks (mean) | SPN > IPN | SPN > IPN | SPN > IPN | NR |
| Iacobelli (2010) | N = 67/40<br>GA < 33 weeks | SPN > IPN | SPN > IPN | SPN > IPN | SPN > IPN |
| Smolkin (2010) | N = 70/70<br>BW ≤ 1,500 g | IPN > SPN | IPN > SPN | NR | NR |
| Kriessl (2016) | 371 prescriptions<br>In 71 preterm/term infants<br>SPN = ready to use triple chamber bag | NE | SPN > IPN | NE | SPN > IPN |

Studies comparing standardized parenteral nutrition (SPN) versus "recommended intakes" or "unit guidelines" are not presented.
SPN: standardized parenteral nutrition; IPN: individualized parenteral nutrition; GA: gestational age; BW: birth weight; NR: not reported; NE: not evaluable.

on a daily basis, reflecting the patient's medical condition and most recent laboratory tests [1]. Recently, one exhaustive review on standard versus individualized PN has been published within the ESPGHAN/ESPEN/ESPR guidelines on pediatric PN [2]. The authors conclude that studies on this topic are very few and not specifically designed and that no randomized controlled trials comparing SPN versus IPN are available in newborn infants. Based on these considerations, the evidence level for recommending one type of preparation over the other is low [2]. SPN should generally be used over IPN in the majority of newborn patients, including VLBW premature infants (LoE 2, RG 0, Conditional recommendation for). IPN should generally be used when the nutritional requirements cannot be met by the available range of SPN formulations (i.e., in very sick and metabolically unstable patients and infants requiring PN for prolonged periods) (LoE 2, RG B, Strong recommendation for). Any study has been specifically designed in order to investigate whether, among all types of standardized preparations (industrially prepared, commercially batch-produced bags delivered by manufacturers or hospital pharmacies), one should be preferred over another, even if it is assumed that large-scale commercial preparations of SPN could assure better pharmaceutical control and decrease the risk of PN-associated infections [3]. From the clinician point of view, it worthwhile to note that, despite the growing number of publications showing that non-individualized PN (by industrially-prepared, premixed bags or standardized formulations by manufacturers) provides optimal nutritional, electrolyte and mineral intakes [4-6], national surveys on PN practice have reported that SPN accounts for less than 50% of the prescriptions of PN in newborn infants and that it is significantly more frequently prescribed by level II than level III units [7, 8]. Moreover, in level III neonatal intensive care units (NICUs), the choice of formulations varies according to gestational age (GA) at birth, postnatal day and weight, and postconceptional age, thus babies born at less than 28 weeks of GA have up to 80% of individualized prescriptions from day 2 of life and they keep on with this type of bags up to 1,200 g, whereas for infants over 32 weeks of gestation, industrially-prepared bags are preferred [8]. Furthermore, it appears that the practice of supplementation in standardized bags occurs in 55% of level III units. One observational trial in 67 infants born less than 33 weeks of gestation [6] administered SPN by 5 different formulations during the first week of life, showed that 7% of infants needed discontinuation of SPN due to metabolic/hydro-electrolytic imbalance or severe morbidities. All the above literature suggests that the specificities of more vulnerable infants should

be taken into account when establishing policies for the choice of PN in III level NICUs, and also that controlled prospective trials are warranted to assess the tolerance of industrially-prepared bags or standardized formulations in sick preterm babies. Finally, ESPGHAN/ESPEN/ESPR guidelines state that computerized provider order entry (CPOE) programs should be used in the prescription process of PN, whether standardized or individualized [2]. We believe that this is a crucial issue, often not measured by previous studies, which have evaluated the tolerance of SPN bags. In particular, we advocate that modern CPOE software integrate the composition of all SPN preparations available in the NICU, in order to suggest the best prescription taking into account nutritional, electrolytic and water intakes administered to the infant in addition of the PN. To conclude, it appears that, a combination of standardized PN bags, industrially prepared or under strict pharmacological control, could satisfy PN needs in NICUs for most VLBW neonates, providing that a small number of individualized PN formulations remain available for more unstable infants, on a daily basis. Of course, the implementation of a quality process in choosing, ordering and administering PN is advocated, and continuous evaluation of practices should accompany this.

## REFERENCES

[1] Riskin A, Shiff Y, Shamir R. Parenteral nutrition in neonatology – to standardize or individualize? Isr Med Assoc J. 2006;8(9):641-5.

[2] Riskin A, Picaud JC, Shamir R. ESPGHAN/ESPEN/ESPR/CSPEN working group on pediatric parenteral nutrition. Standard versus individualized parenteral nutrition. Clin Nutr. 2018 Jun 18. [Epub ahead of print].

[3] Bethune K. The use of standard parenteral nutrition solutions in pediatrics: a UK perspective. Nutrition. 2001;17(4):357-9.

[4] Senterre T, Abu Zahirah I, Pieltain C, de Halleux V, Rigo J. Electrolyte and Mineral Homeostasis After Optimizing Early Macronutrient Intakes in VLBW Infants on Parenteral Nutrition. J Pediatr Gastroenterol Nutr. 2015;61(4):491-8.

[5] Rigo J, Senterre T. Intrauterine-like growth rates can be achieved with premixed parenteral nutrition solution in preterm infants. J Nutr. 2013;43(12 Suppl):2066S-70S.

[6] Iacobelli S, Bonsante F, Vintéjoux A, Gouyon JB. Standardized parenteral nutrition in preterm infants: early impact on fluid and electrolyte balance. Neonatology. 2010;98(1):84-90.

[7] Inspection Générale des Affaires Sociales (IGAS). Evaluation des pratiques en matière de nutrition parentérale pédiatrique. Available at: http://www.ladocumentationfrancaise.fr/var/storage/rapports-publics/154000256.pdf, date of publication: 2015, last access: September 2018.

[8] Lapillonne A, Fellous L, Kermorvant-Duchemin E. Use of standardized parenteral solutions in French neonatal departments: results of a national survey. Arch Pediatr. 2009;16(10):1329-36.

## LECT 9

## PULMONARY HYPERTENSION: ANY NEWS?

M. Sánchez Luna

*University Hospital Gregorio Marañon, Complutense Universityof Madrid, Research Institute, Madrid, Spain*

Persistent pulmonary hypertension of the newborn (PPHN) is defined by the presence of right-to-left shunt across the fetal cardiac channels, generally because a relatively higher right vascular resistance compared to left vascular resistance. Many etiological factors are involved in this entity, but many times it is related to failed in the average decrease of the high *in-utero* high vascular resistance due to anomalies in the vascular endogenous nitric oxide production at delivery. A better gestational and perinatal care today is probably one the causes of fewer cases of the most severe forms of PPHN. Probably, a better understanding of the pathophysiology prevents its appearance because of fewer posterm deliveries, and better monitoring of the deliveries, but still some cases cannot be prevented and still occurs with high associated morbidity. Diagnosis depends on cardiac ultrasound and Doppler analysis of the intracardiac shunts after confirmation of not having a congenital cardiac malformation. The use of oxygen is the first step in the therapy but is controversial. Oxygen is a potent pulmonary vasodilator, but in the absence of good lung recruitment it can only improve transitory the oxygenation, even more, the use of high $FiO_2$ can produce a detrimental effect of the pulmonary pressure increasing it [1]. The use of volumetric capnography helps in the analysis not only of the tidal volume but most important in the measurement of the physiological dead space and perfusion of the lungs. Also, it can be used to analyze the responses to strategies better to increase the lung perfusion, as recruiting strategies or the use of inhaled Nitric Oxide (iNO). Most of the severe hypoxic respiratory failure in the newborn can be treated with a prompt establish of the functional residual capacity (FRC) by the use of continuous distending pressure or invasive mechanical ventilation. Recruitment of the lung with different strategies is key in the therapy of pulmonary hypertension secondary to parenchymal lung disease [2]. The use of HFOV is probably more helpful to recruit the lung than conventional ventilation, and in some situations even without good evidence from clinical trials, the administration of exogenous surfactant can help to

"open the lung". Although most of the severe forms of PPHN due to severe respiratory failure will respond to respiratory support, the early use of iNO is the most important therapy specific. Today, there is good evidence to recommend its use earlier in the therapy of pulmonary hypertension to reduce the need for ECMO, normally after lung recruitment and with oxygenation index higher than 15-25 [3]. In some situations where iNO is not effective the addition of sildenafil, a phosphodiesterase 5 blocker can help. PPHN is also diagnosed in preterm infants in severe RDS, mostly when prolonged rupture of membranes accompany it. In these situations, iNO is also a safe therapy that can help in the management of these preterm infants, decreasing the need of aggressive mechanical ventilation and high $FiO_2$, although there are data that suggest that iNO therapy is not always associated to an increase on survival, but an early use can be effective. Finally, ECMO is still an effective therapy when no response to other medical therapies occurs. Its use is decreasing mostly due to better respiratory management and the earlier use of iNO. The European registry of ECMO in neonates shows a global survival rate of 75% with respiratory failure, being the survival rate of the PPHN diagnosis at 73%. In summary, there is a decrease in the incidence of the most severe forms of PPHN in term neonates, and focus on prompt recruitment strategies and the early use of iNO is probably the main strategy in most of these forms. There are more PPHN cases among preterm infants, mostly in those cases associated with prolonged rupture of membranes, the early use of iNO is also effective in most of these situations.

REFERENCES

[1] Farrow KN, Lakshminrusimha S, Reda WJ, Wedgwood S, Czech L, Gugino SF, Davis JM, Russell JA, Steinhorn RH. Superoxide dismutase restores eNOS expression and function in resistance pulmonary arteries from neonatal lambs with persistent pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol. 2008;295(6):L979-87.

[2] Abman SH, Hansmann G, Archer SL, Ivy DD, Adatia I, Chung WK, Hanna BD, Rosenzweig EB, Raj JU, Cornfield D, Stenmark KR, Steinhorn R, Thébaud B, Fineman JR, Kuehne T, Feinstein JA, Friedberg MK, Earing M, Barst RJ, Keller RL, Kinsella JP, Mullen M, Deterding R, Kulik T, Mallory G, Humpl T, Wessel DL; American Heart Association Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation; Council on Clinical Cardiology; Council on Cardiovascular Disease in the Young; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Surgery and Anesthesia; and the American Thoracic Society. Pediatric Pulmonary Hypertension: Guidelines From the American Heart Association and American Thoracic Society. Circulation. 2015;132(21):2037-99.

[3] Konduri GG, Sokol GM, Van Meurs KP, Singer J, Ambalavanan N, Lee T, Solimano A. Impact of early surfactant and inhaled nitric oxide therapies on outcomes in term/late preterm neonates with moderate hypoxic respiratory failure. J Perinatol. 2013;33(12):944-9.

**LECT 10**

**PERSISTENT DUCTUS ARTERIOSUS: TO CLOSE OR NOT TO CLOSE?**

C. Dani

*Division of Neonatology, Careggi University Hospital of Florence, Florence, Italy*

*Department of Neurosciences, Psychology, Drug Research, and Child Health, University of Florence, Florence, Italy*

The patency of ductus arteriosus (PDA) is a frequent complication in preterm infants occurring up to 70% of preterm infants with respiratory distress syndrome (RDS) born < 28 weeks' gestation. Although the management of PDA is still debated, 60% to 70% of preterm infants of < 28 weeks' gestation receive medical or surgical therapy for a PDA. A persistent left-to-right shunt through the ductus complicating RDS has been associated with a worsening of respiratory failure, lowering of survival rate, increased risk of intraventricular hemorrhage (IVH), and bronchopulmonary dysplasia (BPD) [1]. Currently, there is a lively debate regarding the adequate management of PDA: pharmacological prophylaxis has been found to be effective in decreasing the occurrence of IVH and the need of PDA surgical ligation, but did not decrease NEC and neurodevelopment impairment in extremely preterm infants. Moreover, it exposes many infants, who would not develop hemodynamically significant PDA (hsPDA), to unnecessary drugs that may have concerning renal and gastrointestinal side effects. Therefore, in many centers, the management of PDA is decided from specific selective echocardiographic criteria with the aim of treating only patients with hsPDA at highest risk of BPD. Finally, some centers adopted non-interventional, conservative management of PDA and did not close it at all [2], although an increase in mortality after cessation of PDA treatment has been reported in some centers [3]. Recent data from Vermont Oxford Network detailed that Italian neonatal intensive care units reported in 2015 an occurrence of PDA in infants with gestational age < 30 weeks' gestation of 50.5% (IQR: 33.3-66.7). Among these patients, 32.9% (IQR: 21.1-43.8) were treated with ibuprofen for the pharmacological closures of PDA, while 6.1% (IQR: 0.0-8.2)

required a PDA surgical closure. On the other hand, the occurrence of PDA was higher at the lowest gestational age being 88.9% (IQR: 100.0-100.0) and 81.5% (IQR: 75.0-100.0) at 23 and 24 weeks' gestation, respectively, and ranging between 70.3 to 32.0% from 25 to 29 weeks' gestation. Similarly, the PDA surgical closure was more frequent in infants born at 23 (17.1%, IQR 0.0-28.6) and 24 (14.3%, IQR 0.0-33.3) weeks' gestation than in infants born at 25-29 weeks' gestation (13.6-0.5%). On the other hand, these data are in agreement with Semberova et al., who recently reported that spontaneous closure of PDA occurs less frequently and later in very low birth weight infants born at < 26 weeks' gestation than in very low birth weight infants born at > 26 weeks' gestation [4]. Therefore, infants who were born at 23-24 weeks' gestation might have greatest benefits from the pharmacological closure of PDA because they have the highest occurrence of hsPDA, the highest risk of developing hsPDA refractory to cyclo-oxygenase inhibitors (i.e., indomethacin or ibuprofen) requiring surgical ligation, and highest mortality, IVH, and BPD rate. For these reasons, by a potentially more favorable cost-benefit balance, it might be argued that infants born at these extreme gestational ages deserve an unusual approach to the pharmacological closure of PDA different from more mature infants. Unfortunately, previous studies did not investigate the management of hsPDA in infants who were born at 23-24 weeks' gestation probably due to the great difficulties in planning randomized controlled studies in this area. Therefore, we planned a multicenter retrospective study in 12 third level Neonatal Intensive Care Units for assessing the occurrence of hsPDA in a large cohort of preterm infants born at 23-24 weeks of gestational age and the effect of pharmacological treatment with ibuprofen to evaluate the hypothesis that in these patients the hsPDA rate and the risk of treatment failure are higher than in infants born at 25-28 weeks of gestational age. We calculated a sample size of 63 and 315 infants born at 23-24 or 25-28 weeks of gestational age, respectively, at a power of 80% and α = 0.05, and actually studied 80 and 326 patients of the first and second group, respectively.

## REFERENCES

[1] Schena F, Francescato G, Cappelleri A, Picciolli I, Mayer A, Mosca F, Fumagalli M. Association between Hemodynamically Significant Patent Ductus Arteriosus and Bronchopulmonary Dysplasia. J Pediatr. 2015;166(6):1488-92.

[2] Rolland A, Shankar-Aguilera S, Diomande D, Zupan-Simunek V, Boileau P. Natural evolution of patent ductus arteriosus in the extremely preterm infant. Arch Dis Child Fetal Neonatal Ed. 2015;100(1):F55-8.

[3] Brooks JM, Travadi JN, Patole SK, Doherty DA, Simmer K. Is surgical ligation of patent ductus arteriosus necessary? The Western Australian experience of conservative management. Arch Dis Child Fetal Neonatal Ed. 2005;90(3):F235-9.

[4] Semberova J, Sirc J, Miletin J, Kucera J, Berka I, Sebkova S, O'Sullivan S, Franklin O, Stranak Z. Spontaneous Closure of Patent Ductus Arteriosus in Infants ≤1500 g. Pediatrics. 2017;140(2):e20164258.

## LECT 11

## MONITORING CARDIOVASCULAR PARAMETERS IN NICU

D. Doni, P.E. Tagliabue

*Neonatology and Neonatal Intensive Care Unit, MBBM Foundation, Monza, Italy*

Circulatory failure is a major cause of mortality and morbidity in critically ill newborn infants. Despite the great importance of hemodynamic status in newborn, it is usually assessed by the interpretation of various clinical and biochemical parameters. The most used indicators of circulatory failure are just today blood pressure, heart rate, urine output, capillary refill time, serum lactate concentration, temperature difference, pH, central venous oxygen saturation, and color. In literature, it is reported that a survey questionnaire sent to neonatal intensive care units revealed that these parameters were most used to diagnose hemodynamic status, but cardiac output (CO) measurement was not even mentioned [1]. Objective CO measurement and clinical estimation of systemic blood flow based on the interpretation of routine clinical variables do not always agree with critically ill patients. Many neonates might be under or over-treated and are at risk of iatrogenic injury. Therefore objective CO monitoring may be beneficial for the patient, and it reduces mortality and morbidity. Low CO is associated with increased mortality, periventricular/intraventricular hemorrhage and poor neurodevelopmental outcomes. Many methods of CO monitoring are available, but not all are feasible in neonates. The extremely and low birthweight infants have difficulties in vascular access, size restraints, the potential toxicity of indicators. Noninvasive methods of CO monitoring were spread in neonatal intensive care units. The functional echocardiography was introduced by Kluckow to describe the bedside use of transthoracic echocardiography to assess myocardial function and systemic and pulmonary blood flow. Doppler ultrasound echocardiography can measure stroke

volume (SV) through a vessel and multiple SV by heart rate will result in cardiac output value. The major limitations of echocardiography are significant training required, operator dependent, inaccuracy due to the error in the assessment of cross-sectional area (CSA) of a vessel. Blood flow velocity can be measured in the ascending aorta with a noninvasive ultrasound probe positioned in the sternal notch. The CSA of the aortic valve is derived from an algorithm using height, weight, and age. Thoracic electrical impedance technology is probably the only true non-invasive method of CO monitoring. Major limitations are inaccuracy due to alteration in position or contact of the electrodes, irregular heart rates and acute changes in tissue water content. The cardiac output assessment is also useful for describing the behavior of oxygenation, during incremental/decremental CDP trials in infants receiving High-Frequency Oscillatory Ventilation (HFOV). In a recent article, we evaluated right ventricular output (RVO) during HFOV. The results showed that oxygenation improved during inflation, whereas RVO deteriorated, and that oxygenation remained stable during deflation, whereas RVO improved, suggesting that lung mechanics and RVO are more sensitive than oxygenation to overdistension and they may be useful in clinical practice to guide open lung maneuvers [2]. It is well known that bronchopulmonary dysplasia (BPD) may result in chronic pulmonary artery hypertension and right ventricular (RV) dysfunction. The contribution of left ventricular (LV) dysfunction to the clinical pathophysiology of BPD remains unclear due to the limited number of available reports on the changes in LV ECHO parameters in children with BPD. Changes in the vasculature system can cause hypertension in the neonates. In turn, these changes will increase the LV afterload, leading to increased pressure in the left atrium, and abnormal diastolic filling, which is indicative of impaired diastolic LV function. Indeed, an impaired diastolic function has been shown in studies on infants with BPD. Postcapillary pathophysiology and cardiac dysfunction in BPD it has been suggested in a recent article [3]. Bedside ECHO was used as an easily accessible, non-invasive, and commonly used tool. Close cardiac function monitoring may be included in the assessments of infants with BPD, because it may assist with the clinical management and improve outcomes.

## REFERENCES

[1] de Boode WP. Clinical monitoring of systemic hemodynamics in critically ill newborns. Early Hum Dev. 2010;86(3):137-41.

[2] Zannin E, Doni D, Ventura ML, Fedeli T, Rigotti C, Dellacá RL, Tagliabue PE. Relationship between Mean Airways Pressure, Lung Mechanics, and Right Ventricular Output during High-Frequency Oscillatory Ventilation in Infants. J Pediatr. 2017;180:110-5.

[3] Sehgal A, Malikiwi A, Paul E, Tan K, Menahem S. A new look at bronchopulmonary dysplasia: postcapillary pathophysiology and cardiac dysfunction. Pulm Circ. 2016;6(4):508-15.

## LECT 12

# DO NOT ATTEMPT RESUSCITATION (DNAR) ORDERS, WITHDRAWING/WITHHOLDING CARE IN THE NICU

P. Biban, S. Lauriola, M. Ventola, C. Forcellini, B. Ficial, F. Bissolo, E. Bonafiglia, L. Chini, L. Pecoraro, I. Sibona, R. Frassoldati, R. Beghini

*Neonatal Intensive Care Unit, Department. of Pediatric and Neonatal Critical Care, Verona University Hospital, Italy.*

Several technologic advances and innovations have markedly characterized the neonatal intensive care unit (NICU) care in the last decades. Consequently, many newborns with life-threatening situations, e.g., due to extremely low gestational age or severe congenital and acquired clinical conditions, may ultimately survive with a good outcome. However, some infants will inevitably die, either in the delivery room or within the NICU, despite maximal medical treatment or after complex decisions to limit or withdraw life-sustaining interventions (LSI). In particular, causes and timing of death for the subset of infants who do not survive despite intensive care are evolving, and discussions concerning the withholding or withdrawal of LSI in these patients are progressively increasing in the NICUs [1, 2]. Neonatal deaths are rarely due to severe clinical conditions, which are refractory to maximal treatment, at least in developed countries. Rather, most NICU deaths are more and more often associated with limitation or withdrawal of active therapy, including the extreme choice of not attempting resuscitation in case of cardiopulmonary arrest episodes (do not attempt resuscitation – DNAR). This paper aims to discuss some of the relevant issues related to DNAR orders, as well as some decisions about withholding and withdrawing care in newborns admitted to the NICU. Ethical considerations about the early management of extremely premature babies, born at the limits of viability, are beyond the scope of this paper. Caring for severely ill newborns implies complicated

choices to be made, particularly when facing end-of-life situations. Neonatologists often must deal with infants having no chance of survival, or who may eventually survive, but with a very poor quality of life. A progressive transition from aggressive and life-prolonging medical interventions to a more comfortable and quality-of-life oriented measures may be observed in these cases. In such difficult circumstances, the decision-making process concerning withholding or withdrawing of care may quite vary in different NICUs, depending on local cultural, religious or ethical beliefs, both among healthcare professionals and parents. Similarly, parents' involvement during end-of-life (EOL) discussions can markedly change according to traditional habits. For instance, some centers are adopting a paternalistic approach, where all major decisions are still delegated to the medical (and the nursing) team. This attitude suffers from several limitations and may carry negative consequences, such as complicated mourning process for the family members, legal disputes, risk of moral distress or internal conflicts within the NICU staff. Conversely, nowadays most clinicians are inclined to involve the family as soon as appropriate, in order to better understand parents' views on death and dying, informing them about the beneficial role of analgosedation and palliative care, and sharing the responsibility of withholding or withdrawing specific life-sustaining interventions. To this end, timely and high-quality communication is mandatory, as parents should be fully informed about their baby's prognosis as well as the care options available, including palliative care and EoL procedures [3]. Ideally, healthcare providers should be adequately trained in communication techniques, particularly focused on difficult conversations, active and empathic listening and basic human interactions. However, even more importantly, clinicians should grant a balanced view, tailoring information to the needs of the family and making sure they have fully understood the information provided before making any decision. Concepts such as "DNAR" or "limitation of life-sustaining interventions" may not be obvious for most parents. Therefore a plain language and repeated conversations may be often required. Actually, the precise meaning and interpretation of DNAR orders could be somewhat confusing also to healthcare professionals themselves. In fact, in a recent study by Arzuaga et al., variable expertise and marked discrepancies were reported amongst the NICU staff about interpretation, as well as

appropriate management, of newborns who have a DNAR order [4]. Of note, formal education on discussing or managing patients with a DNAR order appeared not to be systematically provided to the personnel. In addition, as a reflection note, amongst staff members who had previous experiences withholding or withdrawing medical interventions, some considered this practice as acceptable, even without a previous assent of the family [4, 5]. Contrariwise, apart from some rare exception, we believe a full involvement of the family should be mandatory during end-of-life discussions, as well as during any decision-making about life-sustaining interventions. Indeed, parents are usually the best advocates of their baby and may facilitate clinicians in choosing the right option in a more individualized manner.

## REFERENCES

[1] Singh J, Lantos J, Meadow W. End-of-life after birth: death and dying in a neonatal intensive care unit. Pediatrics. 2004;114(6):1620-6.

[2] Hellmann J, Knighton R, Lee SK, Shah PS; Canadian Neonatal Network End of Life Study Group. Neonatal deaths: prospective exploration of the causes and process of end-of-life decisions. Arch Dis Child Fetal Neonatal Ed. 2016;101(2):F102-7.

[3] Janvier A, Barrington K, Farlow B. Communication with parents concerning withholding or withdrawing of life-sustaining interventions in neonatology. Semin Perinatol. 2014;38(1):38-46.

[4] Arzuaga BH, Wraight CL, Cummings CL, Mao W, Miedema D, Brodsky DD. Do-Not-Resuscitate Orders in the Neonatal ICU: Experiences and Beliefs Among Staff. Pediatr Crit Care Med. 2018;19(7):635-42.

[5] Biban P. Do-Not-Resuscitate Orders and Overall Goals of Care in Critically Ill Newborns: Are We Always on the Same Page? Pediatr Crit Care Med. 2018;19(7):676-8.

## LECT 13

## VITAMINS IN THE NEWBORN

C. Romagnoli

*Neonatal Intensive Care Unit, Rome, Italy*

In the last years, attention has been given to the vitamin's intake for newborn infants and children. In particular, many studies have highlighted the role of vitamin K in preventing neonatal hemorrhagic disease, and of vitamin D. This report wants to make a point about the role of some vitamins (B-complex, A, C, D, and E) in the neonatal period both in full-term and in preterm infants. B-vitamins are a fundamental group of micronutrients in the neonatal period, mainly for preterm infants. We know the role of vitamins B9 (folic acid) and B12 (cobalamin),

while the role of the other B-vitamins in the neonatal period have not been studied extensively [1]. B-vitamins play an essential role in almost all anabolic and catabolic processes of the body, and they are essential in the healthy development of the body, especially of the central nervous system. Their role is played in enzymatic activities (as coenzyme) in the synthesis of catecholamine and neurotransmitters, in the endogenous synthesis of many antioxidant compounds, in DNA/RNA synthesis and DNA methylation processes [1]. The suggested intake for B-vitamins in the first six months of life are not always the result of specific studies but are derived from the content of breast milk, which is not always suitable for the needs of the newborn infants, especially if born preterm [1-3]. Furthermore, the safe upper level of intake for many B-vitamins is not yet defined. Infant's status of B1 and B6 vitamins is strongly dependent on maternal intake and status, and maternal supplementation improves the milk's vitamin concentration and the breastfed infant's status. Thiamin, riboflavin, and niacin seem not to be a nutritional problem for neonates in European countries, but specific studies are lacking. Biotin and folic acid should be supplemented in preterm infants, while cobalamin seems to be a nutritional problem only in developing countries. Vitamins A, C, and E are important for their action in the antioxidant systems of the body and their possible role in the prevention of oxygen radical pathologies (ROP and BPD) has been widely demonstrated [2, 3]. Severe or marginal deficiency of vitamin A may affect over 16% of mothers, suggesting the need for vitamin A intake during pregnancy. Maternal status and intake influence its milk concentration. Milk from well-nourished women is an excellent source of vitamin A, but infants prematurely weaned could have vitamin A deficiency. Prematurity and very low birth weight are associated with vitamin A deficiency at birth and during the neonatal period because of insufficient intake, while a positive effect on mortality and chronic lung disease has been proven. Neonatal vitamin C status depends on the maternal vitamin C concentrations in the fetus less than 80% of the maternal one. The vitamin C concentrations in milk samples are in agreement with the reference value. However, storage for 1 months in a freezer and 24 h in a refrigerator will preserve only two-thirds of the initial vitamin C [3]. Neonates have lower vitamin E concentrations than their mothers, indicating that neonatal status is dependent on maternal vitamin E status. Despite the decrease in α-tocopherol from colostrum to mature milk, the exclusively breastfed term infants may have access to adequate serum α-tocopherol. Prematurity and very low birth weight are associated with vitamin E deficiency at birth and during the neonatal period because of insufficient intake. Vitamin D has been the subject of the majority of the studies, and many of its beneficial effects have been re-evaluated especially in light of the non-mineral effects of this vitamin. In more recent reports the vitamin D content of human milk is related to the lactating mother's vitamin D status, being the concentrations higher in the milk of supplemented mothers. However, the daily supply of vitamin D from breast milk is low. Therefore, exclusively breastfed infants should receive a supplement of vitamin D because, otherwise, they could be at risk of developing vitamin D deficiency and, if possible, rickets. Low serum concentrations (< 50 nmol/L) are common in preterm infants during birth hospitalization and at discharge from the neonatal intensive care unit. Indeed, many neonatal nutritional strategies for early preterm infants may be insufficient to achieve recommended vitamin D intake and target serum 25(OH)D concentrations. In conclusion, full-term infants of well-nourished women receive an adequate intake of vitamins except for vitamin D. However, preterm infants should receive adequate supplementation to avoid possible deficiencies at least until the age of the weaning.

REFERENCES

[1] Kennedy DO. B Vitamins and the Brain: Mechanisms, Dose and Efficacy – A Review. Nutrients. 2016;8(2):68.

[2] Elmadfa I, Meyer AL. Vitamins for the first 1000 days: preparing for life. Int J Vitam Nutr Res. 2012;82(5):342-7.

[3] Leaf A, Lansdowne Z. Vitamins – Conventional Uses And New Insights. In: Koletzko B, Poindexter B, Uauy R (Eds.). Nutritional Care of Preterm Infants: Scientific Basis and Practical Guidelines. Basel: Karger, 2014, pp. 152-66.

**LECT 14**

**ANEMIA OF PREMATURITY: WHAT METABO-LOMICS CAN ADD**

V. Fanos[1], R. Pintus[1], F.S. Corbu[2], G. Ledda[1], E. Coni[1], A. Dessì[1], M. Puddu[1], F. Cesare Marincola[2]

[1]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, Azienda Ospedaliera Universitaria, University of Cagliari, Cagliari, Italy

[2]Department of Chemical and Geological Sciences, Cittadella Universitaria, University of Cagliari, Cagliari, Italy

## INTRODUCTION

Iron exhibits a U-shaped risk, typical of essential nutrients, in which risk of adverse outcomes in infants and young children increases not only with low or inadequate availability but also at higher availability. Iron Deficiency Anemia (IDA) is a concern in the infant and young child because of the vulnerable period of brain development in the first 24 months. The differential prioritization of iron to erythropoiesis exacerbates the vulnerability of the brain when the iron is limiting during this critical period. A limited number of studies find impaired cognitive and brain development with IDA. Iron is particularly crucial in preterm infants to meet the high demands for hematopoiesis, growth, and development. Thus, the supplementation with iron is indicated in preterm infants to prevent the anemia of prematurity. It is commonly believed that iron supplementation must also be performed after the discharge, at least 6-12 months of age, depending on the diet.

## PRELIMINARY DATA

Very recently we performed a preliminary investigation during an iron supplementation period of 2 months of the urinary metabolome ($^1$H-NMR spectroscopy) of 23 preterm infants (gestational age between $28^{+3}$ and $36^{+6}$ weeks and birth weight between 790 and 1,890 g) admitted to the Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, Azienda Ospedaliera Universitaria, University of Cagliari, Italy. The infants received prophylaxis with iron pidolate 3 mg/kg/day to prevent anemia of prematurity. Only 19 infants completed the trial. Urine was collected non-invasively with cotton balls at three-time points: at discharge (T0), at 1 (T1) and 2 (T2) months after the iron treatments. $^1$H-NMR spectroscopy and multivariate statistical analysis were used to analyze the urinary metabolic profile. To the best of our knowledge, this is the first metabolomics study on the role of iron treatment on the metabolism of the preterm infant for prevention of anemia of prematurity. Significant differences were observed between samples collected at the three-time points. The betaine is the primary metabolite of choline. It has an essential role as a precursor of membrane phospholipids and lipoproteins. Moreover, it is involved in the synthesis of the neurotransmitter acetylcholine. According to a recent study, pre- and postnatal supplementation of choline are able to reduce anemia-related brain deficits in a murine model. Again, regarding creatine, in animal models, low creatinine levels in CSF preceded brain

metabolic derangement. Myo-inositol presents, although indirectly, protective action on hemolysis, being a structural component of the membrane protein glycosyl-fosfatidyl-inositol (GPI), which is crucial for the protection of complement-mediated hemolysis. Finally, it is well known that a deficit of the enzyme fosfatidil-inositol-glycan-A (PIG-A) is responsible for the paroxysmal nocturnal hemoglobinuria. Dimethylglycine (DMG) is strictly involved in the metabolism of the red blood cells: an increase of DMG is negatively correlated with anemia, is a robust index of glutathione need and/or mitochondrial dysfunction.

## DISCUSSION

Although these results are preliminary, the observed temporal changes in the concentrations of the metabolites could potentially be linked to pathways related to red blood cells. Further studies are needed in order to confirm this hypothesis. Metabolomics seems to be a promising non-invasive technology for the study of anemia of prematurity.

## REFERENCES

• Atzori L, Griffin JL, Noto A, Fanos V. Review metabolomics: a new approach to drug delivery in perinatology. Curr Med Chem. 2012;19(27):4654-61.

• Brannon PM, Taylor CL. Iron Supplementation during Pregnancy and Infancy: Uncertainties and Implications for Research and Policy. Nutrients. 2017;9(12):E1327.

• Dessì A, Cesare Marincola F, Masili A, Gazzolo D, Fanos V. Clinical metabolomics and nutrition: the new frontier in neonatology and pediatrics. Biomed Res Int. 2014;2014:981219.

• Fanos V, Antonucci R, Barberini L, Atzori L. Urinary metabolomics in newborns and infants. Adv Clin Chem. 2012;58:193-223.

• Fanos V, Antonucci R, Atzori L. Metabolomics in the developing infant. Curr Opin Pediatr. 2013;25(5):604-11.

• Fanos V, Van den Anker J, Noto A, Mussap M, Atzori L. Metabolomics in neonatology: fact or fiction? Semin Fetal Neonatal Med. 2013;18(1): 3-12.

• Fanos V, Buonocore G, Mussap M. Neonatomics and childomics: the right route to the future. J Matern Fetal Neonatal Med. 2014;27(Suppl 2):1-3.

• Fanos V. Metabolomics and microbiomics: individualized medicine from the fetus to the adult. San Diego: Academic Press, 2016.

• Noto A, Fanos V, Dessì A. Metabolomics in Newborns. Adv Clin Chem. 2016;74:35-61.

• Pintus R, Cesare Marincola F, Corbu S, Coni E, Dessì A, Puddu M, Fanos V. Iron pidolate in preterm newborns for prevention of anemia of prematurity: first metabolomics results. In: Selected Abstracts of the 2$^{nd}$ International Workshop "Intensive Care of the Newborn"; Verona (Italy); March 9-10, 2018. J Pediatr Neonat Individual Med. 2018;7(1):e070125.

## LECT 15

## IRON STATUS AND LATE PRETERM INFANTS

R. Luciano

*IRCCS Fondazione Policlinico Universitario A. Gemelli, Rome, Italy*

Iron is the element with the highest concentration in blood. It is able to reversibly bind and release oxygen due to the two different oxidation stable configurations $Fe^{++}$ and $Fe^{+++}$. This property allows that iron, besides the presence in hemoglobin and myoglobin heme, plays a crucial role in many processes that are essential for our body, like cellular respiration or enzymatic activity. Iron is also essential in the central nervous system for cognitive and learning processes. Many essential developmental processes as myelinogenesis, dendritogenesis, and synaptogenesis depend on enzymes containing iron. Iron is also necessary for neurotransmitter synthesis, serotonin, dopamine, norepinephrine. Preclinical studies in rodents, as well as clinical studies, showed that iron deficiency (ID) has a strong impact on neurologic development. These effects vary in relation to the developmental period affected by ID, and they are not always reversible with iron supplementation, in case of a delayed correction. In adults, total body iron is equal to 4 grams, of which 65% is hemoglobin iron, 25% storage, 5% cytochrome/enzymatic iron and 5% myoglobin. Transport iron represents less than 0.1% of total iron. In term neonates, body iron is 75 mg/kg, due to the higher blood volume. In adults, recycling from red cell iron provides 95% of total iron, and only 5% comes from diet. In infants, only 70% of red cell iron comes from recycling and 30% from the diet. When iron losses and iron needs outnumber the iron supplies (intake and recycling), then a condition of ID starts, being iron deficiency anemia (IDA) the final stage. Our body reacts to ID increasing iron absorption and starting the use of reserves, i.e., ferritin and haemosiderin to almost storage exhaustion, but at the same time, from the beginning of the process, a small amount of enzyme iron and transport iron is involved as well. As a consequence, it is crucial to prevent ID, instead of treating IDA at a later stage. A child with ID may be asymptomatic or with no specific symptomatology. A single marker to define iron status is not available. Diagnosis of ID is based on red blood count (Hb, Htc, MCV, RDW and, recently, CHr) and serum ferritin and percentage of transferrin saturation. ID is not a rare condition since the ID prevalence in the USA population is as high as 5-10%. Prevalence of IDA in infants between 6 and 9 months is about 2-3%. ID does not occur in healthy term neonates because intrauterine iron stores are sufficient until weaning with complementary foods and fortified formulas. The $Fe^{++}$ contained in the heme of hemoglobin and myoglobin in diet meat is very well absorbed. Preterm neonates (PTn) are at high risk for ID. Iron transport across the placenta increases during gestation is the vast majority (about 80%) transferred during the third trimester, mostly after the 30th week of gestation. Iron supplementation is, therefore, the rule for PTn discharged from the intensive care units. The preterm birth rate has increased by 33% in the last 25 years, almost entirely due to the rise in late preterm births ($34^{+0}$ to $36^{+6}$ week gestation) that represent nowadays 7 to 9% of all live births. Among this population, 1.25% more risk of mental retardation vs. term neonates is reported [1]. After discharge, late PTs are managed in the same way as term neonates, and usually, they do not receive iron prophylaxis. A recent Italian study [2], reported that less than 25% of late PTn discharged from the hospital receive recommendations for iron prophylaxis, although they have insufficient storage of iron compared to term neonates. They receive iron transport across the placenta for a shorter time interval. Moreover, iron requirements for growth are higher than term neonates requirements, due to the significant increase in growth velocity and consequently in blood volume. The late Ptn iron supply is lower in respect to very low birth weight neonates because the prevalence of exclusive breastfeeding is higher (the iron content of breast milk is low), blood transfusions are rarely needed, and iron prophylaxis is not standardized. In recent years, thanks to the studies of Berglund et al. [3], that showed a significantly reduced prevalence of IDA and a more adaptative behavior in moderately low birth weight infants (birth weight between 2,000 and 2,500 grams), the ESPGHAN Committee on Nutrition stated that MLBW should receive iron supplements of 1-2 mg • kg • day. Preliminary evaluation of a RCT trial performed in our institution shows that ID prevalence is high in the placebo group of late preterm infants. Interestingly enough, iron supplemented infants also have a high prevalence of ID at the suggested dosage when exclusively breastfed. Compliance rate in iron prophylaxis is low. Despite the American Academy recommendations, an American study [4] reported that among preterm breastfed and mixed-fed infants none received oral iron supplements 3 times per week before 3 months of age, 2% received them at 3 months, and 13% received them at 10.5 months.

On the light of recent reports, implementing iron prophylaxis is mandatory not only in the very preterm babies but also in the growing population of late preterm infants.

REFERENCES

[1] Petrini JR, Dias T, McCormick MC, Massolo ML, Green NS, Escobar GJ. Increased risk of adverse neurological development for late preterm infants. J Pediatr. 2009;154(2):169-76.

[2] Parodi E, Ferrero A, Perrone B, Saracco P, Giraudo MT, Regoli D. Current practice of iron prophylaxis in preterm and low birth weight neonates: A survey among Italian Neonatal Units. Pediatr Neonatal. 2018 Feb 1. [Epub ahead of print].

[3] Berglund SK, Chmielewska A, Starnberg J, Westrup B, Hägglöf B, Norman M, Domellöf M. Effects of iron supplementation of low-birth-weight infants on cognition and behavior at 7 years: a randomized controlled trial. Pediatr Res. 2018;83(1-1):111-8.

[4] Dee DL, Sharma AJ, Cogswell ME, Grummer-Strawn LM, Fein SB, Scanlon KS. Sources of supplemental iron among breastfed infants during the first year of life. Pediatrics. 2008;122(Suppl 2):S98-104.

**LECT 16**

**NEONATAL TRANSFUSION PRACTICE**

C. Franco, F. Petrillo, K. Del Vecchio, G. D'Amato, A. Del Vecchio

*Department of Women's and Children's Health, Neonatal Intensive Care Unit, ASL Bari, Bari, Italy*

INTRODUCTION

Blood transfusions are important supportive therapy in neonatal intensive care units (NICUs). Sick neonates, in particular, preterm neonates, are deeply transfused, especially within the first week of life, when they receive at least one red blood cell (RBC) transfusion and even multiple transfusions during their hospital stay. Transfusion practice is not without risk since preterm neonates are particularly prone to transfusion complications, including transfusion-associated circulatory overload (TACO) and transfusion-associated acute lung injury (TRALI), which are often underdiagnosed. Thus, in current neonatology practice, especially in extremely low birth weight neonates, avoidance of unnecessary transfusions becomes the major objective. Therefore, the definition and refining of the most clinically appropriate protocols for blood product use in neonates remain a continuing task, in order to improve practice and avoid unnecessary transfusions [1].

RED BLOOD CELLS TRANSFUSION

One of the main difficulties with deciding when to transfuse is the lack of a clear and agreed definition of severe anemia in neonates. The majority of RBC transfusions to neonates are given to maintain hematocrit (Hct) or blood hemoglobin concentration at a satisfactory level to optimize oxygenation, even though there is insufficient knowledge on the effects of limiting oxygen delivery to the neonatal tissues. Actually, Hct level is a poor indicator of tissue oxygenation, and, although it motivates the transfusion, this level may be arbitrarily decided or related to the results of the very few trials conducted on infants. The same arbitrariness in deciding when to transfuse a neonate exists when we consider some surrogate markers of anemia, such as apnea, retarded growth, lethargy or tachycardia, that are present not only in anemic infants but may accompany many other clinical conditions other than anemia [2]. In addition to the well-known risks associated with transfusion (e.g., transmission of infections, alloimmunization, febrile reactions, hemolytic reactions, allergic reactions, additional donor exposure), necrotizing enterocolitis or intraventricular hemorrhage (IVH) may follow an RBC transfusion in neonates, although a causal relationship has not yet been demonstrated. The important question is whether the adverse effects are from anemia itself or the transfusions. For this reason, we continue to study all the possible ways to avoid transfusions and delayed cord clamping or cord milking, drawing all initial laboratory blood tests using fetal cord blood at delivery, limiting phlebotomy losses become valid alternatives to neonatal RBC transfusions [2-6].

PLATELET TRANSFUSIONS

Platelets transfusions, next to RBCs transfusions, are commonly used in neonates, although very often improperly. This frequent use of platelet transfusions in NICUs lacks a substantial evidence base, and the benefits of these transfusions remain speculative. As a matter of fact, 98% of platelet transfusions administered to NICU patients are given prophylactically, in the absence of bleeding, with the assumption that this reduces the risk of severe hemorrhage, but without a clear relationship between low platelet counts and consequential clinical bleeding or significant hemorrhage. On the contrary, only 2% of all neonates in NICUs receive an appropriate and lifesaving platelet transfusions because of thrombocytopenic bleeding [4, 5]. It is necessary to consider that, besides the severity of thrombocytopenia, a large number of factors, mainly including the degree of prematurity, may predict the bleeding risk in neonates. Thus, indications and thresholds for prophylactic platelet transfusions

remain based on clinical experience, despite various efforts to develop improved tests to assess primary hemostasis and bleeding risk in neonates. At present the NeoBAT, a neonatal bleeding assessment tool, is also being used by the PlaNeT2 study (http://www.planet-2.com), an ongoing randomized prophylactic threshold trial randomly assigning neonates to a 25,000/mL versus 50,000/mL transfusion threshold. It is necessary to accurately determine when to transfuse platelets because of adverse outcomes from platelet transfusions which are well documented and include transmission of bacterial and nonbacterial infections, alloimmunization, febrile reactions, hemolytic reactions, allergic reactions, TRALI, transfusion-related gut injury, and donor exposure. Growing evidence indicates that multiple platelet transfusions increase the mortality rate of thrombocytopenic NICU patients. It has been clearly reported that every platelet transfusion was given increases the odds of death by 5% above what it would be had no platelet transfusion been administered [7].

PLASMA TRANSFUSION

Plasma transfusion is a relatively frequent intervention in the NICU, although there are a very few studies investigating plasma use in neonates. Similarly to platelet transfusion, although there is limited evidence to support the effectiveness of this practice, most plasma transfusions are given prophylactically to neonates with no evidence of active bleeding [8]. Indications and rationale for the use of plasma in neonates include abnormal coagulation profile, active bleeding or before invasive procedures in patients at high risk of bleeding. The recent British Society of Hematology (BSH) guidelines do not recommend the prophylactic plasma administration to "non-bleeding" neonates with minor prolongation of PT/aPTT and before surgery. Furthermore, BSH guidelines definitely do not recommend plasma transfusion as a means for volume replacement or prevention of bleeding in neonates [9]. Adverse outcomes from plasma transfusion are substantially the same as reported for platelet transfusions [10].

CONCLUSIONS

Based on small studies and not on statistically valid clinical trials, guidelines for neonatal transfusions remain controversial, and practices vary greatly. Consequently, since recommendations derive from insufficient literature, they continue to generate poor compliance and frequent violations [4, 5].

REFERENCES

[1] Christensen RD, Carroll PD, Josephson CD. Evidence-based advances in transfusion practice in neonatal intensive care units. Neonatology. 2014;106(3):245-53.

[2] Del Vecchio A, Henry E, D'Amato G, Cannuscio A, Corriero L, Motta M, Christensen RD. Instituting a program to reduce the erythrocyte transfusion rate was accompanied by reductions in the incidence of bronchopulmonary dysplasia, retinopathy of prematurity and necrotizing enterocolitis. J Matern Fetal Neonatal Med. 2013;26(Suppl 2):77-9.

[3] Christensen RD, Ilstrup S. Recent advances toward defining the benefits and risks of erythrocyte transfusions in neonates. Arch Dis Child Fetal Neonatal Ed. 2013;98(4):F365-72.

[4] Del Vecchio A, Franco C, Petrillo F, D'Amato G. Neonatal Transfusion Practice: When do Neonates Need Red Blood Cells or Platelets? Am J Perinatol. 2016;33(11):1079-84.

[5] Goel R, Josephson CD. Recent advances in transfusions in neonates/infants. F1000Res. 2018;7(F1000 Faculty Rev):609.

[6] Baer VL, Lambert DK, Carroll PD, Gerday E, Christensen RD. Using umbilical cord blood for the initial blood tests of VLBW neonates results in higher hemoglobin and fewer RBC transfusions. J Perinatol. 2013;33(5):363-5.

[7] Baer VL, Lambert DK, Henry E, Snow GL, Sola-Visner MC, Christensen RD. Do platelet transfusions in the NICU adversely affect survival? Analysis of 1600 thrombocytopenic neonates in a multihospital healthcare system. J Perinatol. 2007;27(12):790-6.

[8] Motta M, Del Vecchio A, Perrone B, Ghirardello S, Radicioni M. Fresh frozen plasma use in the NICU: a prospective, observational, multicentred study. Arch Dis Child Fetal Neonatal Ed. 2014;99(4):F303-8.

[9] New HV, Berryman J, Bolton-Maggs PH, Cantwell C, Chalmers EA, Davies T, Gottstein R, Kelleher A, Kumar S, Morley SL, Stanworth SJ; British Committee for Standards in Haematology. Guidelines on transfusion for fetuses, neonates and older children. Br J Haematol. 2016;175(5):784-828.

[10] Karam O, Demaret P, Shefler A, Leteurtre S, Spinella PC, Stanworth SJ, Tucci M; Canadian Critical Care Trials Group (CCCTG); Pediatric Acute Lung Injury and Sepsis Investigators (PALISI); BloodNet; PlasmaTV Investigators. Indications and Effects of Plasma Transfusions in Critically Ill Children. Am J Respir Crit Care Med. 2015;191(12):1395-402.

**LECT 17**

## INDIVIDUALIZED DEVELOPMENTAL CARE STABILIZING SLEEP, MOVEMENT, POSTURE AND CARDIORESPIRATORY CONTROL PROTECTS THE BRAIN IN THE NICU

F. Ferrari, N. Bertoncelli, E. Garetti, E. Della Casa, M.F. Roversi, L. Ori, L. Lucaccioni

*Neonatal Intensive Care Unit, Department of Medical and Surgical Sciences of the Mother, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy*

Numerous experimental and clinical studies have shown that the brain plasticity is at its most soon after birth and that the brain development depends on genes and environmental inputs.

In the NICUs, preterm infants are exposed to a large number of noxious stimuli such as frequent

painful and stressing procedures, disruption of sleep, excessive noise and light levels, daily routine handling, and parental separation. Significant findings have provided evidence of the detrimental impact of the aforementioned stimuli on the developing human brain. Strategies aiming at preventing this impact along with neuroprotective strategies, early neurodevelopmental support and the promotion of bonding and attachment should be implemented in the NICUs. Free parents access and their early involvement, sleep protection, regulation of the NICU macro and micro-environment could be considered the basis of individualized developmental care and may have short and long-term effects on neurodevelopment of preterm infants. Kangaroo-mother care (KMC) is an evidence-based practice that provides short and mid-term benefits on survival, neurodevelopment, breastfeeding and the quality of mother-infant interaction. The Newborn Individualized Developmental Care and Assessment Program (NIDCAP) is an approach to environmental support and care based on reading each preterm infant's behavioral cues, and on the formulation of care recommendations aiming to enhance the infant's strengths, and supports the infant vulnerabilities. The literature to date reports numerous studies regarding the effectiveness of NIDCAP on neurodevelopmental outcome at 2 weeks corrected age (CA), and 9 months, at 1, 2, 3 and 5 years CA and school age.

REFERENCES

• Als H, Duffy FH, McAnulty G, Butler SC, Lightbody L, Kosta S, Weisenfeld NI, Robertson R, Parad RB, Ringer SA, Blickman JG, Zurakowski D, Warfield SK. NIDCAP improves brain function and structure in preterm infants with severe intrauterine growth restriction. J Perinatol. 2012;32(10):797-803.

• Charpak N, Tessier R, Ruiz JG, Hernandez JT, Uriza F, Villegas J, Nadeau L, Mercier C, Maheu F, Marin J, Cortes D, Gallego JM, Maldonado D. Twenty-year Follow-up of Kangaroo Mother Care Versus Traditional Care. Pediatrics. 2017;139(1):e20162063.

• Roué JM, Kuhn P, Lopez Maestro M, Maastrup RA, Mitanchez D, Westrup B, Sizun J. Eight principles for patient-centred and family-centred care for newborns in the neonatal intensive care unit. Arch Dis Child Fetal Neonatal Ed. 2017;102(4):F364-8.

## LECT 18

## POINT OF CARE TESTING (POCT) INNOVATION, CONNECTED HEALTH AND BEYOND – HOW DIGITAL TECHNOLOGY IS TRANSFORMING LABMED, HEALTH, AND SOCIAL CARE?

S. Bernardini

*Department of Experimental Medicine, University of Tor Vergata, Rome, Italy*

Laboratory Medicine is encompassing a new dimension: the so-called 6P Medicine: Personalized, Predictive, Preventive, Participative and care Pathway, Proof-based Medicine (EBM). It is dealing with medical, demographic, environmental, technological and social challenges. Technological advances and new tools: nano/biosensors (NBIC: Nanotech, Biotechnologies, IT, Cognitive sciences), selective robotics, Informatics, mobile health, and Internet connectivity. This will lead to a real revolution both in professional practice and the organization of laboratories with two trends: consolidation of technical facilities versus externalization to patients with increased interoperability requirements via information systems and e-health. Many factors are stimulating point of care testing (POCT) demand: Healthcare reform and patient-centered care, technological advancements (faster, easier-to-use devices), laboratory staff shortages, increasing older population and more chronic disease, rising incidence of lifestyle diseases (e.g., cardiac, diabetes, infectious diseases, etc.), increase in home-based POCT usage, increasing trend toward health care decentralization, long-term savings, rural locations with limited lab services and prevalence of diseases in developing countries. WHO has provided guidelines for those developing POCT devices (for the detection of sexually transmitted infections, such as Chlamydia and HIV): Affordable – for those at risk of infection; Sensitive – minimal false negatives; Specific – minimal false positives; User-friendly – minimal steps to carry out test; Rapid & Robust – short turnaround time and no need for refrigerated storage; Equipment-free – no complex equipment; Delivered – to end users.

The future balance between testing in laboratories and self-testing or testing at the point of care is difficult to forecast accurately. Several themes emerged from the predictions relating to POC and in particular a prominent role for mobile health (mHealth) and associated real-time medical data, the emergence of wearable analytical devices, and new types of POC analyzers (e.g., medical tricorders, diagnostic toilets, printed paper diagnostics). The potential of a smartphone for healthcare applications was quickly realized, and it has been used in many different ways. Medical apps for fitness and health have been very popular and are expected to grow almost exponentially in the next future. A device that plugged-into a smartphone to create a medical test device was the next phase of development,

and current capabilities range from glucose testing to ultrasound scanning. The expanding scope of smart devices for mHealth is exemplified by the growing number of smart wearables, i.e., clothing or accessories that have sensors integrated or weaved into their structure and that can provide health information unobtrusively during daily living. Wearables includes devices worn on the wrist (e.g., Apple Watch® for monitoring heart rate and rhythm; Embrace smart band to detect epileptic seizures), a mouthguard (e.g., measure linear and rotational acceleration, impact location and direction, and counts every impact for concussion assessment) (Prevent Biometrics 2018); clothing (e.g., CardioInsight Noninvasive 3D Mapping System) (Medtronic 2018); and different types of wireless patches (e.g., Smartcardia – vital signs temperature, pulse, blood pressure, blood oxygen levels, cardiac rhythm and cardiac electrical activity) (SmartCardia 2018) that in some cases are flexible and stretchable (e.g., e-skin with pressure and thermal sensors). It seems likely that the amount and modes of access to POC testing will increase soon. Perhaps mHealth is best positioned because of the ubiquity of smartphones, their connectivity, which underpins a future vision of widespread telehealth with data sent in real-time to a patient's medical record for assessment, and interpretation by a medical professional.

**LECT 19**

**MICROBIOME IN FORENSIC SCIENCE**

B. Benedetti*, P. Roberti*, E. d'Aloja

*Legal Medicine Unit, Department of Medical Sciences and Public Health, University and Teaching Hospital of Cagliari, Cagliari, Italy*

*\*Equally contributing Authors*

A new field of interest in microbiology with high potential in many disciplines, including forensic medicine, is microbiome. This term refers to the set of microorganisms, their genomes and environmental interactions that they establish in an ecosystem. The purpose of this review is to highlight some of the possible applications of the microbiome in forensic sciences.

POST-MORTEM INTERVAL ESTIMATION

In the phases of decomposition of a corpse, an active role of bacterial communities in their temporal succession well marked in time (so-called "microbial clock") has been described, which forms the basis for establishing the time of death, especially in peculiar conditions such as late stages of decomposition. These shifts in microbial populations are due to changes in environmental factors, such as the presence/absence of oxygen, temperature, humidity and pH and a variation in homeostasis within bacterial communities themselves. There are biological interactions which don't produce disequilibrium in bacterial communities, such as neutralism, mutualism, and commensalism; while others – such as amensalism, competition, predator-prey – may throw the equilibrium off balance [1]. Pechal et al. showed how the passage of time involves changing in microbial population not only in the number of bacteria that tends to increase but also in their taxonomic diversity which – in contrast – shows a decrease, due to a greater homogenization among taxa. The study identified in all sample sites (eyes, nose, ears, mouth, and rectum) a post-mortem interval (PMI) threshold of about 48 hours for the appearance of this modification, except for the rectum. The best prediction with the lowest error rate (± 2-5 days) is reached 20-25 days post-mortem [2].

PERSONAL IDENTIFICATION

Several studies showed potentiality to identify an individual within a population, based on salivary or skin microbiome because the microbial communities have a sufficient level of inter-individual variability. Furthermore, the microbiome of an individual tends to remain unvarying over time. The microbiota profile of the skin can be helpful to link a person to a specific object that has been touched. Consequently, this could be useful to demonstrate or exclude the presence of an individual in a crime scene when DNA is not sufficient to determine a genetic profile, leading to the creation of the so-called "microbial fingerprint". Where personal identification is not decisive, microbiome – especially of the skin – can be used for intelligence purposes, providing clues that are potentially relevant for identifying lifestyle, personal habits and even the ethnic group of an individual [3].

MICROBIOME AND HEALTH STATE

An interesting role provided by the study of the microbiome is the possibility to give information about the state of health of individuals before death. As shown by Pechal et al. [2], the taxonomic diversity of the first 48 hours after death – which still reflects the ante-mortem microbiome populations – and the biological functions investigated by KEGG ortholog (KO) pathways make possible to hypothesize that the microbial composition in the

first hours after death is a potential indicator of health conditions before death. It has been shown that an increase of biodiversity is a sign of a good general state of health, and a reduction thereof is related instead to the presence of heart pathologies. It also highlighted that the mouth represents the best sampling site. Forensic microbiome limitations and future perspectives, However, there are limitations to the study of microbiome that could be overcome by new research. First of all, the anatomical variability of the sampling site is a factor to consider, both *in vivo* and in post-mortem conditions, especially in the early stages of decomposition. In the latter case the taxonomic composition is also influenced by external factors such as humidity, temperature, and entomological fauna – both in the sense of cooperation and competition – and it varies according to the sampling method (such as swab vs. scrapes). Furthermore, data from the animal model cannot be transferred in all to humans, and few studies using human corpses have been developed because of lacking donors. An important point to consider in the development of future research will be the formulation of an appropriate consent to human subjects involved in microbiome sampling *in vivo*, capable of highlight ethical implications of giving samples potentially able to provide several personal information, such as sexual behavior, health state, etc. Interesting future studies could be based on investigations of PMI on human corpses, in which a cross-study of microbiology, entomology, and chemistry should be carried out to clarify their interactions. The creation of standardized analytical tools and protocols with a well-established error rate would allow a sufficiently accurate prediction of the PMI and application in the forensic field, with a sufficient level of scientific reliability in the court.

REFERENCES

[1] Zuñiga C, Zaramela L, Zengler K. Elucidation of complexity and prediction of interactions in microbial communities. Microb Biotechnol. 2017;10(6):1500-22.

[2] Pechal JL, Schmidt CJ, Jordan HR, Benbow ME. A large-scale survey of the postmortem human microbiome, and its potential to provide insight into the living health condition. Sci Rep. 2018;8(1):5724.

[3] Metcalf JL, Xu ZZ, Bouslimani A, Dorrestein P, Carter DO, Knight R. Microbiome Tools for Forensic Science. Trends Biotechnol. 2017;35(9):814-23.

## LECT 20

## MICROBIOME AND PROBIOTICS IN THE PERINATAL ARENA

N. Giovannini

*Department of Obstetrics and Gynecology, Policlinico di Milano, Milan, Italy*

"Non-communicable diseases" (NCD) are nearly associated with over 75% of mortality within EU. Pregnancy is one of the leading critical and crucial independent phases of NCD prevention. The "first one thousand days of life" – entailed between conception and second year of life impose a new multidisciplinary approach in order to modulate proactively future adult's epigenetic potential. Microbiome discovery – super organ that entirely engrains the somatic and genetic human kit – is being accounted as the best variable on the intricate balances comprehension within systems, organs, even topographically distant. Pregnancy, the type of birth, nutrition are decisive in defining the microbiota formation of the newborn and, then, the trajectories of cardiometabolic health, immune in adult life. The best neonatal biotic expenditure occurs through direct contact with the *Lactobacilli* along the birth canal, exclusive breastfeeding and, then, contact with family antigens (and pets!). Probiotics are living organisms that produce positive health effects in the host. Their administration during pregnancy to the woman and in the puerperium can improve the outcomes from the immunity and metabolic profile, partly preventing the "burden" of some rapidly growing last decades diseases – as atopy and cardiometabolic disorders. Preliminary studies are encouraging to move to a more refined systemic medicine of understanding (top-down system biology), freeing itself from the reductionistic paradigm.

## LECT 21

## MICROBIOTA, PROBIOTICS AND PREBIOTICS

F. Indrio, V. Dargenio

*Department of Pediatrics, Gastroenterology Unit, University of Bari, Bari, Italy*

In all vertebrates, the skin and the mucous membranes are colonized by a myriad of microorganisms, including bacteria, mainly strict anaerobes, but also viruses, protozoa, archaea, and fungi, commonly known as microbiota. In humans, more than 100 trillion microorganisms, especially bacteria, colonize the oral-gastrointestinal tract, and most of these micro-organisms reside in the colon.

Though gut microbiota comprises a wide variety of bacterial species and strains whose composition and density vary along the gastrointestinal tract, it is defined mainly by 2 bacterial phylotypes, *Bacterioidetes* (*Bacteroides*) and *Firmicutes* (*Lactobacilli*, *Clostridium*, *Enterococcus*), with *Proteobacteria* (*Enterobacteriaceae*, *E. coli*), *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* phyla present in relatively low numbers. The main contributions of the microbiota to the host include digestion and fermentation of carbohydrates, the production of vitamins (biotin, folic acid, vitamin K, vitamin B-complex group), the development of Gut-Associated Lymphoid tissues (GALT), the polarization of the immune response and the prevention of colonization by pathogens. The protective action is aimed at preventing colonization and subsequent translocation of pathogenic bacteria to the underlying bloodstream. The microbiota is essential for the development of the GALT and plays an important role in shaping the immunological response through the stimulation of the synthesis and secretion of immunoglobulin A and the production of balanced T-helper cell response. Furthermore, over the last decade, numerous scientific discoveries have highlighted that the microbiota is involved in a complex bidirectional communication system between the Central Nervous System (CNS) and the gut. The brain can alter the microbiota through modulation of intestinal secretion, permeability, and motility, removing excessive bacteria from the lumen and preventing bacterial overgrowth. On the other side, the visceral information from the gastrointestinal tract can influence the brain function [2]. In recent years, growing evidence suggests that supplementation with probiotics and prebiotics can modulate intestinal bacterial patterns by aiding the colonization of beneficial bacteria. Probiotics are defined by the FAO and the WHO as live microorganisms that, when administered in adequate amounts, confer health benefits on the host. Probiotics can suppress intestinal inflammation by preventing the overgrowth of inflammation-inducing microbes and gas-forming coliforms. They might play an important role in maintaining gut homeostasis by modulating intestinal barrier function, immunity, motility and influencing the gut-brain interaction. They have an antimicrobial effect modifying the microflora, secreting antibacterial substances, competing with pathogens to prevent their adhesion to the intestinal epithelium, competing for nutrients necessary for pathogen survival, producing an antitoxin effect and

reversing some of the consequences of infection on the intestinal epithelium, such as secretory changes and neutrophil migration. Probiotics reduce the pro-inflammatory status by immunomodulation and protecting tissues against microbial infection; their mechanism of action consists of modifying the production of cytokines in different cell populations. Probiotics are also capable of modulating the immune system and regulating the allergic immune cell response of the body. The manipulation of the microbiota through probiotic supplementation is an important and expanding field in the prevention and management of functional gastrointestinal diseases, infant colics, inflammatory bowel diseases, irritable bowel syndrome, acute diarrhea and antibiotic-associated diarrhea and Necrotizing Enterocolitis [2]. Prebiotics are defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit. Prebiotics transit undigested by human enzymes through the small intestine and eventually reach the colon where they stimulate the growth and/or the activity of beneficial bacteria, mostly *Bifidobacteria* and lactic acid bacteria. Established health benefits of some specific prebiotics relate to improving calcium absorption, modifying the glycemic index, reducing blood lipid levels, producing metabolites that influence brain function, energy, and cognition, and enhancing colonic bacterial fermentation thereby reducing gut transit time. Such physiological benefits have positive effects on osteoporosis, diabetes, and colorectal cancer, respectively [3].

## REFERENCES

[1] Krishnan S, Alden N, Lee K. Pathways and functions of gut microbiota metabolism impacting host physiology. Curr Opin Biotechnol. 2015;36:137-45.

[2] Hungin APS, Mitchell CR, Whorwell P, Mulligan C, Cole O, Agréus L, Fracasso P, Lionis C, Mendive J, Philippart de Foy JM, Seifert B, Wensaas KA, Winchester C, de Wit N; European Society for Primary Care Gastroenterology. Systematic review: probiotics in the management of lower gastrointestinal symptoms – an updated evidence-based international consensus. Aliment Pharmacol Ther. 2018;47(8):1054-70.

[3] Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017;14(8):491-502.

## LECT 22

## URINARY MICROBIOTA: IS THERE ANY DIFFERENCE IN CHILDREN WITH URINARY TRACT DISEASE?

G. Masnata[1], M. Limone[2], V. Manca[1]

[1]*Pediatric Urology and Urodynamics, G. Brotzu Hospital, Cagliari, Italy*

[2]*School of Pediatrics, University of Cagliari, Cagliari, Italy*

Urinary tract was considered to be sterile in normal conditions since standard urine culture has been introduced in clinical practice. The Human Microbiome Project, launched in 2008, did not include the bladder and urinary tract because of the "sterile urine" dogma. With the improvement in cultivation and technologies able to accurately detect nucleic acids from bacteria, it is clear that a variable microbiome is present in the bladder. "Uncultivated bacteria", defined as bacteria not detectable in standard urine culture, are common in urine samples from healthy individuals regardless of the method used for sample collection [1]. The techniques in use to identify resident bladder microbiota are 16S rRNA sequencing and Expanded Quantitative Urine Culture (EQUC). It has been demonstrated that many of the organisms identified in urine by 16S rRNA gene sequencing are cultivable with EQUC protocol. These methods confirmed that there are live bacteria with a negative standard urine culture even in the bladders of pregnant women. Considering normal urinary microbiota is present, the classic (standard) definition of asymptomatic bacteriuria and Urinary Tract Infection (UTI) has to be challenged. Asymptomatic bacteriuria is universal and not a rare event. An association between composition and decreased diversity of the urinary microbiota has been made with clinical UTI and host antimicrobial peptides (AMPs) even before clinical diagnosis. Communities of functional bacteria could, therefore, increase the risk of or give protection against recurrent UTIs. In children with recurrent febrile UTI or Vesicoureteral Reflux with or without renal scarring *Dorea-* and *Escherichia*-dominant microbiota was found more frequently than in healthy controls, where *Prevotella-* and *Lactobacillus*-dominant clusters were more common. Stable colonization seems to be a fundamental step in establishing a connection between microbiota and urinary tract disease. Particular patterns have been identified in patients with Neurogenic Bladder (NB) compared to healthy subjects with significant differences in *Enterobacteriaceae* and *Lactobacillaceae* presence. The genus *Actinobaculum* was present in specific NB microbiota, it was not detected by cultivation, and it was strongly associated with pyuria [2]. Neurogenic bladder is often associated with neurogenic bowel

dysfunction depending on the cause and severity of the underlying neurologic disease. The frequent presence of *Enterobacteriaceae* in the urinary tract of these patients may be explained because fecal incontinence or bowel care regimes may induce colonization of perineum by fecal flora. Clinical experience suggests that patients with neurogenic bladder have frequent "positive" standard urine culture without a concurrent symptomatic UTI, thus not requiring antimicrobial therapy. Bacteria commonly cultivated in these patients are "standard" uropathogens easy to detect, while in healthy subjects microbiome seems more diverse and not visible in the routine urine culture. Bacteria have been found in cancerous kidneys, but could chronic inflammation associated with a particular resident microbiota also lead to urinary symptoms? Examining other conditions, Bladder and Bowel Dysfunction (BBD) is frequent in children (15-20%) and describes many Lower Urinary Tract Symptoms (LUTS) such as increased urinary frequency urgency, daytime incontinence, enuresis, dysuria, voiding postponement and urinary retention. Overactive bladder (OAB), underactive bladder and dysfunctional voiding can be part of this clinical scenario. It is clear that a condition which alters bladder filling or emptying such as BBD, NB, or Congenital Abnormalities of Kidneys and Urinary Tract (CAKUT), can increase the probability of having UTIs, but what is the role of the microbiota? (**Fig. 1**) Might normal microbiota have a protective role because of its interaction
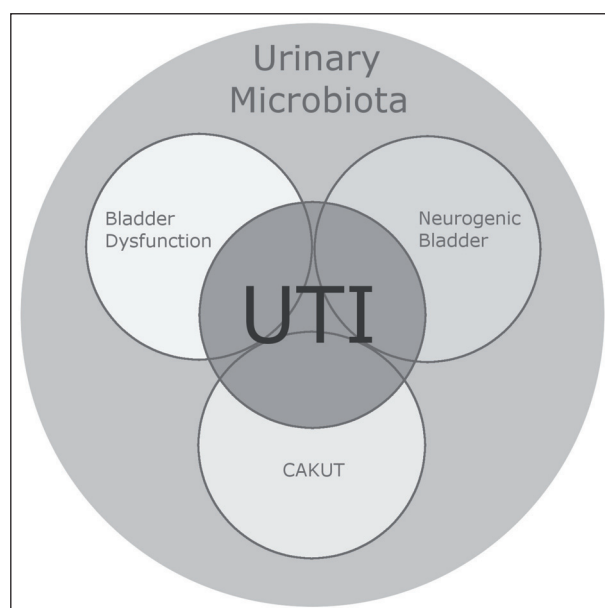


**Figure 1 (LECT 22).** The possible relationship between Urinary Tract Infection (UTI), diseases of the urinary tract and bladder microbiota.

with urothelium? Might pathological changes in the resident population affect bladder function? Do urinary physical characteristics such as pH, electrolytes or density affect urinary microbiota? The answer is probably yes, but we still do not know how. In adults with urge incontinence, with or without detrusor overactivity, *Gardnerella*, *Staphylococcus*, *Streptococcus*, *Actinomyces*, *Aerococcus*, *Corynebacterium*, *Oligella* are present in the urinary tract, while *Lactobacillus*, *Prevotella*, and others are more commonly reported in healthy patients. Learning urinary microbiota's natural history since its acquisition and studying its variations during growth in the pediatric population may be relevant [3]. Even if publications about urinary microbiome are rapidly increasing, we lack understanding about its natural evolution and its association with disease in children. Comprehending the behavior of bacteria in urine could offer tools to manipulate them for the benefit of children with urinary tract diseases, affecting renal outcomes and reducing traditional antimicrobial therapy use.

REFERENCES

[1] Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, Mueller ER, Schreckenberger P, Dong Q, Nelson DE, Brubaker L. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol. 2012;50(4):1376-83.

[2] Groah SL, Pérez-Losada M, Caldovic L, Ljungberg IH, Sprague BM, Castro-Nallar E, Chandel NJ, Hsieh MH, Pohl HG. Redefining Healthy Urine: A Cross-Sectional Exploratory Metagenomic Study of People With and Without Bladder Dysfunction. J Urol. 2016;196(2):579-87.

[3] Gerber D, Forster CS, Hsieh M. The Role of the Genitourinary Microbiome in Pediatric Urology: a Review. Curr Urol Rep. 2018;19(1):13.

**LECT 23**

**METABOLOME AND MICROBIOME IN AUTISM: THE LAST DATA**

M. Mussap[1], V. Fanos[1,2]

[1]Department of Surgery, University of Cagliari, Cagliari, Italy

[2]Neonatal Intensive Care Unit, Neonatal Pathology, and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy

Autism Spectrum Disorder (ASD) is a set of neurodevelopmental impairments clinically marked by social deficits and unusual sensory-motor and repetitive stereotypic behaviors [1]. Several comorbid conditions are often associated with autism. The origin of ASD cannot be referred to a single, unique etiological factor; rather, ADS derives from the combination of genetic, environmental and biological risk factors mainly operating during pregnancy and perinatal age. On the one hand, *de novo* mutations, common variants, and short nucleotide polymorphisms account for roughly 50% of the disorder [2, 3]. On the other hand, prenatal and neonatal chemical exposure, drug administration, infections, and metabolic imbalances are implicated in the etiology and pathogenesis of ASD. Approximately 50% of children with ASD suffer from at least one gastrointestinal (GI) symptom [4]; constipation and diarrhea are those most frequently observed. Additional detectable GI symptoms in ASD are abdominal pain, gaseousness and flatulence [5]. Notably, the severity of GI symptoms is associated with that of autism symptoms [6]. Accumulating evidence indicates the key role of gut microbiota in the pathogenesis of ASD [7]. Gut microbiota is composed of approximately $10^{14}$ prokaryotic organisms represented by thousands of species living in a symbiotic manner, with overall biomass of > 1 kg [8]. The colonization of the infant's gut takes place prenatally by the so-called 'vertical transmission *in utero*' even though the mechanism(s) and the sequence of the transgenerational transmission of microbes (from the mother to the fetus) remain still unclear [9]. What is known is that microbes colonize the placenta at the time of placental decidua implantation; subsequently, amniotic fluid and cord blood are also colonized by maternal bacteria coming from oral cavity, gut, vagina and endometrial uterine tissue [10]. As a result, gut microbiota composition at birth depends on maternal and postnatal factors, such as changes in maternal gut microbiome during pregnancy, maternal vaginal infection, antibiotic and drug exposure, delivery mode, host genetics, breastfeeding, diet [11]. The gut microbiota of infants with ASD significantly differs from that of neurotypical individuals, leading to a perturbation in microbial homeostasis, namely dysbiosis. When compared with that of neurotypical children, the gut microbiota of children with ASD is characterized by lower levels of *Bifidobacterium* and *Firmicutes* and higher levels of *Lactobacillus*, *Bacteroidetes*, *Sarcina*, *Desulfovibrio*, *Caloramator*, *Sutterella* and mostly *Clostridium* [12]. Indeed, in ASD children *Clostridium histolyticum* group (*Clostridium* clusters II and I) has been found significantly increased in fecal samples [13]. The persistence of *Clostridium* spores after the discontinuance of the antibiotic therapy may be a key factor for the relapse of GI symptoms and the increased prevalence of ASD [14]. However,

this hypothesis should be confirmed by further studies. Interestingly, in autistic children exhibiting GI symptoms further genera such as *Prevotella*, *Coprococcus*, and unclassified *Veillonellaceae* are present in lower abundance [15]. It remains still unclear whether GI symptoms cause dysbiosis in ASD or whether it contributes to the manifestation of GI symptoms. The potential relationship between autism and gut microbiota takes its origin from the influence of dysbiosis on the gut-brain axis, a bidirectional communication system by which the two organs modulate their functions to each other. Gut microbiota is crucial for brain development and behavior: in a hierarchy of reflexes, several multiple parallel systems and circuits orchestrate the gut-brain axis, including the two branches of the autonomic nervous system (ANS); the enteric nervous system, recently classified as the third branch of the ANS and containing between 200 and 600 millions neurons; the neuroendocrine system; the neuroimmune signaling network; the release of toxins and neurotransmitters by gut bacteria [16]. The observation that immune cell pathways, both within the central nervous system (CNS) and in peripheral lymphatic tissues, are strongly implicated in mediating microbial modulation of brain function and behavior supports the primary role of the immune system for all these complex interactions [17]. Albeit no absolute gut microbial signature has been identified in ASD, perturbations in the gut microflora ecosystem promote inflammation that, in turn, leads to alterations in gut motility by cytokine-mediated vagal activation of the hypothalamus and related limbic brain regions, and to the increase in mucosal permeability, referred to as a 'leaky gut'. With the loss of the mucosal integrity, some metabolites such as toxins, neurotransmitters, and products of bacterial fermentation of host dietary resistant starch and non-starch polysaccharides move easily into the bloodstream and may cross the blood-brain barrier reaching the CNS and interfering in neuroactivity. Microbial-derived bioactive neuropeptides, such as serotonin (5-hydroxytryptamine), gamma-aminobutyric acid (GABA), 5-hydroxytryptamine acid (5-HT), nor-epinephrine, dopamine, and acetylcholine might act either by reaching their respective receptors or eliciting host response via long-distance neural signaling. In addition, the bottom-up modulation of gut microbiota – CNS is mediated by short-chain fatty acids (SCFAs), phenol compounds, secondary bile acids, and free amino acids (i.e., Tryptophan). Signals are propagated by the interaction between these molecules with enteroendocrine and enterochromaffin cells, and with the mucosal immune system. Among SCFAs, acetic acid, propionic acid (PPA), butyrate, isobutyric acid, valeric acid, and isovaleric acid play a critical role in patients with ASD. In particular, the prevalence of *Clostridium* and *Desulfovibrio spp.* entails an increase in PPA concentration with effects on cognition and social behavior miming ASD. PPA is beneficial at appropriate levels mainly by exerting an influence on mitochondrial and lipid metabolism; however, when abnormally elevated, PPA reinforces its direct effects on GI physiology and ultimately interferes with GI activity leading to some negative effects on health and behavior. Moreover, abnormal abundance in PPA is deleterious for CNS because of the blockage of fatty acid oxidation, the deficiency of energy carriers and, ultimately, the gap junction closing. Given that the dramatic increase in ASD prevalence is mainly due to environmental factors, a paradigm shift in the diagnosis and management of the disease is an immediate priority. It should be based on the system biology approach, embracing genetics, epigenetics, transcriptomics, proteomics, and metabolomics in order to reveal the complex pathogenesis of the disease, to establish a very early diagnosis and to initiate an early treatment [18]. In this context, metabolomics represents an extraordinary tool for achieving this objective: it allows to assess the metabolic phenotype, the interactions between metabolites and alterations in metabolic pathways leading to the onset of the disease [19].

## REFERENCES

[1] Lord C, Elsabbagh M, Baird G, Veenstra-Vanderweele J. Autism spectrum disorder. Lancet. 2018;392(10146):508-20.

[2] Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, Mahajan M, Manaa D, Pawitan Y, Reichert J, Ripke S, Sandin S, Sklar P, Svantesson O, Reichenberg A, Hultman CM, Devlin B, Roeder K, Buxbaum JD. Most genetic risk for autism resides with common variation. Nat Genet. 2014;46:881-5.

[3] Iossifov I, Levy D, Allen J, Ye K, Ronemus M, Lee YH, Yamrom B, Wigler M. Low load for disruptive mutations in autism genes and their biased transmission. Proc Natl Acad Sci USA. 2015;112(41):E5600-7.

[4] Holingue C, Newill C, Lee LC, Pasricha PJ, Daniele Fallin M. Gastrointestinal symptoms in autism spectrum disorder: A review of the literature on ascertainment and prevalence. Autism Res. 2018;11:24-36.

[5] Chaidez V, Hansen RL, Hertz-Picciotto I. Gastrointestinal problems in children with autism, developmental delays or typical development. J Autism Dev Disord. 2014;44:1117-27.

[6] Gorrindo P, Williams KC, Lee EB, Walker LS, McGrew SG, Levitt P. Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. Autism Res. 2012;5:101-8.

[7] Vuong HE, Hsiao EY. Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder. Biol Psychiatry. 2017;81(5):411-23.

[8] Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology. 2009;136:65-80.

[9] Walker RW, Clemente JC, Peter I, Loos RJF. The prenatal gut microbiome: are we colonized with bacteria in utero? Pediatr Obes. 2017;12(Suppl 1):3-17.

[10] Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonization may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep. 2016;6:23129.

[11] Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. Nat Med. 2016;22(7):713-22.

[12] Li Q, Han Y, Dy ABC, Hagerman RJ. The Gut Microbiota and Autism Spectrum Disorders. Front Cell Neurosci. 2017;11:120.

[13] Parracho HM, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol. 2005;54(Pt 10):987-91.

[14] Cao X, Lin P, Jiang P, Li C. Characteristics of the gastrointestinal microbiome in children with autism spectrum disorder: a systematic review. Shanghai Arch Psychiatry. 2013;25:342-53.

[15] Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, Krajmalnik-Brown R. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One. 2013;8(7):e68322.

[16] Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The Central Nervous System and the Gut Microbiome. Cell. 2016;167(4):915-32.

[17] Fung TC, Olson CA, Hsiao EY. Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci. 2017;20:145-55.

[18] Fanos V, Noto A, Mussap M. The juniper bush of autism spectrum disorder (ASD): metabolomics, microbiomics, acetaminophen. What else? J Pediatr Neonat Individual Med. 2018;7(2):e070205.

[19] Mussap M, Noto A, Fanos V. Metabolomics of autism spectrum disorders: early insights regarding mammalian-microbial cometabolites. Expert Rev Mol Diagn. 2016;16(8):869-81.

## LECT 24

## MODE OF CHILDBIRTH AND EFFECT ON NEONATAL AND MATERNAL HEALTH AND DISEASES

A. Ragusa[1], S. Rugolotto[2], A. Svelato[1], C. Incarnato[1]

[1]*Maternal and Neonatal Department, Massa Carrara Apuane Hospital, Massa Carrara, Italy*

[2]*Division of Pediatrics, Rovigo Hospital, Rovigo, Italy*

We know that neonatal and maternal health depends on the interaction between the environment and DNA itself. Even though DNA sequence does not change over the years, some genetic characteristics of the human being can be affected by silencing or activation of some nucleotide sequences. In one word, today epigenetics does matter much more than we used to think in 1942 when Waddington (1905-1975) used for the first time the word "epigenetics". The environment can modify the activation of DNA sequence, with changes in protein productions and the phenotype.

A good example of the close relationship between environment, genotype and phenotype is the so-called Small for Gestational Ages (SGA) newborns or IUGR (Intra Uterine Growth Restriction) fetuses. These infants have a low birth weight because the uterine environment was very poor, and did not provide so much energy for different reasons. Only those fetuses who could adjust to this hostile environment can survive. After birth, if these newborns grow up in an environment rich in energy, they develop cardiovascular disease in a higher percentage than the newborns with appropriate birth weight. Also, maternal weight before gestation affects the newborn who adjusts himself to this environment. One more example of how the environment can also affect complex characters like behavior is the following about mice. Mice with good parenting/nursing skills give birth to mice with good parenting skills. Mice with bad parenting skills give birth to mice with bad parenting skills. When one of the mice with bad parenting after birth are grown by mice with good parenting skills, they acquire new good parenting skills, showing that DNA methylation can change after birth if they are born in a different environment. More examples of epigenetics are within the mode of delivery and drugs administered during labor. Even the modalities of assistance at the time of delivery can have long-term consequences, for example cord clamp timing at birth, either delayed (> 180 sec) or early (< 10 sec) affect motor and social skills at 4 years of age, mainly in males, showing that boys with delayed cord clamping have better motor and social performances.

Fentanyl and synthetic oxytocin administration during labor decrease neonatal suction during the first hour of breastfeeding and the probability of a successful skin to skin phase.

When synthetic oxytocin was given to mice perinatally an "hormone imprinting" phenomenon was observed changing their social and sexual behavior. Hormone imprinting theory is a good scientific basis, for explaining the permanence of some biological effects at a considerable distance from birth. Cesarean section has been proved to increase maternal death, maternal morbidity, peripartum hysterectomy, hospital admissions during puerperium, amniotic fluid embolism, placental malposition during the

following pregnancies. Therefore, the cesarean section has always to be cautiously considered. If we consider neonatal health, cesarean section increases perinatal iatrogenic lacerations, respiratory disorders, more difficult cardiovascular adaptation, lower breastfeeding prevalence, anemia, asthma, laryngitis, gastroenteritis, ulcerative colitis, coeliac disease, lower respiratory tract infections, idiopathic juvenile arthritis, death, obesity, type 1 diabetes, metabolic syndrome, cancer, leukemia. One of the most important factors to explain the aforementioned diseases is probably the lack of "bacterial contamination" through the birth canal. However, this mechanism cannot be the only one to act, as, for example, in cesarean section newborns, who have been exposed to labor, many of the previously described negative effects, which occur in adult life, are much less pronounced. We know that almost 95% of the cells of the human body is made of non-human cells, mainly microbes. These microorganisms constitute the so-called "microbiome". Microorganisms are important for the development of the human immune system. Lacking a normal microbiome, or having different types of bacteria as it happens during cesarean section, might affect the normal development of the human immune system. This is why when a cesarean section is done researchers try to see if adding specific bacteria to the neonatal nutrition might improve the building of neonatal microbiome and reduce some of the illnesses written above.

## REFERENCES

• Barker DJ, Gluckman PD, Robinson JS. Conference report: fetal origins of adult disease – report of the First International Study Group, Sydney, 29-30 October 1994. Placenta. 1995;16(3):317-20.

• Fall CH, Osmond C, Barker DJ, Clark PM, Hales CN, Stirling Y, Meade TW. Fetal and infant growth and cardiovascular risk factors in women. BMJ. 1995;310(6977):428-32.

• Godfrey KM, Barker DJ. Maternal nutrition in relation to fetal and placental growth. Eur J Obstet Gynecol Reprod Biol. 1995;61(1):15-22.

• Martyn CN, Barker DJ, Jespersen S, Greenwald S, Osmond C, Berry C. Growth in utero, adult blood pressure, and arterial compliance. Br Heart J. 1995;73(2):116-21.

• Waddington CH. Organisers and Genes. Cambridge: Cambridge University Press, 1940.

## LECT 25

## PRETERM LABOR FROM THE SOCIETY FOR MATERNAL-FETAL MEDICINE (SMFM) POINT OF VIEW: AN UPDATE

T. Frusca

*Obstetrics and Gynecology Unit, University of Parma, Parma, Italy*

In recent years, many efforts have been made by Maternal-Fetal Medicine (MFM) researchers in order to better define the diagnosis of preterm labor (PTL), to identify a population at risk of PTL and preventive strategies. Given the importance of correctly identifying a real PTL as a strategy to limit the steroids for prevention of RDS only to patients who will deliver within 7-10 days, the approach to the diagnosis is based not only on symptoms (contractions) and evidence of cervical dilatation, but on the measurement of cervical length by transvaginal ultrasound. In the majority of European Ob Unit the evidence of contractions and cervical length < 20 mm is considered an indication for tocolysis and steroids, while with a cervical length between 20 and 29 mm is considered an indication for further testing with fibronectin and only patients with positive fibronectin test will be candidate for steroids [1, 2]. Since the publication of Iams et al. on *NEJM* [3], who correlated cervical length at 20-24 weeks to the incidence of preterm labor more than 100 publications appeared in the literature aimed to better define the population at risk, given that the majority of PTL occurs in a patient with no clinical risk factors. A universal transvaginal cervical length screening program has been advocated by many Authors [4] and suggested by the Society of Maternal and Fetal Medicine in 2012, although universal screening is not being formally implemented, as many Societies have not accepted these recommendations, measurement of US cervical length is generally performed by many physicians during the routine US scan at 20 weeks. Differences in the role of screening have been pointed out regarding twins as far as cervical length correlated to PTL and preventive strategies and we will limit our review on singleton pregnancies.

Controversies still exist about preventive strategies with progesterone even in singleton, whether intramuscular versus vaginal and which population will benefit from this prevention (only those with previous PTL?). Moreover, not only progesterone but also cervical cerclage and more recently Arabin pessary have been suggested as preventive strategies in patients at risk either for previous history of PTL or identification of short cervix at US.

Recently an individual meta-analysis showed that Vaginal progesterone significantly reduces the risk of preterm birth < 33 weeks of gestation in women with a singleton gestation and a cervical length < 25 mm, regardless of their history of spontaneous

preterm birth [5]. The general policy advocated by most obstetricians is to give progesterone in patients with previous history starting from the first trimester and to suggest vaginal progesterone in patients with short cervix at 20-24 weeks of gestation as preventive strategies, the use of progesterone for maintenance therapy after an episode of threatened PTL is not considered useful by the few data reported in literature [6]. In singleton gestations without prior spontaneous preterm birth but with TVS-CL < 25 mm in the second trimester, cerclage does not seem to prevent preterm delivery or improve neonatal outcome; however, a subgroup analysis found that among women with a very short cervical length (cervical length < 10 mm) and with no previous history, cerclage was associated with a significant decrease in the risk of preterm birth < 35 weeks of gestation (RR, 0.68; 95% CI, 0.47-0.98). The data are limited and no consensus at the moment exists about this strategy [7]. Controversies also exist about the possible preventive role of Arabin pessary in patients at risk for short cervical length as a recent metanalysis taking into account all the publications on this subject was not able to confirm previous data published in singleton pregnancies [8].

Our suggestions from current literature are:

- to perform universal transvaginal cervical length screening at 18 and 24 weeks of gestation in women with a singleton gestation and to offer vaginal progesterone to those with a cervical length < 25 mm, regardless of the history of spontaneous preterm birth;
- to consider cerclage in women with singleton gestation, history of spontaneous preterm birth, and a cervical length < 25 mm;
- to discuss with patients, the choice of cerclage, if CV L is very low (< 10 mm) even in the absence of history;
- to use TV US cervical length and fibronectin in the triage of patients with symptoms of contractions.

Role of the pessary, although potentially promising, is still under evaluation as well as identification of risk and preventive strategies in twin pregnancies.

## REFERENCES

[1] Berghella V, Saccone G. Fetal fibronectin testing for prevention of preterm birth in singleton pregnancies with threatened preterm labor: a systematic review and metaanalysis of randomized controlled trials. Am J Obstet Gynecol. 2016;215(4):431-8.

[2] Alfirevic Z, Allen-Coward H, Molina F, Vinuesa CP, Nicolaides K. Targeted therapy for threatened preterm labor based on sonographic measurement of the cervical length: a randomized controlled trial. Ultrasound Obstet Gynecol. 2007;29(1):47-50.

[3] Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, Thom E, McNellis D, Copper RL, Johnson F, Roberts JM. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med. 1996;334(9):567-72.

[4] Orzechowski KM, Boelig RC, Baxter JK, Berghella V. A universal transvaginal cervical length screening program for preterm birth prevention. Obstet Gynecol. 2014;124(3):520-5.

[5] Romero R, Conde-Agudelo A, Da Fonseca E, O'Brien JM, Cetingoz E, Creasy GW, Hassan SS, Nicolaides KH. Vaginal progesterone for preventing preterm birth and adverse perinatal outcomes in singleton gestations with a short cervix: a meta-analysis of individual patient data. Am J Obstet Gynecol. 2018;218(2):161-80.

[6] Suhag A, Saccone G, Berghella V. Vaginal progesterone for maintenance tocolysis: a systematic review and metaanalysis of randomized trials. Am J Obstet Gynecol. 2015;213(4):479-87.

[7] Berghella V, Ciardulli A, Rust OA, To M, Otsuki K, Althuisius S, Nicolaides KH, Roman A, Saccone G. Cerclage for sonographic short cervix in singleton gestations without prior spontaneous preterm birth: systematic review and meta-analysis of randomized controlled trials using individual patient-level data. Ultrasound Obstet Gynecol. 2017;50(5):569-77.

[8] Saccone G, Ciardulli A, Xodo S, Dugoff L, Ludmir J, Pagani G, Visentin S, Gizzo S, Volpe N, Maruotti GM, Rizzo G, Martinelli P, Berghella V. Cervical Pessary for Preventing Preterm Birth in Singleton Pregnancies With Short Cervical Length: A Systematic Review and Meta-analysis. J Ultrasound Med. 2017;36(8):1535-43.

## LECT 26

## BIG DATA, ULTRASOUND, AND NEONATAL RESPIRATORY DISTRESS: ANYTHING IN COMMON?

F. Raimondi

*Division of Neonatology, Department of Translational Medical Sciences, Università "Federico II" di Napoli, Naples, Italy*

The powerful and fast development of Computer Science and Information Technology is making a broad impact in the medical world. Medical institutions are building very large patients databases that provide the background for clinical research purposes. The digital revolution is bringing together Radiology, Obstetrics, and Neonatology to provide innovative solutions to a key clinical problem: the diagnosis and monitoring of neonatal respiratory distress.

## ULTRASOUND FOR NEONATAL RESPIRATORY DISTRESS

Lung ultrasound (LUS) is a novel imaging technique that has shown initially promising results in adult emergency medicine. An ultrasound beam crossing

the chest generates images from the superficial structures (the subcutaneous and muscular planes, the ribs and the pleura) and artifactual images related to the air-to-fluid ratio of the lung. Ultrasound profiles combining real images and artifacts have been found to have a good to excellent diagnostic accuracy for several adult respiratory diseases [1]. Compared to the conventional radiogram and CT, normally used for this kind of diagnostics, lung ultrasound is radiation-free, repeatable at the patient's bedside and inexpensive. Neonatal publications are now available to describe the ultrasound appearance of congenital pulmonary airway malformations, pneumonia, atelectasis, transient tachypnea of the neonate (TTN) and respiratory distress syndrome (RDS) [2]. A prospective international study has found an absolute diagnostic accuracy of ultrasound for detecting neonatal tension pneumothorax.

Besides the purely descriptive approach, LUS can monitor the changes in neonatal respiratory conditions. This functional or dynamic strategy has been applied to a cohort of 154 term or near-term neonates who were serially by LUS soon after birth. It was then described the transition to an air-filled lung, and it was demonstrated that the presence of substantial amount of fluid in the lung beyond the first two hours of life was significantly related to the subsequent need of respiratory support. A semiquantitative, functional approach has been applied to LUS by introducing an aeration score. This strategy has allowed the use of LUS to predict the failure of CPAP and the need for surfactant administration in neonates with RDS.

## INTRODUCTION TO MACHINE LEARNING

Machine Learning (ML) is part of the vast field of artificial intelligence dealing with algorithm construction to predict the data. Modern computer technology has allowed the application of ML to many different domains (from financial data analysis to face recognition) sharing the need for large databases. ML can analyze an ultrasound image dataset. The main current strategies include supervised ML, where a classifier is trained on a database of ultrasound images labeled with desired classification output. Once trained, the classifier can be used to label new images correctly. As an example, this approach has been used to distinguish malignant tumors from benign lesions; unsupervised ML, where no preset labels are given and the algorithm will find similarities in data [3].

## PUTTING ALL TOGETHER

Computer-aided diagnosis is one important application of ML to LUS. Obstetricians were the

first to apply these technologies to predict neonatal respiratory conditions from fetal lung data.

In a monocentric investigation based on 900 fetal images, Bonet-Carne et al. were able to predict respiratory morbidity on a cohort of 144 terms and near-term neonates with 86% sensitivity and 87% specificity. The same group later coordinated a prospective, international study to evaluate the performance of the ultrasound texture analysis test to predict neonatal respiratory morbidity with comparable results. With a similar, supervised ML approach, our group has recently studied a cohort of 75 preterm neonates with different causes of respiratory distress building a database of 600 images. We showed a significant correlation between the computer-assisted analysis and the degree of respiratory distress as expressed by validated blood gases indexes (i.e., the oxygenation ratio and the alveolar-arterial oxygen gradient).

## CONCLUSIONS

Artificial intelligence applications to computer-aided diagnosis are exploding. They will likely contribute to change our diagnostic and monitoring standards of neonatal respiratory distress.

## REFERENCES

[1] Volpicelli G, Elbarbary M, Blaivas M, Lichtenstein DA, Mathis G, Kirkpatrick AW, Melniker L, Gargani L, Noble VE, Via G, Dean A, Tsung JW, Soldati G, Copetti R, Bouhemad B, Reissig A, Agricola E, Rouby JJ, Arbelot C, Liteplo A, Sargsyan A, Silva F, Hoppmann R, Breitkreutz R, Seibel A, Neri L, Storti E, Petrovic T; International Liaison Committee on Lung Ultrasound (ILC-LUS) for International Consensus Conference on Lung Ultrasound (ICC-LUS). International evidence-based recommendations for point-of-care lung ultrasound. Intensive Care Med. 2012;38(4):577-91.

[2] Raimondi F, Yousef N, Migliaro F, Capasso L, De Luca D. Point-of-care lung ultrasound in neonatology: classification into descriptive and functional applications. Pediatr Res. 2018 Jul 20. [Epub ahead of print].

[3] Brattain LJ, Telfer BA, Dhyani M, Grajo JR, Samir AE. Machine learning for medical ultrasound: status, methods, and future opportunities. Abdom Radiol (NY). 2018;43(4):786-99.

## LECT 27

## GESTATIONAL DIABETES

F. Mecacci

*High-Risk Pregnancy Unit, Women and Children Health Department, Careggi University Hospital, Florence, Italy*

## INTRODUCTION

Hyperglycemia is one of the most common pregnancy complications; it occurs in one in six pregnant women worldwide, and the majority

of cases are due to gestational diabetes mellitus (GDM). Hyperglycemia in pregnancy is associated with higher incidence of maternal complication (hypertensive disorders, cesarean section, birth trauma and subsequent development of type 2 diabetes) but also perinatal morbidity and mortality. Long-term data show the importance of *in utero* imprinting in increasing the risk of diabetes and cardio-metabolic disorders in the offspring of mothers with HIP and increased maternal risk of future diabetes and cardiovascular disorders [1].

## FETAL COMPLICATIONS

Hyperglycemia during the second and third trimester 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. 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. 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 severe 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. Stillbirth is another well-known complication of diabetic pregnancies; 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.

## NEONATAL COMPLICATIONS

Infants of diabetic mothers are at increased risk for mortality and morbidity compared with infants born to nondiabetic mothers. Preterm delivery both spontaneous and medically indicated occurs more frequently in diabetic than nondiabetic 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. Metabolic complications (hypoglycemia, hypocalcaemia, 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 percent have respiratory distress or signs of reduced cardiac output or heart failure [2].

## 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. While the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study demonstrated that fetal growth and neonatal morbidity are related to maternal glucose levels, the effect of maternal glycemic levels on long-term offspring outcome (childhood obesity and fat mass) is less studied. A recent follow-up study of the offspring showed that maternal glycemia was associated with anthropometric measures of obesity (total skinfolds and subscapular/triceps ratio) and not with childhood BMI [3]. The HAPO Follow-up Study is in progress, it collects data of from the cohort of women and their offspring who participated in the HAPO Study 8-12 years later to determine associations of maternal glucose levels during pregnancy with measures of adiposity in offspring and the associations of maternal glucose levels during pregnancy and maternal metabolic disorders 8-12 years later. Consequently, addressing hyperglycemia in pregnancy and a tight maternal glycemic control can prevent not only short-term fetal and neonatal morbidity but also long-term complications and is, therefore, a key to reducing the burden of non-communicable diseases. With this aim, in 2015 FIGO published evidence-based guidelines on the diagnosis, management, and care of women with gestational diabetes and develop strategies to disseminate and implement GDM screening and managing [1].

## REFERENCES

• Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, Cabero Roura L, McIntyre HD, Morris JL, Divakar H. The International Federation of

Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. Int J Gynaecol Obstet. 2015;131(Suppl 3):S173-211.

• Weindling MA. Offspring of diabetic pregnancy: short-term outcomes. Gynaecol Obstet. 2015;131(Suppl 3):S173-211.

• Landon MB, Mele L, Varner MW, Casey BM, Reddy UM, Wapner RJ, Rouse DJ, Tita ATN, Thorp JM, Chien EK, Saade G, Grobman W, Blackwell SC, VanDorsten JP; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units (MFMU) Network. The relationship of maternal glycemia to childhood obesity and metabolic dysfunction. J Matern Fetal Neonatal Med. 2018;18:1-9.

**LECT 28**

## GESTATIONAL DIABETES FROM A PEDIATRIC VIEWPOINT

R. Antonucci, C. Locci

*Pediatric Clinic, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy*

### INTRODUCTION

Gestational Diabetes Mellitus (GDM) is one of the most common pregnancy disorders, being present in approximately 2-6% of all pregnancies in Europe [1]. The WHO defines GDM as either diabetes or glucose intolerance that is detected for the first time during pregnancy [2]. During pregnancy, fetal growth and development require an increasing maternal-to-fetal glucose supply. Consequently, the mother develops a condition of insulin resistance in order to ensure a positive maternal-fetal gradient of glucose. In early pregnancy, insulin resistance does not substantially change. From the mid-second trimester onwards, in response to increasing placental mammasomatotropin, the requirement for insulin increases by 2.0-2.5-fold in order to maintain maternal euglycemia. GDM occurs when maximum insulin secretion cannot match the level of insulin resistance. When this condition occurs, both the mother and fetus are exposed to hyperglycemia and its detrimental effects. Several risk factors have been implicated in the development of GDM, including maternal obesity, advanced maternal age, ethnic background, family history of type 2 diabetes (T2DM) and prior GDM. Previous adverse pregnancy outcome, previous macrosomia, glycosuria, polyhydramios, large fetus in present pregnancy, nutritional imbalances, and multiple pregnancies may also increase the risk for GDM [2].

### SCREENING, DIAGNOSIS, AND MANAGEMENT OF GDM

Detection of GDM lies on screening, followed by diagnostic tests, as necessary. Screening can be selective, based on risk stratification, or universal. Approaches for screening tests comprise either fasting glucose, random glucose or, more often, glucose challenge test. In 2010, the International Association of Diabetes and Pregnancy Study Groups recommended GDM screening for all women at 24-28 weeks' gestation. Timely testing enables the obstetrician to evaluate glucose tolerance and allows treatment to be started quickly. Once a diagnosis of GDM has been made, close perinatal surveillance is warranted. The management of women with GDM, during the prenatal period, is aimed at reducing fetal and maternal morbidity and mortality, and consists of medical nutrition therapy and weight management, physical exercise, self-monitoring of blood glucose and pharmacological treatment if required. The options for pharmacological therapy are insulin, which is the first choice, or oral agents.

### MATERNAL MORBIDITY

The adverse maternal outcome can be short-term (preeclampsia, hypertension and increased risk of a cesarean section) and especially long-term (T2DM and metabolic syndrome later in life) [3].

Although maternal glucose tolerance frequently normalizes shortly after pregnancy, women with GDM are at increased risk of developing T2DM later in life. Studies have documented that women are more than 7 times as likely to develop T2DM after GDM, and that about 50% of mothers with GDM will develop T2DM within 10 years, thus making GDM one of the strongest predictors of T2DM. In women with previous GDM, development of T2DM can be prevented or delayed by lifestyle interventions and/or medical treatment [1].

### OFFSPRING MORBIDITY

Short-term morbidity. The infants of GDM women have an increased risk for stillbirth, preterm birth (PTB), excessive fetal growth, birth trauma as well as various metabolic and electrolyte disturbances [2]. The relationship between hyperglycemia during pregnancy and PTB is controversial. Some authors did not report an increased risk for PTB among women with GDM, and others documented that GDM is itself a risk factor for prematurity. Fetal hyperinsulinemia, in response to the high placental transfer of glucose from the mother, results in increased fat and fetal protein stores leading to macrosomia [2] and its perinatal complications including cesarean section, shoulder dystocia and

brachial plexus injury [3]. Neonatal hypoglycemia occurs in 25% of neonates and is influenced by the maternal glycemic control at the time of delivery [3]. It results from the fetal hyperinsulinemia and can cause severe central nervous system and cardiopulmonary disturbances [2]. Neonatal hypocalcemia is reported in 10-20% of infants born to GDM mothers. It is related to the severity of maternal diabetes [3] and has little clinical importance [2].

Neonatal polycythemia is found in 5% of infants of GDM mothers and may be a contributor to neonatal hyperbilirubinemia [2, 3]. In infants born to diabetic mothers, neonatal respiratory distress may be caused by respiratory distress syndrome (RDS), transient tachypnea of the newborn, and other disorders [3]. RDS may be the consequence of fetal hyperinsulinemia interfering with the action of cortisol on the synthesis of surfactant. Long-term morbidity. The long-term morbidity in the offspring of mothers with GDM includes adverse neurological and cognitive outcomes and principally early onset metabolic syndrome. In offspring of GDM mothers, the risks of obesity, metabolic syndrome, T2DM and impaired insulin sensitivity and secretion are 2-8-fold those in offspring of mothers without GDM. The long-term effects of *in utero* exposure to GDM are not always evident in early childhood while emerging during puberty. The pathogenic mechanisms underlying the abnormal metabolic risk profile in offspring are not precise, but epigenetic modifications induced by exposure to maternal hyperglycemia during fetal life are implicated. Animal studies show that treatment in offspring can prevent long-term metabolic complications, but this observation remains to be confirmed in humans. Therefore, diabetes begets diabetes, and it seems likely that GDM plays a relevant role in the global diabetes epidemic [1].

## REFERENCES

[1] Damm P, Houshmand-Oeregaard A, Kelstrup L, Lauenborg J, Mathiesen ER, Clausen TD. Gestational diabetes mellitus and long-term consequences for mother and offspring: a view from Denmark. Diabetologia. 2016;59(7):1396-99.

[2] Mirghani Dirar A, Doupis J. Gestational diabetes from A to Z. World J Diabetes. 2017;8(12):489-511.

[3] Ashwal E, Hod M. Gestational diabetes mellitus: Where are we now? Clin Chim Acta. 2015;451(Pt A):14-20.

## LECT 29

## EXCESSIVE GESTATIONAL WEIGHT GAIN: INTERVENTIONS AIMED TO REDUCE THE

## EFFECTS ON MATERNAL AND OFFSPRING HEALTH

L. Di Cerbo, D. Menichini, F. Facchinetti

*Mother-Infant Department, University of Modena and Reggio Emilia, Modena, Italy*

The prevalence of overweight and obesity has increased over the past few decades and has become a global health problem. Obese women have a higher risk of miscarriage, gestational diabetes, venous thromboembolism, hypertension, preeclampsia, induced labor, cesarean section, anesthetics complications and wound infections, and their babies are at increased risk of stillbirth, congenital anomalies, prematurity, macrosomia or large for gestational age and experience birth trauma or neonatal death [1]. A recent systematic review conducted in ten clinical trials showed a consistent association between excessive gestational weight gain (GWG) and the development of newborns adiposity or other metabolic diseases early in life, during adolescence or adulthood [2]. Evidence from epidemiological studies and animal models indicate that maternal undernutrition/overnutrition and hormone imbalance are critical factors in the process known as "intrauterine fetal programming" [3]. Prenatal maternal weight and GWG interfere irreversibly in the development of organs involved in the control of food intake and metabolism, and may also influence the prevalence and severity of obesity and other metabolic diseases in future generations.

To define the optimal GWG the United States' Institute of Medicine (IOM) set important criteria in 2009, which are based on maternal pre-conception body mass index (BMI). Approximately 40-70% of women gain more than the IOM recommendations with those most at-risk being already overweight or obese at conception [4]. According to the current position of the Academy of Nutrition and Dietetics, intervention type and intensity seem to affect the efficacy of the programs; effective programs tended to last six weeks or longer, focus on improving both dietary intake and physical activity intensity, and actively engage women, through routine monitoring of weight gain and/or food intake and physical activity intensity. In general, as shown by the latest published meta-analysis, an intensive diet and exercise intervention during pregnancy reduces GWG with a level of evidence from moderate to high than control groups, as well as cesarean section rate [5]. Some interventions (customized low-calories

**Table 1 (LECT 29).** Effects of lifestyle interventions on gestational diabetes.

| Lifestyle interventions | n | GDM |
|---|---|---|
| Not adherent to lifestyle changes | 99 | 35 (35%) |
| Fully adherent to diet only | 25 | 3 (12%) |
| Adherent both to diet and physical activity | 24 | 1 (4.2%) |

GDM: gestational diabetes mellitus.

diet plus physical activity) are also able to reduce gestational diabetes mellitus (GDM). However, the main factor is represented by the degree of compliance as reported in **Tab. 1** [6].

In conclusion, interventions aimed at preventing excessive GWG has a high potential. However, the characteristics of interventions (quality, timing, intensity) as well as compliance affect success. This has important implications for designing future interventions that may benefit more women.

REFERENCES

[1] Siega-Riz AM, Viswanathan M, Moos MK, Deierlein A, Mumford S, Knaack J, Thieda P, Lux LJ, Lohr KN. A systematic review of outcomes of maternal weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. Am J Obstet Gynecol. 2009;201(4):339.e1-14.

[2] Pérez-Morales ME, Bacardi-Gascon M, Jimenez-Cruz A. Association of excessive GWG with adiposity indicators and metabolic diseases of their offspring: systematic review. Nutr Hosp. 2015;31(4):1473-80.

[3] Barker DJ, Osmond C, Law CM. The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. J Epidemiol Community Health. 1989;43(3):237-40.

[4] Simas TA, Liao X, Garrison A, Sullivan GM, Howard AE, Hardy JR. Impact of updated Institute of Medicine guidelines on prepregnancy body mass index categorization, gestational weight gain recommendations, and needed counseling. J Womens Health (Larchmt). 2011;20(6):837-44.

[5] [No authors listed]. Effect of diet and physical activity based interventions in pregnancy on gestational weight gain and pregnancy outcomes: meta-analysis of individual participant data from randomised trials. BMJ. 2017;358:j3991.

[6] Bruno R, Petrella E, Bertarini V, Pedrielli G, Neri I, Facchinetti F. Adherence to a lifestyle programme in overweight/obese pregnant women and effect on gestational diabetes mellitus: a randomized controlled trial. Matern Child Nutr. 2017;13(3).

## LECT 30

## "TOO MUCH" AND "NOT ENOUGH" WEIGHT IN PREGNANCY

A. Dessì, M. Puddu, V. Fanos

The intrauterine growth restriction (IUGR) affects from 5% up to 10% of pregnancies, and it is diagnosed during prenatal age, whether the fetus, at a given gestational age, can't reach its growth potential, which is genetically determined, for one or more reasons. There are several risk factors, and among them, there is maternal smoke followed by malnutrition during pregnancy and maternal, fetal and uteroplacental pathologies [1]. The term diabetes mellitus, on the other hand, identifies a group of metabolic diseases characterized by hyperglycemia associated with disorders of secretion or the insulin sensitivity or both. Type 1 diabetes (TD1) that represents the 3-6% of the total cases of diabetes is classified among the so-called autoimmune diseases, and generally, it occurs during infancy or adolescence with a peak of incidence between age 10 and 14. Sardinia, together with Finland, is the region that presents the highest number of new TD1 cases per year, reaching an incident of over 50 cases per 100,000 inhabitants. Type 2 Diabetes (TD2) is the most common form of diabetes (about 90% of cases), and it manifests generally after 30-40 years of age. Lastly, every case in which there is a high level of circulating glucose for the first time during pregnancy is defined as gestational diabetes (GDM): this condition occurs in 4% of pregnancies. Due to the short- and long-term complications of diabetes (among them: cardiovascular diseases, neuropathy and diabetic nephropathy), the healthcare, social and economic impact has imposed on a worldwide level the search for organizational pathways that minimize as much as possible the incidence of the acute events or the debilitating complications that imply extremely high costs both direct and indirect. In Italy, nowadays, the consumption of the healthcare resources of diabetic people is 2.5 times higher than that of non-diabetic people of the same sex and age. TD1, together with GDM, is one of the diseases that worsen the course of pregnancy, increasing the risk of maternal-fetal complications in the short and long term. Referring to the neonatal health, diabetes in pregnancy, related to a higher risk of congenital malformations, mortality, and morbidity represents the most frequent cause of embryo-fetopathy. The incidence of malformations in neonates of diabetic mothers is estimated to be from 2 to 4 times higher than that found in the general population, and the most affected organs are heart, central nervous system, kidney and the skeleton. In particular, the incidence of congenital cardiopathy is about 5 times higher when compared to the general population. Moreover, even neonates of diabetic mothers without congenital cardiac malformations, sonographic studies showed hypertrophy of the intraventricular septum and the ventricular walls. Nevertheless, according to the data in the literature, it is not possible to evaluate the incidence and the specific

risk of cardiomyopathy in patients with hypertrophy of the septum at birth. IUGR neonates, like children of diabetic mothers, represent today a challenge for the neonatologist due to the perinatal complications (such as mortality, hypoglycemia, hypothermia) that are associated with these pathologies. But they represent a challenge for pediatricians as well, that have to face not only the complications of infancy, such as asthma and early obesity but also with the necessity to prevent, since childhood, the future late complications, meaning chronic diseases such as metabolic syndrome and coronaropathy. Several studies on animal models highlighted that the exposition to a hyperglycemic and hypoglycemic environment in the uterus could lead to a reduced glucose tolerance at birth that persists in adulthood, regardless of genetic predisposition of the subjects. Although metabolic alterations such as hypo/hyperinsulinemia and hypoglycemia had been observed, metabolic pictures that can be considered "specific" of these pathologies had never been described in neonates of diabetic mothers or IUGR. Several studies in literature affirm that environmental factors that affect the fetus can influence its prenatal development determining structural and functional alterations that could be irreversible and persist in the post-natal life increasing the risk of developing metabolic diseases in adulthood such as obesity, TD2, and metabolic syndrome. In this scenario, it seems clear that the development of analytical strategies more and more sensitive and useful for the early diagnosis in subjects at risk of developing diabetes can provide essential contributions to reduce the enormous clinical, social and economic costs of this pathology. The new "omics" sciences such as metabolomics and microbiomics seem to be able to characterize the metabolic phenotype of neonates of diabetic mothers or IUGR and detect a "biochemical fingerprint" useful for the recognition of potential predictive biomarkers [2]. The ultimate goal of these sciences is to design a nutritional and therapeutic program different for each patient.

REFERENCES

[1] Dessì A, Corona L, Pintus R, Fanos V. Exposure to tobacco smoke and low birth weight: from epidemiology to metabolomics. Expert Rev Proteomics. 2018;15(8):647-56.

[2] Fanos V, Pintus R, Dessì A. Clinical Metabolomics in Neonatology: From Metabolites to Diseases. Neonatology. 2018;113(4):406-13.

**LECT 31**

**UNRAVELING THE COMPLEX GENETICS OF CHILDHOOD AND ADOLESCENT PSYCHO-**

**PATHOLOGY, INCLUDING AGGRESSION, THROUGH INTERNATIONAL COLLABORA-TIONS: AN UPDATE ON THE CAPICE AND ACTION PROJECTS**

M. Manchia[1,2], V. Fanos[3]

[1]Section of Psychiatry, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy

[2]Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada

[3]Neonatal Intensive Care Unit, Neonatal Pathology, and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy

Psychological and behavioral alterations, including aggression, are increasingly prevalent in the general population. Typically, their onset is during childhood and adolescence with a developmental trajectory that, in the majority of cases, lead to the manifestation of full-blown symptoms and the diagnosis of a severe mental disorder. These conditions have a complex diathesis, with a strong heritable component setting a liability threshold on which environmental risk (and protective) factors exert their modulatory effect. Given the polygenic genetic architecture of these traits, under the common disease-common variant hypothesis only samples of adequate size (in the order of tens of thousands) can reach the statistical power to detect true signals of genetic association. In this context, researchers have striven to pool together resources creating international consortia for the collection and analysis of "omics" and phenotypic data. Illustrative of this scenario is the EU funded initiatives ACTION (Aggression in Children: Unraveling gene-environment interplay to inform Treatment and InterventiON strategies) and CAPICE (Childhood and Adolescence Psychopathology: unraveling the complex etiology by a large Interdisciplinary Collaboration in Europe). The ACTION project aims at dissecting the etiology and pathogenesis of aggression by integrating accurate clinical data with extensive "omics" coverage, including metabolomics, of longitudinally collected cohorts [1]. This will lead to the development of novel diagnostic tools and causative targets and guide the development of treatment and prevention strategies, ultimately reducing the impact of aggression on society. For instance, ACTION clinical findings have provided further insights on the co-occurrence of aggression with oppositional and ADHD-related problems, as well as with anxiety-depression and other internalizing symptoms from six large population-

based European cohort studies from the Netherlands (2x), the UK, Finland and Sweden (2x) [2]. Of note, these data have been implemented in an online tool to visualize associations between aggression and psychopathology as a function of rater, gender, instrument, and cohort [2]. Genetic findings from ACTION pointed to high stability and heritability (60-80%) of aggressive behavioral problems [3], while epigenetic gene-ontology (GO) analysis highlighted that genes involved in developmental and central nervous system processes are enriched among the top list genes the epigenome-wide meta-analysis [4]. Another key aspect in collaborative international projects is to ensure adequate and standard training of research personnel by facilitating the exchange of experiences and interactions among groups. In this context, CAPICE is a network that takes advantage of data collected within the EArly Genetics and Lifecourse Epidemiology (EAGLE) consortium, a well-established collaboration of the many European birth and adolescent population-based (twin and family) cohorts with unique longitudinal information on lifestyle, family environment, health, and emotional and behavioral problems. Using phenotypic and genome-wide genotypic data of over 60,000 children, in addition to genome-wide genotypes for over 20,000 mothers and epigenome-wide data for over 6,000 children, the early stage researchers involved in CAPICE will contribute to improving later outcomes of young people in European countries with child and adolescent psychopathology. ACTION and CAPICE are a testament of the importance of putting together robust international networks to collect and integrate large phenotypic and "omics" datasets. This will permit to test new research hypotheses, to create proper analytical pipelines and to facilitate the clinical implementation of findings, ultimately leading to a decreased severity of the outcomes.

REFERENCES

[1] Boomsma DI. Aggression in children: unravelling the interplay of genes and environment through (epi)genetics and metabolomics. J Pediatr Neonat Individual Med. 2015;4(2):e040251.

[2] Bartels M, Hendriks A, Mauri M, Krapohl E, Whipp A, Bolhuis K, Conde LC, Luningham J, Fung Ip H, Hagenbeek F, Roetman P, Gatej R, Lamers A, Nivard M, van Dongen J, Lu Y, Middeldorp C, van Beijsterveldt T, Vermeiren R, Hankemeijer T, Kluft C, Medland S, Lundström S, Rose R, Pulkkinen L, Vuoksimaa E, Korhonen T, Martin NG, Lubke G, Finkenauer C, Fanos V, Tiemeier H, Lichtenstein P, Plomin R, Kaprio J, Boomsma DI. Childhood aggression and the co-occurrence of behavioural and emotional problems: results across ages 3-16 years from multiple raters in six cohorts in the EU-ACTION project. Eur Child Adolesc Psychiatry. 2018;27(9): 1105-21.

[3] Porsch RM, Middeldorp CM, Cherny SS, Krapohl E, van Beijsterveldt CE, Loukola A, Korhonen T, Pulkkinen L, Corley R, Rhee S, Kaprio J, Rose RR, Hewitt JK, Sham P, Plomin R, Boomsma DI, Bartels M. Longitudinal heritability of childhood aggression. Am J Med Genet B Neuropsychiatr Genet. 2016;171(5):697-707.

[4] van Dongen J, Nivard MG, Baselmans BM, Zilhão NR, Ligthart L; BIOS Consortium, Heijmans BT, Bartels M, Boomsma DI. Epigenome-Wide Association Study of Aggressive Behavior. Twin Res Hum Genet. 2015;18(6):686-98.

**LECT 32**

## THE ROLE OF BIG DATA IN NEUROPSYCHIATRIC DISORDERS: A FOCUS ON METABOLOMICS

H.S.R. Rajula[1,2], M. Manchia[3,4], V. Fanos[1]

[1]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, University of Cagliari, Cagliari, Italy

[2]PhD student Marie Sklodowska-Curie CAPICE Project

[3]Section of Psychiatry, Department of Medical Science and Public Health, University of Cagliari, Cagliari, Italy

[4]Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada

### BACKGROUND

Neuropsychiatric disorders are a heterogeneous group of conditions with a multiple diatheses. Indeed, the trajectory leading to a diagnosis of a neuropsychiatric disorder is likely modulated by the interplay of genetic and environmental factors. Specific criteria guide the identification of a specific neuropsychiatric phenotype (diagnosis), the use of psychometric tools (scale and/or questionnaires), and, to a certain extent, by biomarkers, including those derived by "omics" approaches. The large datasets obtained by the integration of clinical and omics data need specific analytical pipelines. Indeed, the term big data refers to highly complex, heterogeneous and high-dimensional large-scale datasets. Big Data approaches are hypothesis-generating and discovery-oriented, with the goal of revealing the hidden patterns or information behind complex data using computer science and, statistical approaches [1]. In this context, the question of how to exploit these resources for the investigation of the pathophysiological mechanisms of neuropsychiatric disorders appears of interest.

### OBJECTIVE

Here we aimed at performing a systematic review of the literature-pertaining to Big Data approaches in neuropsychiatric disorders, with a specific focus on metabolomics.

## METHODS

Computerized databases (e.g., Pubmed, PsychInfo and Google Scholar) were used to access English-language articles published using the following search terms: "Big Data" AND "metabolomics" AND "psychiatry" or "Big Data" AND "metabolomics" AND "neurology". Papers were selected from the initial search (performed in August 2018) if the primary outcome(s) of interest was (were) categorized in any of the following domains: (i) Big Data, (ii) neuropsychiatric disorders, (iii) metabolomics.

## RESULTS

Our systematic search identified 10 studies. Big Data at multiple levels are being generated and integrated to study network failures in Alzheimer's disease (AD). They used metabolomics as a global biochemical approach to identify peripheral metabolic changes in AD patients and correlate them with cerebrospinal fluid pathology markers, imaging features, and cognitive performance [2]. The challenge is to combine big data provided by the genomics, transcriptomics, proteomics, and metabolomics with complex systems science, systems biology, and system medicine the body [3]. There is a vast amount of data on the role of environmental determinants of risk in schizophrenia (SZ). Quantitative data-synthesis approaches have provided estimates of the effect size of such environmental determinants on the risk of developing SZ [4]. In the literature exposure to environmental risk factors during, but not only gestational AGE increases the risk of SZ significantly. Individuals at high genetic risk might, therefore, have a higher likelihood of developing the illness if exposed to one or more of these risk factors, compared with unexposed subjects [4]. Several environmental determinants appear to modulate the risk of Bipolar disorder (BD), particularly when exposure occurs in prenatal and/or perinatal phases. Among these, the winter season of birth, urbanicity of place of birth, parental loss, and paternal age, as well as malnutrition during the gestational period, appear to increase the risk of developing BD [4] significantly.

## CONCLUSIONS

Our work identified key resources available for psychopathological studies and call for the application and development of Big Data approaches to dissect the causes and mechanisms of neuropsychiatric disorders [1]. Around the world, unprecedented amounts of data are being collected with diverse content ranging from the genetic and molecular "omics" to the clinical phenotypes of patients in their doctor's office. Big Data could revolutionize the development of effective treatments for the AD but only if such data are turned into actionable knowledge [5]. Integrative mechanism-based predictive platforms using complexity science have successfully led to scientific advances in other fields. Such advanced algorithms, when combined with big data information could similarly advance AD research and development by creating a systems-based understanding of this heterogeneous disease to predict which molecular targets (and similar drugs) will yield clinical benefit in which patients and to improve the clinical development success rate [5]. A Big Data approach that integrates phenotypic/behavioral/personal information with vast amounts of 'omics' provides a unique strategic opportunity to provide actionable knowledge in identifying those at risk for BD. Until a Big Data approach is empirically validated, it could not be considered applicable to the clinical setting at this time. Large clinical/research networks dedicated to the treatment and study of BD that integrate pediatric and adult populations will be required to sufficiently enroll adequate numbers of individuals at risk for BD to realize the opportunities of Big Data in the prediction and prevention of BD [6]. These findings highlight the need for proper methodology to handle large datasets of "omics" and phenotypic data. Indeed, recent technological advances have brought a dramatic increase in (epi)genomic, transcriptomic, proteomic and metabolomics data as well as a structural and functional brain imaging data. Understanding how genetic and environmental interactions impact brain structure and function modulating the continuum between health and dysfunction is now the central question in medicine and more so in neuropsychiatry.

## ACKNOWLEDGMENTS

## REFERENCES

[1] Zhao Y, Castellanos FX. Annual Research Review: Discovery science strategies in studies of the pathophysiology of child and adolescent psychiatric disorders – promises and limitations. J Child Psychol Psychiatry. 2016;57(3):421-39.

[2] Toledo JB, Arnold M, Kastenmüller G, Chang R, Baillie RA, Han X, Thambisetty M, Tenenbaum JD, Suhre K, Thompson JW, John-Williams LS, MahmoudianDehkordi S, Rotroff DM, Jack JR, Motsinger-Reif A, Risacher SL, Blach C, Lucas JE, Massaro T, Louie G, Zhu H, Dallmann G, Klavins K, Koal T, Kim S, Nho K, Shen L, Casanova R, Varma S, Legido-Quigley C, Moseley MA, Zhu K, Henrion MYR, van der Lee SJ, Harms AC, Demirkan A, Hankemeier T, van Duijn CM, Trojanowski JQ, Shaw LM, Saykin AJ, Weiner MW, Doraiswamy PM, Kaddurah-Daouk R; Alzheimer's

Disease Neuroimaging Initiative and the Alzheimer Disease Metabolomics Consortium. Metabolic network failures in Alzheimer's disease: A biochemical road map. Alzheimers Dement. 2017;13(9):965-84.

[3] Rajula HSR, Mauri M, Fanos V. Scale-free networks in metabolomics. Bioinformation. 2018;14(3):140-4.

[4] Faa G, Manchia M, Pintus R, Gerosa C, Marcialis MA, Fanos V. Fetal programming of neuropsychiatric disorders. Birth Defects Res C Embryo Today. 2016;108(3):207-23.

[5] Geerts H, Dacks PA, Devanarayan V, Haas M, Khachaturian ZS, Gordon MF, Maudsley S, Romero K, Stephenson D; Brain Health Modeling Initiative (BHMI). Big data to smart data in Alzheimer's disease: The brain health modeling initiative to foster actionable knowledge. Alzheimers Dement. 2016;12(9):1014-21.

[6] McIntyre RS, Cha DS, Jerrell JM, Swardfager W, Kim RD, Costa LG, Baskaran A, Soczynska JK, Woldeyohannes HO, Mansur RB, Brietzke E, Powell AM, Gallaugher A, Kudlow P, Kaidanovich-Beilin O, Alsuwaidan M. Advancing biomarker research: utilizing 'Big Data' approaches for the characterization and prevention of bipolar disorder. Bipolar Disord. 2014;16(5):531-47.

**LECT 33**

## SHARING RESEARCH: COMMUNICATION CAN HELP

M. Mauri[1], V. Fanos[2]

[1]University of Cagliari, Cagliari, Italy

[2]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy

Research and Computer Science (or Informatics) have a lot in common. It is quite clear that Informatics can grow only if supported by new technological research findings, and that Research can proliferate if supported by customized tools that allow to simulate models, extract and process data. However, these are just the most obvious things. When we say that Research and Informatics have a lot in common, we mean that they also share one of their primary missions: the goal of sharing. Research is not fruitful without the exchange of information through scientific works, workshops, conferences, international meetings among researchers, peer-reviewed processes, travels and other comparison opportunities. Informatics would be just an algorithmic, enigmatic and self-referential game if stripped of its broader goal: the capability of instantly break with new information and new findings, making them available from the coldest part of the world to the warmest; Informatics is the reign of sharing. The word "informatics" is inherently related to the term "information" and,

consequently, communication. As research can do, also communication can change everything in a second. Both can save millions of lives in a second: turning an epidemic into a controlled disease, in the first case; stopping a war, in the second case. What would happen if research and communication were misaligned? This is not a rare situation. It happened in the past where the research mostly ignored for more than 100 years some intuitions to contain the puerperal fever, although those intuitions were in some cases also adequately communicated [1, 2]. It is happening today when communication is not correctly driving the science in the knowledge gap between vaccines [3]. Research and Communication: both can save millions of lives in a second, we said before. Both can cause the contrary, especially if not aligned. Without doubts, dissemination and communication of scientific results are now acknowledged as crucial parts of the research process [4]. The European Union (EU), through Research & Innovation Framework Programmes, has a clear position about that: the dissemination of scientific research projects is nowadays mandatory and governed by contracts between the European Union and the recipients of funding. Besides, EU recommends a diversified science divulgation, which stimulates creativeness and requires a digital presence [5]. That is why we started this article comparing Research and Computer Science. In this context, the University of Cagliari, Department of Surgical Science, has a significant role; it is currently leading the dissemination of two important EU funded projects focused on childhood mental health. UNICA is trying to share the research findings of these projects in an innovative and easy-readable way, hoping to give a consistent contribution in the understanding of the causes and comorbidities of some critical childhood mental health problems. The ACTION project (Aggression in Children: Unraveling gene-environment interplay to inform Treatment and InterventiON strategies, FP7/2007-2013, grant agreement no. 602768) works to improve the understanding of the causes of individual differences in aggression among children in order to better inform the development of prevention and treatment strategies. One of the most relevant activities done within this project, exploiting at best the possibilities of Informatics, is the release of an interactive tool (**Fig. 1**) that shows the comorbidities of child aggression with other 22 childhood psychopathologies [6, 7]. The CAPICE project (Childhood and Adolescence
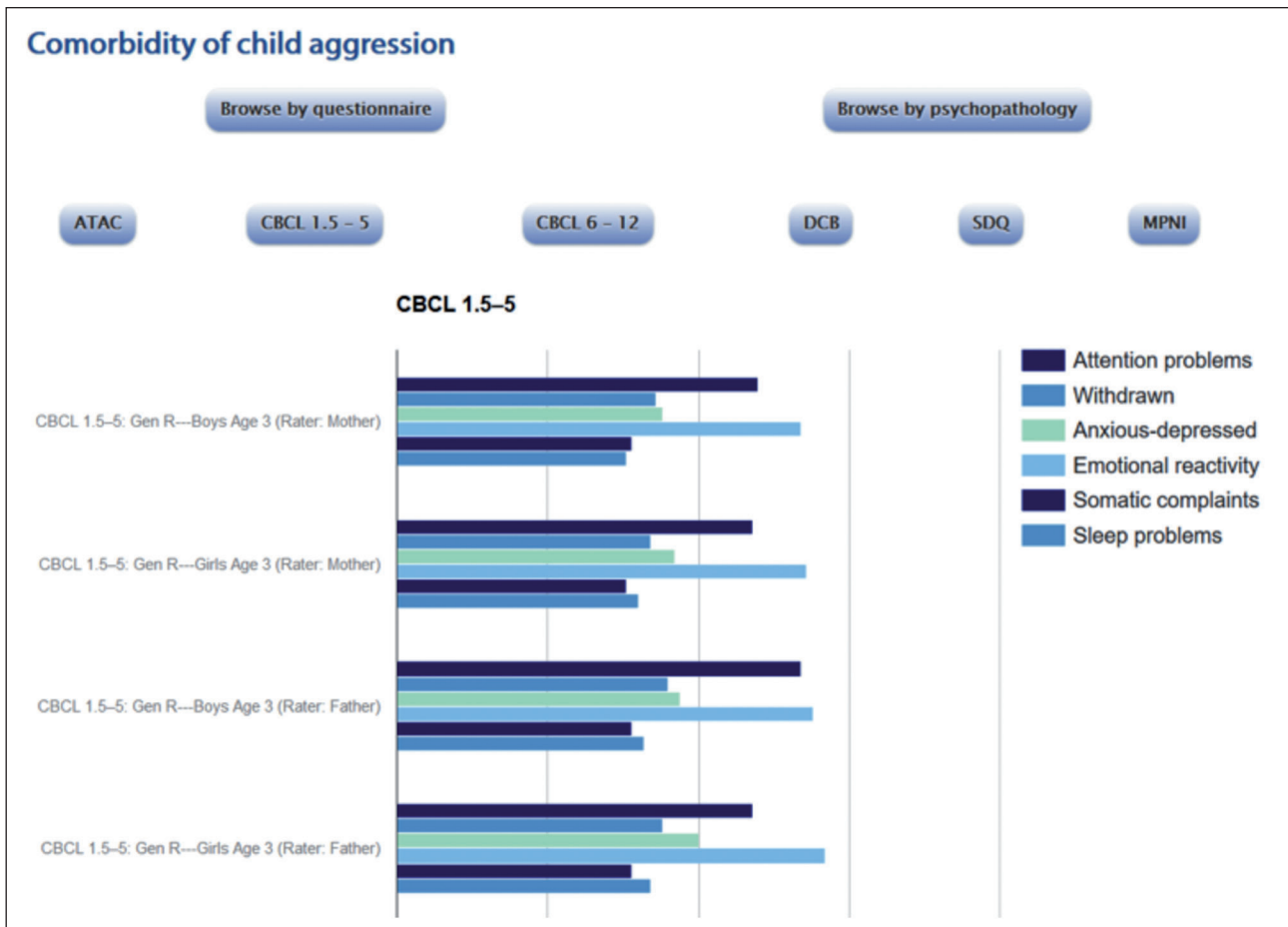
**Figure 1 (LECT 33).** A page of the website of the project ACTION.

Psychopathology: unravelling the complex etiology by a large Interdisciplinary Collaboration in Europe, H2020 Marie Curie Actions, grant agreement no. 721567): with a focus on common and debilitating problems in childhood and adolescence, including depression, anxiety, and Attention Deficit Hyperactivity Disorder, CAPICE is trying to contribute to improving later outcomes of young people in European countries with child and adolescent psychopathology. One of the most relevant activities done within this project, exploiting at best the possibilities of Informatics, is a research blog (**Fig. 2**) weekly populated with experiences narrated from 12 Early Stage Researchers, hosted in 12 prestigious research institutes across Europe [8, 9] (**Fig. 1** and **Fig. 2**).

REFERENCES

[1] Hallett C. The attempt to understand puerperal fever in the eighteenth and early nineteenth centuries: the influence of inflammation theory. Med Hist. 2005;49(1):1-28.

[2] Burch D, When Childbirth Was Natural, and Deadly. Available at: https://www.livescience.com/3210-childbirth-natural-deadly.html, last access: August 2018.

[3] Grant RP. Why scientists are losing the fight to communicate science to the public. Available at: https://www.theguardian.com/science/occams-corner/2016/aug/23/scientists-losing-science-communication-skeptic-cox, last access: August 2018.

[4] Crosswaite C, Curtice L. Disseminating research results-the challenge of bridging the gap between health research and health action, Health Promotion International. 1994;9(4):289-96.

[5] European Commission, Communicating EU Research & Innovation. A guide for project participants. Luxembourg: Publications Office of the European Union, 2012.

[6] http://www.action-euproject.eu/ComorbidityChildAggression, last access: August 2018.

[7] Bartels M, Hendriks A, Mauri M, Krapohl E, Whipp A, Bolhuis K, Conde LC, Luningham J, Fung Ip H, Hagenbeek F, Roetman P, Gatej R, Lamers A, Nivard M, van Dongen J, Lu Y, Middeldorp C, van Beijsterveldt T, Vermeiren R, Hankemeijer T, Kluft C, Medland S, Lundström S, Rose R, Pulkkinen L, Vuoksimaa E, Korhonen T, Martin NG, Lubke G, Finkenauer C, Fanos V, Tiemeier H, Lichtenstein P, Plomin R, Kaprio J, Boomsma DI. Childhood aggression and the co-occurrence of behavioural and emotional problems: results across ages 3-16 years from multiple raters in six cohorts in the EU-ACTION project. Eur Child Adolesc Psychiatry. 2018;27(9):1105-21.

[8] http://www.capice-project.eu, last access: September 2017.

[9] http://www.capice-project.eu/index.php/blog, last access: September 2017.

**Figure 2 (LECT 33).** A page of the website of the project CAPICE.

**LECT 34**

## ENDOCRINE DISRUPTORS AND NEURO-DEVELOPMENT

M.E. Street[1], F. Cirillo[1], C. Catellani[1], P. Palanza[2], G.C. Panzica[3], E. Grossi[4], P. Lazzeroni[1], C. Sartori[1]

[1]*SS Endocrinologia e Diabetologia Pediatrica, AUSL-Reggio Emilia-IRCCS, Reggio Emilia, Italy*

[2]*Department of Medicine and Surgery, University of Parma, Parma, Italy*

[3]*Department of Neuroscience, University of Turin, Turin, Italy*

[4]*Villa Santa Maria Institute, Tavernerio (CO), Italy*

Neurodevelopment refers to the processes which determine the growth and development of the brain or central nervous system. The term also refers to brain function reflecting emotion, learning ability, self-control and memory throughout growth. Neurodevelopment is profoundly affected by the effect of hormones and various growth factors. Therefore, any substance that affects the production, action or effect of a hormone can affect neurodevelopment. To date, we are aware of approximately 1,000 chemicals out of 85,000 that can affect the endocrine system. These include plasticizers as phthalates and bisphenol A (BPA), flame retardants, pesticides, industrial chemical as alkylphenols, dioxins, metals, and air pollutants. Endocrine disruptors (EDC) are by definition single exogenous chemicals or a better mixture of chemicals in everyday life, that can interfere with

any aspect of hormone action. Data from ecological and animal models and observations in humans prove that EDC can significantly affect wildlife and human health [1]. Emerging research on maternal EDC exposure and child neurodevelopmental outcomes have recently described significant associations between gestational levels of plasticizers as BPA and phthalates with alterations of emotional behavior, aggressive behavior, cognitive impairment and Attention Deficit Hyperactivity Disorder (ADHD) in children [2]. Animal studies have shown that maternal exposure to BPA during gestation and/or lactation can induce long-term alterations in offspring behavior, including anxiety and exploration, learning and memory, and socio-sexual behaviors. In addition, treatment with EDCs can also affect mothers' behavior. BPA is the most studied EDC in this regard. BPA in the brain has been shown to act primarily as a weak estrogen receptor agonist and as an antiandrogen, and to cause epigenetic changes altering gene expression in different regions [1] at very low doses (below the reference dose of 50 μg/kg/day traditionally considered the tolerable daily intake or TDI) via maternal treatment. The BPA-induced effects on anxiety behaviors have been associated with altered mesolimbic dopaminergic signaling, increased expression of glucocorticoid receptors in the hippocampus, or reduction in estrogen-dependent gene expression in the amygdala [1]. The brain alterations associated with BPA exposure and increased anxiety, are generally sex-dependent and/or alter normal sex differences observed in the control population [1]. Although there are relatively few studies in humans, findings from epidemiological studies are consistent with data in animal models associating maternal BPA levels to internalizing behavior in children, including anxiety and depression [1]. Prenatal and early postnatal BPA exposure has been associated with changes in cognitive responses, socio-sexual interactions, play behavior and parental care in rodents, non-human and human primates [2]. Although there is scarce experimental evidence, studies suggest that BPA may impair memory formation by interfering with neural plasticity processes. Concerning Socio-Sexual Behaviour, some studies have evidenced a reduction of social interactions in BPA-exposed animals. A study in mice reported increased play behavior and social investigation in BPA-exposed juveniles; in addition, the observed effect was transgenerationally transmitted up to the third generation, suggesting an epigenetic effect of BPA

exposure via the germ line [3]. Age at testing, the developmental stage of exposure, sex and other variables can influence BPA effects. Human studies suggest sex-dependent associations between gestational BPA or phthalate exposure and changes in social and aggressive behaviors in children and adolescents [4]. In animals, direct exposure to EDCs can affect maternal behavior in females altering mother-pup interactions [4]. Maternal exposure during pregnancy to a low, but environmentally relevant BPA dose, through a non-stressful administration procedure, determines subtle alterations in maternal behavior and the behavioral development of their offspring [4]. In fact, mice fed BPA during late pregnancy show a reduction in maternal nursing behavior and an increase in the time spent away from the nest over the first 2 weeks post-partum. The findings in the Literature overall suggest that pregnancy and lactation represent "vulnerable periods of development" for the mother and that maternal brain, physiology, and behavior are highly sensitive to endocrine disruption. In humans, the most common neurodevelopmental disorders in humans include learning disabilities, sensory deficits, developmental delays, attention deficit and hyperactivity disorder and autism, which is the most severe and costly [1]. Autism disability is at present very common affecting more than 10% of children [5]. The role of genetic abnormalities in autism has stimulated a huge amount of research; however, genetic factors alone account for approximately 20-30% of all cases, whereas 70-80% of cases are likely the result of complex interactions between environmental risk factors and inherited or *de novo* genetic susceptibility [5]. Recent studies suggest an equal contribution of environmental factors, part, and genetic susceptibility to ASD [6]. Only a few industrial chemicals (e.g., lead [Pb], methylmercury, polychlorinated biphenyls [PCBs], arsenic [As], and toluene) are recognized causes of neurodevelopmental disorders and subclinical brain dysfunction. That heavy metals such as cadmium (Cd), As, mercury (Hg), nickel (Ni), and Pb may exhibit endocrine-disrupting activity in animal models, probably by interfering with zinc-fingers of nuclear estrogen receptors, is a recent discovery [6]. Neuroendocrine circuits control food Intake and Energy Metabolism. EDCs are also metabolic disrupting chemicals (MDCs) and can target peripheral targets too (e.g., fat tissue, liver, pancreas, skeletal muscle, intestine), besides the hypothalamus [7] which has an important role in energy balance regulation and food intake expressing receptors

for peripheral hormones signalling the energy status of the body (insulin, leptin and ghrelin). The NPY system represents one of the neuroendocrine circuits and has been implicated in obesity and T2D in humans [1]. All the components of the systems implicated (neuropeptides, receptors, signaling molecules) may be targets of MDCs [7]. Among the most studied MDCs are BPA and Tributyltin (TBT). The exposure to low doses of BPA in mice during the prenatal period alters food intake as well as leptin and insulin levels [8]. TBT, an organotin compound, has obesogenic effects, mediated by the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) receptors on the fat tissue [9]. Prenatal exposure to TBT in mice induces hypothyroidism in the progeny; in pregnant females, a dose-dependent increase in Triiodothyronine (T3)-independent TRH transcription levels has also been reported [9]. Furthermore, acute exposure to TBT has been shown to activate neurons in a crucial region for the regulation of food intake, the ARC, suggesting a direct action on the central nervous system [10]. In rats, TBT can induce a functional dissociation between CRH, Adrenocorticotropic hormone (ACTH) and corticosterone, and an increase in nitric oxide synthase in the hypothalamus [9].

## REFERENCES

[1] Street ME, Angelini S, Bernasconi S, Burgio E, Cassio A, Catellani C, Cirillo F, Deodati A, Fabbrizi E, Fanos V, Gargano G, Grossi E, Iughetti L, Lazzeroni P, Mantovani A, Migliore L, Palanza P, Panzica G, Papini AM, Parmigiani S, Predieri B, Sartori C, Tridenti G, Amarri S. Current Knowledge on Endocrine Disrupting Chemicals (EDCs) from Animal Biology to Humans, from Pregnancy to Adulthood: Highlights from a National Italian Meeting. Int J Mol Sci. 2018;19(6):E1647.

[2] Palanza P, Nagel SC, Parmigiani S, Vom Saal FS. Perinatal exposure to endocrine disruptors: sex, timing and behavioral endpoints. Curr Opin Behav Sci. 2016;7:69-75.

[3] Wolstenholme JT, Edwards M, Shetty SR, Gatewood JD, Taylor JA, Rissman EF, Connelly JJ. Gestational exposure to bisphenol a produces transgenerational changes in behaviors and gene expression. Endocrinology. 2012;153(8):3828-38.

[4] Palanza PL, Howdeshell KL, Parmigiani S, vom Saal FS. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environ Health Perspect. 2002;110(Suppl 3):415-22.

[5] Van Naarden Braun K, Christensen D, Doernberg N, Schieve L, Rice C, Wiggins L, Schendel D, Yeargin-Allsopp M. Trends in the prevalence of autism spectrum disorder, cerebral palsy, hearing loss, intellectual disability, and vision impairment, metropolitan atlanta, 1991-2010. PLoS One. 2015;10(4):e0124120.

[6] Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet. 2006;368(9553):2167-78.

[7] Heindel JJ, Blumberg B, Cave M, Machtinger R, Mantovani A, Mendez MA, Nadal A, Palanza P, Panzica G, Sargis R, Vandenberg LN, Vom Saal F.

Metabolism disrupting chemicals and metabolic disorders. Reprod Toxicol. 2017;68:3-33.

[8] Angle BM, Do RP, Ponzi D, Stahlhut RW, Drury BE, Nagel SC, Welshons WV, Besch-Williford CL, Palanza P, Parmigiani S, vom Saal FS, Taylor JA. Metabolic disruption in male mice due to fetal exposure to low but not high doses of bisphenol A (BPA): evidence for effects on body weight, food intake, adipocytes, leptin, adiponectin, insulin and glucose regulation. Reprod Toxicol. 2013;42:256-68.

[9] Merlo E, Podratz PL, Sena GC, de Araújo JF, Lima LC, Alves IS, Gama-de-Souza LN, Pelição R, Rodrigues LC, Brandão PA, Carneiro MT, Pires RG, Martins-Silva C, Alarcon TA, Miranda-Alves L, Silva IV, Graceli JB. The Environmental Pollutant Tributyltin Chloride Disrupts the Hypothalamic-Pituitary-Adrenal Axis at Different Levels in Female Rats. Endocrinology. 2016;157(8):2978-95.

[10] Bo E, Viglietti-Panzica C, Panzica GC. Acute exposure to tributyltin induces c-fos activation in the hypothalamic arcuate nucleus of adult male mice. Neurotoxicology. 2011;32(2):277-80.

## LECT 35

## MATERNAL-INFANT HEALTH CARE: QUESTIONS AND ISSUES ON AN INTEGRATED PRACTICE

G. Perricone

*Società Italiana di Psicologia Pediatrica, University of Palermo, Palermo, Italy*

The contribution assumes a health model as mental health and therefore as a psychological well-being condition, even in organic pathology, syndromes, pregnancies at risk, etc. and/or in critical outcomes in prenatal diagnosis path [1]. These are conditions that characterize the daily life of the maternal and child hospital and non-hospital. It's a health model that, going beyond the bio-psycho-social one, underlines the importance of moderating variables, that are constituted by cognitive and socio-emotional processes, which allow the person and reference contexts to cross the psychosocial risk condition induced by pathology, by the syndrome, etc. [2]. The development of these moderating variables is the motivation for the development of a functional relationship between cure and care. The contribution underlines how the promotion of the relationship between cure and care, finds a privileged path in an integrated multi-professional work [3, 4]. The integration is presented as a specific type of relationship based on a) the communication, which promotes listening and which allows us to "make place" and "make space" to the other, to get in touch with the emotional world of the

other, which allows closeness and distance, refuse the fear appeal; b) the mediation, which promotes and allows to look for new relational ways with the patient, with contexts. On the other side, the mediation facilitates some significant functions in healthcare, such as the solution of the conflict, the reduction of time and costs; the promotion of spaces for the construction of a way of thinking and a shared model among different professionals. In this sense, the integration gives life to a System, which in one direction "protects" all the subjects involved, and on the other side "safeguards" them, allowing everyone to continue to be a person as well as a patient, a family member, an operator. The reflection underlines a specific "collocation" of the integration within a System Organizational Model, in that it leads the individual to a network of relationships between Services, subjects, and actions, promoting the allostasis of this system. In this sense, there are identified specific epistemological antinomies, which allow us to focus on how the integration becomes a language of the relationship allowing everyone to feel the other colleague/patient with his humanity, and to recover the affectivity; at the same time, the integration becomes the possibility to identify itself as an operator who works in the relationship to obtain system results related to the management of the cure/care relationship. The contribution questions itself on the possibility of a specific multiprofessional practice of the integration identifying some operational criteria that should be traced in taking on the responsibility of the patient and/or the family, giving rise to the first form of plasticity in presenting and "postponing" to each other. Moreover, among the operational criteria, there is the construction of a turn-over based on the complementarity between professionals according to rules, relationships, etc. Finally, there is the criterion relating to the management of a separation between professionals that allows the individual to connect, through his professional intervention, with the intervention of the other; between criteria there is also the transition from a consultancy and a call model based on delegation to a team model, based on the complementarity. Finally, particular attention must be placed to the possible critical issues when in the working model is confused with the person of the other professional, when the sharing of professional choices of the other becomes a "weight", when the "flattening" on the other becomes the disappearance of the difference, when the intersubjectivity is eclipsed and disappears the strategic "distance", not getting the criticalities of the functioning of the ward and/or of relationships. The contribution asks about the possibility of seeing the multidisciplinary integrated work in the maternal and child department according to an idea of beauty in the sense of harmony, consonance, complicity, alliances, and synergy. It is highlighted how the "Aesthetics of integration" allows going beyond the sharing of a common goal of cure [5]; in this sense, we see the integration as a "connective tissue" of the relationship between professionals and between them and the patients with their contexts. In addition, some effects of the integrated work are underlined in terms of a positive transformation perspective of organizational symptoms (absenteeism, conflicts and maladaptive behaviors with respect to the departmental mission and vision) and behavioral symptoms (indecision, restlessness, distrust, impulsivity, isolation); and still psychological symptoms, such as anxious state, poor concentration, dispersion of identity, and not the last physical/psychosomatic symptoms: sleep disorders, gastro-intestinal disorders, etc. [5]. The contribution clearly traces the path that the integration allows, from the research of the patient and his family well-being to the well-being of operators; a way that will enable to respond to the essential levels of assistance and to improve the quality of health services.

The proposed reflection orients the representation of the integrated work as a community work; in this sense, the contribution presents an experience of integrated practice in the maternal and child department.

REFERENCES

[1] Alaimo M. L'unione fa la forza. L'équipe multi-professionale. Available at: https://www.nurse24.it/infermiere/dalla-redazione/lunione-fa-la-forza-lequipe-multiprofessionale.html, date of publication: 2017, last access: September 2018.

[2] Perricone G. L'Estetica dell'Integrazione nel Materno-Infantile. Lecture at 1st S.I.P.Ped. International Congress, Maternal-Infant Health Care: Questions, issues and procedures on integrated practice. Palermo, 30th November-2nd December, 2017.

[3] Perricone G, Polizzi C, Morales MR, Carollo A, Rotolo I, Caldarella R. Corso di psicologia dello sviluppo e dell'educazione con elementi di psicologia pediatrica. Milan: McGraw-Hill, 2018.

[4] Seligman MEP. Flourish: A visionary new understanding of happiness and well-being. New York: Free Press, 2012.

[5] Zwarenstein M, Goldman J, Reeves S. Interprofessional collaboration: effects of practice-based interventions on professional practice and healthcare outcomes. Cochrane Database Syst Rev. 2009;(3):CD000072.

**LECT 36**

**URINARY GC-MS METABOLOMICS COM-PARISON BETWEEN TWO COHORTS OF**

# TODDLER AND ADOLESCENT SUBJECTS AFFECTED BY AUTISM SPECTRUM DISORDER

A. Noto[1], C. Fattuoni[2], M. Mussap[3], L. Barberini[1], M. Siracusano[4], L. Mazzone[4], R. Francavilla[5], P. Curatolo[4], V. Fanos[3]

[1]*Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy*

[2]*Department of Chemical and Geological Sciences, University of Cagliari, Cagliari, Italy*

[3]*Department of Surgical Sciences, University of Cagliari, Cagliari, Italy*

[4]*Child Neurology Unit, Systems Medicine Department, Tor Vergata University Hospital of Rome, Rome, Italy*

[5]*Department of Medicine-Paediatric Section, University of Bari, Bari, Italy*

The diagnosis of autism dates back to the mid-twentieth century when, independently and almost simultaneously, Leo Kanner in 1943 and Hans Asperger in 1944 described this pathology using both the adjective "autistic" [1]. The term "autism", coined by the Swiss psychiatrist Eugen Bleuler, indicated an unconscious state of early childhood, and sometimes persistent over time, tending to isolation in such a way to exclude anything outside one's self. The diagnosis of autism is based on the description and observation of behavior, nowadays, is evident that autism spectrum disorder (ASD) is a complex disease caused by genetic predisposition, epigenetic modification and by the influence of several environmental factors. Nonetheless, biological indicators or biological tests have not yet been identified to confirm the presence of the disease [2]. The identification of sensitive and specific biomarkers still constitutes one of the main challenges to investigate specific biomarkers for ASD could help clinicians to make a diagnosis and hence enabling earlier interventions. In this regard, the use of metabolomics could help to obtain an unbiased analysis of ASD patients aiming to identify a characteristic metabolic fingerprint [3-5]. Metabolomics is one of the most innovative scientific disciplines with the purpose to unveil the metabolic processes within cells, tissues, and organs [6]. Metabolomics aims to discover new biomarkers for a better comprehension of complex diseases. Biological fluids, such as urine and plasma, contain a high number of metabolites (several hundred, with a molecular weight ranging from 50 to 1,000 Dalton) and thus robust, high-sensitive analytical methods are required for identifying the metabolome associated with a specific disease and for comparing it with that associated with health [7]. The purpose of our study is to compare the urinary metabolic signature of ASD toddler with a cohort of ASD adolescents to elucidate any metabolic analogy between these subjects.

## MATERIALS AND METHODS

*Patient selection*

99 subjects (57 toddlers and 42 adolescents) were enrolled in the study: 52 ASD patients, and 47 healthy controls. Parents of both ASD children and healthy controls gave written informed consent before the inclusion in the study. ASD patients were recruited at the Children Psychiatry Unit of the University Hospital of Rome Tor Vergata (Italy) and the Pediatric Division of University of Bari (Italy). Exclusion criteria for ASD included genetic syndromes, neurological disorders, ongoing acute diseases and known inborn errors of metabolism.

*Sample collection and preparation*

First-morning urine samples were collected at home by parents, by using sterile bags, and then brought as soon as possible to the Children Psychiatric Unit of the University Hospital of Rome Tor Vergata and the Pediatric Division of the University of Bari. Urine sample was then transferred in dry ice and sent to the University of Cagliari, where samples were stored at -80° until analysis. Urine samples were thawed at 4°C; subsequently, 150 μL were treated with 800 μL of urease solution (1 mg/mL) and sonicated for 30 min. 800 μL of cold methanol was added, samples were centrifuged, and the supernatant was evaporated to dryness overnight in an Eppendorf vacuum centrifuge. 30 μL of 0.24 M methoxyamine hydrochloride in pyridine were added to each sample and left to react for 17 h at room temperature. 30 μL of MSTFA (N-methyl-N-trimethylsilyl trifluoroacetamide) were added and left to react for 1 h at room temperature. The derivatized samples were diluted with 600 μL of tetracosane in hexane (0.01 mg/mL) just before GC-MS analysis. Samples were analyzed using an Agilent 5977B interfaced to the GC 7890B equipped with a DB-5ms column (J&W), injector temperature at 230°C, detector temperature at 280°C, a helium carrier gas flow rate of 1 ml/min. The GC oven temperature program was set at 90°C for 1 min ramping at 10°C/min to a final temperature of 270°C with 7 min hold time. 1 μL of each sample was injected in split mode (1:20). Mass spectra were acquired in full scan mode using 2.28 scans/s with a mass range of 50-700 Amu. Each acquired chromatogram was analyzed using the free software AMDIS (Automated Mass Spectral Deconvolution and Identification System; http://chemdata.nist.gov/mass-spc/amdis). Subsequently, each chromatographic peak was identified by comparing the

**Table 1 (LECT 36).** Clinical parameters and important metabolites of the population study.

| | Adolescent subjects | Toddler subjects |
|---|---|---|
| **Population** | 42 patients, Bari University Hospital, Italy | 57 patients, Rome University Hospital, Italy |
| | 21 ASD, aged 4-16, 21 controls, aged 4-17 | 31 ASD, aged 2-11, 26 controls, aged 2-6 |
| **Sample collection** | First morning urine | First morning urine |
| **Storage** | At -80°C | At -80°C |
| **Metabolites ↑ in ASD (increased)** | Phenylalanine Tryptophan Tyrosine 4-Hydroxyphenylacetic acid Hippuric acid | Phenylalanine Tryptophan Tyrosine 4-Hydroxyphenylacetic acid Hippuric acid |

ASD: autism spectrum disorder.

relative mass spectrum and retention time with those stored in an in-house made library including 234 metabolites. Other metabolites were identified using NIST08 (National Institute of Standards and Technology's mass spectral database), and Golm Metabolome Database (GMD; http://gmd.mpimp-golm.mpg.de/): the metabolite was considered positively identified with a match factor 70%. This strategy allowed for the detection of 164 compounds: 134 accurately identified, 3 unknown compound matching equally unknown compounds in GMD, and 27 unknown molecules recurring in every sample. AMDIS analysis produced an electronic sheet data matrix (Microsoft® Excel®, Microsoft Co, Redmond Washington DC, USA) used to perform univariate and multivariate statistical analysis.

RESULTS

Urine samples collected from both ASD and healthy controls cohorts were analyzed and compared through multivariate statistical analysis. 99 samples (31 ASD cases and 26 controls and 21 ASD cases and 21 controls) underwent OPLS-DA analysis. Among the detected metabolites, those shared between groups are reported in **Tab. 1**, on the basis of the ASD trends (increased in ASD children). Among them, phenylalanine, tryptophan, tyrosine, 4-hydroxyphenylacetic acid, hippuric acid were identified.

DISCUSSION AND CONCLUSIONS

The main finding of this study is the presence of a reliable urinary metabolic fingerprint of toddler and adolescent ASD subjects. The key metabolic changes consisted of perturbations in various pathways: phenylalanine, tyrosine, 4-hydroxyphenylacetic acid, hippuric acid, and tryptophan. Most of them may be closely related to alterations of the microbial metabolism. High level of phenylalanine, tyrosine, and their conversion product 4-hydroxyphenylacetic

acid are possible signs of intestinal microbiota dysbiosis amenable to *Clostridium* species overgrown [8]. Hippurate is the glycine conjugate of benzoic acid, its role in ASD could be related with the abnormal presence of clostridial species [9]. Finally, tryptophan is known to be associated with behavioral abnormalities of infantile autism [10]. It is not known if the presence of this panel of metabolites is causative or associative; anyway, it is possible to speculate hypothesized that in the next years, the presence of specific metabolites will be used for deciphering an appropriate treatment.

REFERENCES

[1] Kanner L. Autistic disturbances of affective contact. Acta Paedopsychiatr. 1968;35(4):100-36.

[2] Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet. 2008;9(5):341-55.

[3] Noto A, Fanos V, Barberini L, Grapov D, Fattuoni C, Zaffanello M, Casanova A, Fenu G, De Giacomo A, De Angelis M, Moretti C, Papoff P, Ditonno R, Francavilla R. The urinary metabolomics profile of an Italian autistic children population and their unaffected siblings. J Matern Fetal Neonatal Med. 2014;27(Suppl 2):46-52.

[4] Mussap M, Noto A, Fanos V. Metabolomics of autism spectrum disorders: early insights regarding mammalian-microbial cometabolites. Expert Rev Mol Diagn. 2016;16(8):869-81.

[5] Lussu M, Noto A, Masili A, Rinaldi AC, Dessì A, De Angelis M, De Giacomo A, Fanos V, Atzori L, Francavilla R. The urinary 1 H-NMR metabolomics profile of an italian autistic children population and their unaffected siblings. Autism Res. 2017;10(6):1058-66.

[6] Fiehn O. Metabolomics – the link between genotypes and phenotypes. Plant Mol Biol. 2002;48(1-2):155-71.

[7] Wang JH, Byun J, Pennathur S. Analytical approaches to metabolomics and applications to systems biology. Semin Nephrol. 2010;30(5):500-11.

[8] Dyck LE, Kazakoff CW, Dourish CT. The role of catecholamines, 5-hydroxytryptamine and m-tyramine in the behavioural effects of m-tyrosine in the rat. Eur J Pharmacol. 1982;84(3-4):139-49.

[9] Parracho HM, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol. 2005;54(Pt 10):987-91.

[10] Hoshino Y, Yamamoto T, Kaneko M, Tachibana R, Watanabe M, Ono Y, Kumashiro H. Blood serotonin and free tryptophan concentration in autistic children. Neuropsychobiology. 1984;11(1):22-7.

**LECT 37**

**LUNG MICROBIOTA**

U. Pelosi[1], R. Pintus[2]

[1]Past Director Department of Pediatrics, Iglesias, Italy

[2]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, Azienda Ospedaliera Universitaria, University of Cagliari, Cagliari, Italy

For a long time, it was believed that healthy lung was sterile and that could be colonized only in case of lung diseases. This belief came from conceptual mistakes: a) the limits of the microbial identification tests, b) the methods of collecting the samples (elevated risk of contamination with bacteria coming from upper respiratory tract using the examination of the sputum and the material collected by bronchoscopy), c) the natural contamination of the lower respiratory tract by inhaled material. This misconception led to the exclusion of lungs from the Microbiome Human Project. Only successively, since 2010, numerous studies highlighted, thanks to the new technologies of DNA and RNA investigation, that the lungs of a healthy subject are not sterile, but it is colonized by several microorganisms: bacteria, viruses, fungi (**Fig. 1**). These new modalities of investigation allowed to identify several species of bacteria: a) Phylum: *Firmicutes*, *Bacteroides* and *Proteobacteria*, 2) Genus: *Veillonella*,
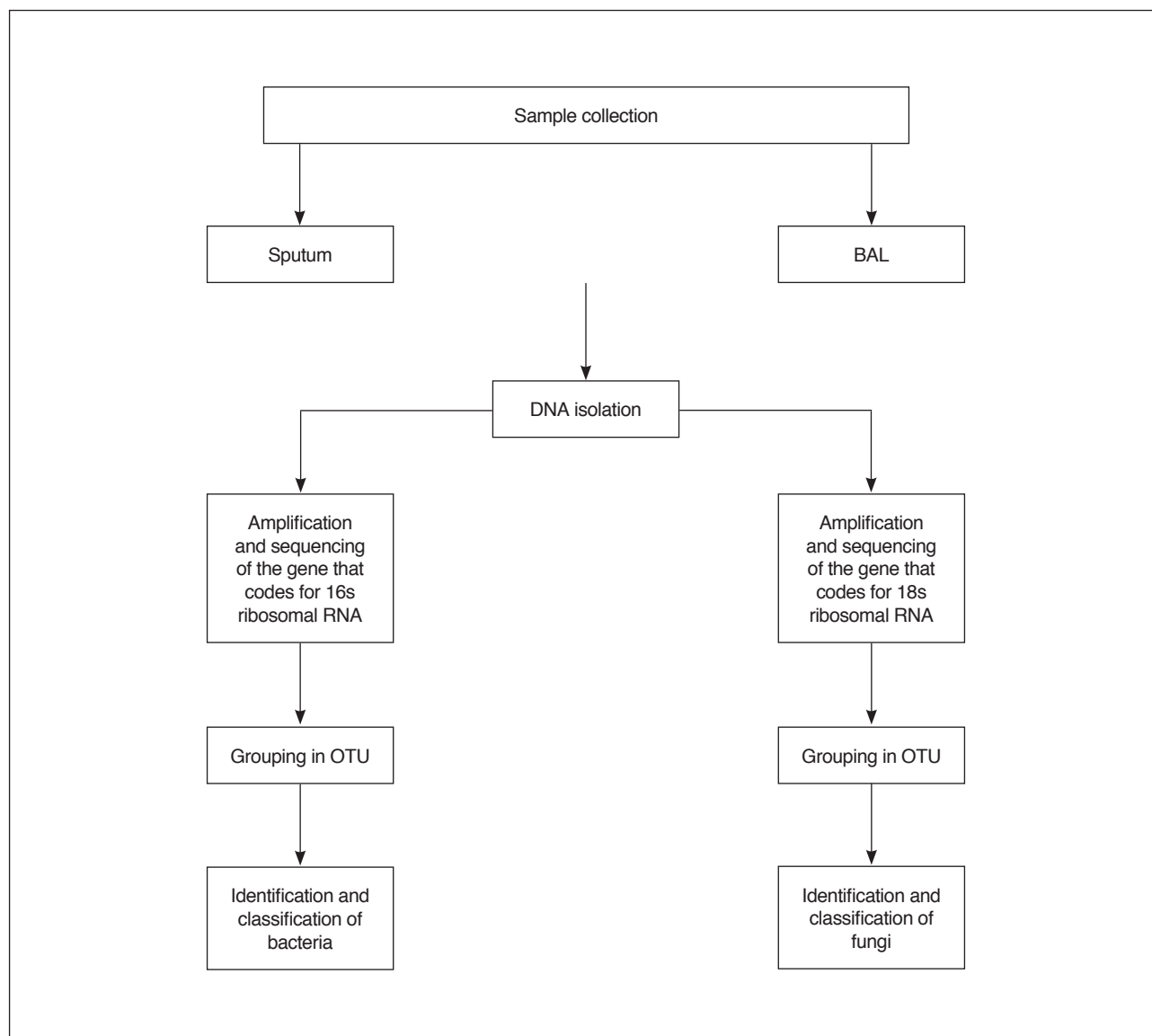


**Figure 1 (LECT 37).** New methods to study the microbiota.

*Prevotella*, *Fusobacteria* and *Streptococcus* with the presence of small quantities of potential pathogens such as *Haemophilus* and 3) with respect to fungi: *Aspergillus*, *Cladosporium*, *Penicillium* and *Eurotium*. The composition of lung microbiota depends on a multitude of factors, some of particular importance, such as: 1) microbial migration (micro-aspiration, inhalation of microorganism, direct mucosal dispersion), 2) microbial elimination (cough, mucociliary clearance, innate and adaptive immunity), 3) conditions of regional growth (availability of nutrients, temperature, $O_2$ tension, local microbial competition, concentration and activity of inflammatory cells). The balance of these factors and the first two, in particular, it is currently considered the critical element of the microbiota composition in healthy lungs. Factors of less importance are represented by: host genetics, living environment, spatial heterogeneity in the different lung districts concerning the anatomy of respiratory apparatus, drugs administration, antibiotics, anti-inflammatory, and corticosteroids. The inappropriate use of these drugs can lead to significant alterations of the microbiota: 1) return of the original composition (resiliency), 2) permanent alteration similar from the functional point of view (reactance), 3) permanent alteration of both composition and function. The composition and the function of lung microbiota are particularly crucial during the neonatal period. Today, there are several pieces of evidence, such as the finding of bacteria in the placenta, in the amniotic fluid, in fetal membranes, and in the cord blood, showing that lung microbiota is present since birth, disproving the belief that fetal environment is sterile. Since the first days of life, bacterial communities have been found in the oral cavity and the pharyngeal nose tube of at term neonates (*Staphylococcus*, *Streptococcus*, and *Moraxella*) and the respiratory tract of intubated preterm neonates (*Proteobacteria*). Furthermore, the composition seems to be influenced by the anatomical features of the lungs and other factors as well, such as the type of birth (cesarean or vaginal), feeding (breastfeeding or formula), antibiotics administration in the first weeks of life. Recent studies evidenced that the integrity of the composition and the correct maturation of the microbiota in the first period of life can influence the prevention of several pulmonary illnesses or can, in case of alteration, induce different pathological states. Its influence on the gut microbiota represents another particularly important aspect of the function of lung microbiota. The observation that lung microbiota is very similar to that of the oropharynx validates the concept that material coming from the mouth colonizes the respiratory tract of healthy subjects through the mechanism of micro-aspiration and some microorganisms present in the lung derive from the migration of germs from the guts. The predominant Phyla in the lung are *Bacteroides* and *Firmicutes* that are in the same time the first microorganisms that characterize the guts microbiota. Nowadays, it is attributed to the gut microbiota, in addition to this effect on the low respiratory tract, the possibility to affect the functional behavior of lung microbiota through its direct immune-modulation.

**LECT 38**

**THE EXCEPTION CONFIRMING THE RULE**

G. Ottonello[1], F. Bardanzellu[1], M.E. Trudu[1], G.Z. Trapletti[1], G. Masnata[2]

[1]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy

[2]Special Service of Pediatric Urology and Urodynamics, Complex Structure of Pediatrics, Brotzu Hospital, Cagliari, Italy

INTRODUCTION

1. Transfusion-related acute lung injury (TRALI) is a massive noncardiogenic pulmonary edema which leads to acute hypoxemia ($SaO_2 <$ 90%), occurring within six hours after blood component transfusion, in patients without others risk factors for acute lung injury, cardiac dysfunction or fluid overload, pneumonia or severe sepsis; such condition seems to be triggered by antineutrophil cytoplasmic and/or anti-HLA antibodies in donor blood, which can bind to recipient's antigens stimulating an excessive immune response. Its prevalence is not known, due to the small and heterogeneous study samples and the frequent under-diagnosis, especially in premature neonates [1, 2].

2. Prenatal ultrasound diagnosis of fetal urinary tract dilation (UTD) occurs in about 1-5% of pregnancies. It can be associated with several functional or structural uropathies (Congenital Anomalies of the Kidney and the Urinary Tract – CAKUT). Prenatal diagnosis may orient the

clinicians to strict clinical surveillance and precocious treatment, avoiding potential acute complicances, even acute kidney injury, and long-term sequelae, including chronic renal failure [3].

CASE REPORT 1

M. is a male neonate, born at 38 weeks of gestational age (GA) by urgent cesarean section for cardiotocographic anomalies, Apgar 8-8, 2.690 g. Immediately after parturition, he underwent nasopharyngeal aspiration, stimulation and supplementary oxygen administration ($FiO_2$ 0.25). Since birth, M. presented cutaneous and mucous pallor, generalized hypotonia, tachypnea (about 65/min), tachycardia (about 170/min) and intercostal returns. His first hemoglobin (Hb) value was 4.8 g/dl, with a hematocrit of 14%, allowing the diagnosis of severe neonatal anemia. Maternal Hb value was in range, there was not hemogroup incompatibility, and direct Coombs exam was negative. Echocardiography performed in the first phases of life showed iso-systemic pulmonary hypertension, a patent ductus arteriosus with bidirectional shunt and a normal left ventricular contractility. Chest radiography at one hour of after birth showed an increase in lung volume

and inflation, diffuse parenchymal mild opacities (**Fig. 1**). Abdominal and cerebral ultrasound examination resulted normal. During the following hours, M. suffered from hypoglycemia, treated with dextrose, and its respiratory situation resulted clinically stationary, requiring to continue $O_2$ administration ($FiO_2$ 0.28-0.30%). At 3 hours of life, he received a transfusion of 30 ml of leukodepletion concentrate erythrocytes of the compatible group, through an umbilical venous catheter. Unfortunately, after one hour since the end of transfusion, M. presented a severe sudden worsening of respiratory distress, desaturation ($SaO_2$ reaching 70%), a severe diffuse reduction of vesicular murmur and marked jugular, intercostal and epigastric returns; therefore, he needed supplementary $FiO_2$ of 0.60 to reach a $SaO_2$ value of 90%. C-reactive protein and blood cultures were negative, electrolytes, hepatic and renal functionality were normal. Chest radiography was repeated and showed the severe worsening of diffuse parenchymal opacities and infiltrates (**Fig. 2**). Due to such findings, we suspected a TRALI; a supportive treatment was provided, and the newborn was transferred in neonatal intensive unit care to start mechanical ventilation support.
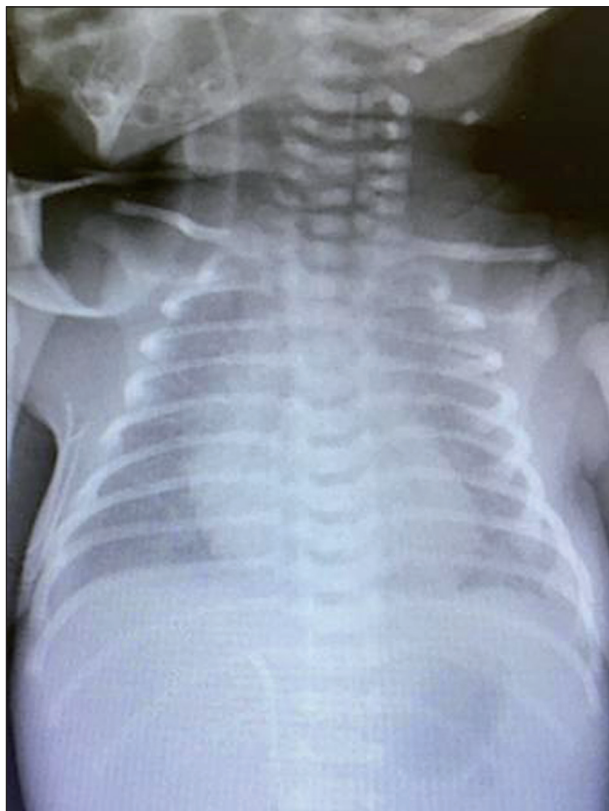


**Figure 1 (LECT 38).** Chest radiography performed at one hour after birth. Increase in lung volume and inflation, diffuse parenchymal mild opacities.
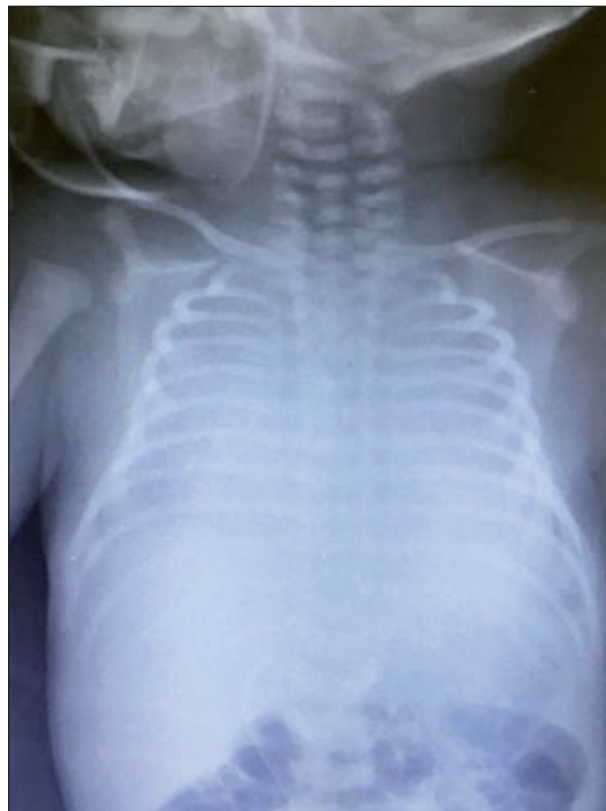


**Figure 2 (LECT 38).** Chest radiography performed after one hour from the end of the transfusion. Severe worsening of diffuse parenchymal opacities and infiltrates.

CASE REPORT 2

S. is a male neonate, born at 37 weeks by cesarean section, after a pregnancy complicated by gestational diabetes and fetal overdevelopment (3,790 g); Apgar 8-10. At 34 wks GA, prenatal ultrasound diagnosis of right kidney malformation CAKUT was proposed, due to the description of a hypertrophic left kidney and a right kidney resembling a multicystic dysplastic kidney (MCDK), even if the differential diagnosis with a UTD could not be excluded; the bladder showed a partial emptying while the quantity of amniotic fluid was normal. After birth, an ultrasound exam confirmed a diffuse right MCDK, a left hypertrophic kidney (maximum diameter of 5.5 cm, 97th centile) and a dilated bladder without emptying. To avoid acute urinary retention, a bladder catheter was positioned. The urine exam resulted in range, and prophylaxis with amoxicillin/clavulanic acid at the dose of 20 mg/kg was started. In such conditions and with the vesical catheter *in situ*, at 3 days of life, he was transferred to our neonatal pathology section. Through an ultrasound exam, we did not confirm the previous findings, since we described a UTD characterized by a severe right urethra-calycopielyc dilation (stage T3) with reduced parenchymal thickness, hypertrophic left kidney with normal morphology and a significant increase in mucosal bladder thickness and trabeculation (**Fig. 3**).

Considering these elements, we suspected the presence of posterior urethral valve resected during catheterization, since the newborn showed urinary retention until this procedure. However, in this perspective, the reason of monolateral UTD instead of bilateral damage remains unclear. S. will be evaluated in follow-up. Up to now, he shows a preserved renal function in the absence of urinary tract infections.

CONCLUSIONS

1. TRALI is a rare life-threatening and potentially fatal cause of transfusion-related morbidity and mortality. Early recognition allows precocious treatment, usually requiring ventilation and hemodynamic support. Resolution generally occurs within 96 h, although death may occur [2].

2. Among the CAKUT, the presence of posterior urethral valve (PUV) is the most frequent cause of lower urinary tract obstruction in boys, associated to bilateral UTD and bladder hypertrophy, potentially leading to chronic renal failure. PUV can be suspected in fetal life through ultrasound evaluation; after birth,

**Figure 3 (LECT 38).** Bladder. Great increase in mucosal thickness and trabeculation.

primary valve ablation is the treatment of choice [4].

REFERENCES

[1] Cho MS, Sharma S. Transfusion, Acute Lung Injury. [Updated 2018 Jun 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018.

[2] Muhammad TJ, Zehra D, Qalab A, Humaira J, Farheen KM, Anwarul H. Transfusion-related acute lung injury in a paediatric intensive care unit of Pakistan. J Ayub Med Coll Abbottabad. 2017;29:4.

[3] Perlman S, Roitman L, Lotan D, Kivilevitch Z, Pode-Shakked N, Pode-Shakked B, Achiron R, Dekel B, Gilboa Y. Severe fetal hydronephrosis: the added value of associated congenital anomalies of the kidneys and urinary tract (CAKUT) in the prediction of postnatal outcome. Prenat Diagn. 2018; 38:179-83.

[4] Sung JK, Jaeyoon J, Chanwoo L, Sejun P, Sang HS, MD, Hye-Sung W, Kun SK. Long-term outcomes of kidney and bladder function in patients with a posterior urethral valve. Medicine. 2018;97:23.

**LECT 39**

## DIARRHEA, GASTROENTERITIS, AND INFLAMMATORY BOWEL DISEASE: THE RATIONALE OF PROBIOTICS

M. Corpino

*Pediatrics and Rare Diseases Clinic, Microcitemico A. Cao Pediatric Hospital, AOB Cagliari, Cagliari, Italy*

Probiotics are defined as live non-pathogenic microorganisms that, when administered in adequate amounts, can replicate and colonize the gastrointestinal tract in sufficient numbers and may confer health benefits to the host. Probiotics are considered biomodulators of the intestinal microbiota.

Microorganisms, to be defined as probiotics, have to be of human origin, resists 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. Probiotics compete for nutrients used by the other incoming bacteria thus providing an antimicrobial protective barrier; they may also interfere with the adherence of pathogenic bacteria, increase the physical and immunological barrier function of the intestine, increase mucus production, decrease ischemic injury and modulate the inflammatory response.

Acute gastroenteritis is usually defined as a decrease in the stool consistency (loose or liquid) and/or an increase in the frequency (typically > 3 stools/day), with or without vomiting or fever. Diarrhea typically lasts less than 7 days and not longer than 14 days [1].

The ESPGHAN guidelines recommend for the management of acute gastroenteritis in children the use of *L. rhamnosus GG* and *S. boulardii*. There is strong evidence in decreasing duration and severity of acute gastroenteritis with the use of these probiotics [1]. Similar recommendations have been published in the United States, in South America, in Latin America, and the Asia-pacific region. Antibiotic-associated diarrhea (AAD) may be a frequent complication during antibiotic therapy; it is defined as diarrhea that occurs concerning antibiotic treatment after the exclusion of other etiologies. A 2015 *Cochrane* review acknowledged to the *L. rhamnosus GG* and *S. boulardii* a preventive role in diarrhea associated with antibiotic therapy. In 2016 the ESPGHAN Working Group (WG) on Probiotics strongly recommend the use of *L. rhamnosus GG* and *S. boulardii* for the prevention of AAD in children. The WG suggests the use of *S. boulardii* for preventing *C. difficile*-associated diarrhea [2]. Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disorder of the gastrointestinal tract, comprising ulcerative colitis (UC) and Crohn's disease (CD). There is limited evidence favoring the use of VSL#3 or *L. reuteri ATCC 55730* as an adjuvant to standard therapy for induction of remission in mild to moderate pediatric UC. There is evidence favoring the use of VSL#3 or *E. coli Nissle* as an alternative to 5-ASA therapy in the maintenance of remission in mild to moderate pediatric UC, especially in mesalazine intolerance. In adults, VSL#3 has shown efficacy in preventing pouchitis and maintaining antibiotic-induced remission of pouchitis. The use of probiotics in the induction or the maintenance of remission of the pediatric CD is not recommended [3]. Future studies are needed to confirm whether probiotics have a definite role in the induction or maintenance of remission in CD and UC. The effectiveness of 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. There is insufficient data concerning 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.

## REFERENCES

[1] Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H; European Society for Pediatric Gastroenterology, Hepatology, and Nutrition; European Society for Pediatric Infectious Diseases. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update 2014. J Pediatr Gastroenterol Nutr. 2014;59(1):132-52

[2] Szajewska H, Canani RB, Guarino A, Hojsak I, Indrio F, Kolacek S, Orel R, Shamir R, Vandenplas Y, van Goudoever JB, Weizman Z; ESPGHAN Working Group for Probiotics Prebiotics. Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Children. J Pediatr Gastroenterol Nutr. 2016;62(3):495-506.

[3] Miele E, Shamir R, Aloi M, Assa A, Braegger C, Bronsky J, de Ridder L, Escher JC, Hojsak I, Kolaček S, Koletzko S, Levine A, Lionetti P, Martinelli M, Ruemmele F, Russell RK, Boneh RS, van Limbergen J, Veereman G, Staiano A. Nutrition in Paediatric Inflammatory Bowel Disease: A Position Paper on Behalf of The Porto IBD Group of ESPGHAN. J Pediatr Gastroenterol Nutr. 2018;66(4):687-708.

**LECT 40**

## IRON AND GASTROINTESTINAL DISEASES

M.G. Clemente

*Pediatric Clinic, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy*

Duodenal mucosa represents a key step in the regulation of body iron homeostasis. Unsurprisingly, in several gastrointestinal diseases, intestinal mucosa damage leads to iron deficiency anemia (IDA). Iron deficiency reduces appetite, impairs children's potential growth, physical activity, school performance and the overall quality of life. However, the complexity of the clinical pictures makes a diagnosis of iron deficiency sometimes challenging, especially for selecting the most appropriate treatment. First level laboratory analysis of blood, urine and stool samples should include all the markers to assess the nutritional and iron status, plus the markers of inflammation [1]. All children with iron-refractory IDA, in whom genetic and hematological causes have been excluded, might need to be investigated for gastrointestinal diseases, including infections, as *G. lamblia* and *H. pylori*, cow milk proteins intolerance or other food allergies, celiac disease (CD), inflammatory bowel diseases (IBD), autoimmune atrophic gastritis and cystic fibrosis (CF).

Malabsorption is the main underlying mechanism in *G. lamblia* infected children and celiac disease. Hypochlorhydria and chronic inflammation are among the factors leading to IDA in *H. pylori* infection and autoimmune atrophic gastritis. In IBD, anemia is most often due to iron deficiency, for both blood loss and malabsorption, and/or chronic inflammation; important to note that the severity of anemia in IBD is a known prognostic factor indicating more extensive and more severe disease. Inadequate nutrition, malabsorption, chronic inflammation, and infection are among the many factors responsible for anemia in CF children. Iron absorption is highly regulated at duodenal mucosa. Iron transporters are located both at the apical (divalent metal transporter 1, DMT1) and basal (ferroportin, FPN) membrane of the enterocytes [2]. The hepatic hepcidin is the main hormone acting on intestinal iron absorption. Erythropoiesis rate and total body iron status influence hepcidin's expression, which results low when the body iron level is low and high when the body iron level is high. By binding to FPN, hepcidin effectively blocks iron absorption by inhibiting the transfer of intracellular iron from enterocyte, hepatocytes, and macrophages into circulation: the total body iron amount is normal or high, but it remains in storage. In the presence of iron shortage, low erythropoiesis rate and the consequent tissue hypoxia inhibit hepatic hepcidin production, FPN activity increases as well as iron absorption. Tissues expressing transferrin receptors can catch iron bound to transferrin in circulation. Beside genetic defects associated with high hepcidin expression, chronic inflammatory states and infections are associated with high hepcidin levels and characterized by IDA-like microcytic anemia despite normal or high iron storage. Serum ferritin, total iron binding capacity, transferrin saturation and, when available, serum transferrin receptors and the transferrin receptors-ferritin index are the laboratory tests that are helpful to determine the true iron status and to differentiate between IDA and anemia secondary to chronic inflammation, or the co-existence of both. Treating the underlying gastrointestinal disease might reverse a mild iron deficiency status. However, iron supplementation, oral or intravenous, could be recommended, depending on etiology and severity. Over the last decade, the availability of oral liposomal iron preparations by which iron can enter into the lymphatic circulation through the M cell lying on the surface of the entire small bowel mucosa, bypassing the sophisticate regulated mechanisms of the duodenal mucosa, has overcome the majority of oral iron adverse-effects, showing, at the same time, a high bioavailability. Promising results from clinical trials (NCT0186416; NCT02390921) have shown oral liposomal iron as a safe and efficacious alternative to intravenous iron, at least at short term [3].

REFERENCES

[1] Bouri S, Martin J. Investigation of iron deficiency anemia. Clin Med (Lond). 2018;18(3):242-4.

[2] Prentice AM. Clinical Implications of New Insights into Hepcidin-Mediated Regulation of Iron Absorption and Metabolism. Ann Nutr Metab. 2017;71(Suppl 3):40-48.

[3] Pisani A, Riccio E, Sabbatini M, Andreucci M, Del Rio A, Visciano B. Effect of oral liposomal iron versus intravenous iron for treatment of iron deficiency anaemia in CKD patients: a randomized trial. Nephrol Dial Transplant. 2015;30(4):645-52.

**LECT 41**

## HOW TO FEED THE GUT MICROBIOTA

G. Biasucci

*Mother and Child Health Department, Pediatrics and Neonatology Unit, "Guglielmo da Saliceto" City Hospital, Piacenza, Italy*

Host genetics, maternal epigenetic positive factors and vaginal delivery work in concert to shape the neonatal gut microbiota homeostasis, also known as "eubiosis". The type of feeding also plays a key role in the development of an infant's gut microbiota. Indeed, breastfeeding is undoubtedly the best way to promote the healthy development of a human offspring and also of infants' gut eubiosis, modulating the infant's early gut colonization, both by human milk microbiome and other unique nutritional components contained in human milk, e.g. oligosaccharides and lactoferrin, also known as prebiotic or bifidogenic factors. Consequently, breastfed infants are more colonized by *Bifidobacteria spp.* than their formula-fed counterparts, who have a higher microbial diversity, including *Clostridium*, *Bacteroides*, *Prevotella*, *Lactobacillus* and *E. coli spp.* [1]. The human milk microbiota composition may, in turn, be influenced by the bacterial entero-mammary pathway, as well as by the infant coming into contact with the mother's breast skin. Based on this, some infant formulae have been supplemented either with prebiotics such as galacto- and fructo-oligosaccharides and/or probiotics, in order to achieve a gut microbial composition more similar to that of breastfed babies. Despite evidence of some favorable clinical effects, these supplementations are still considered as "optional" ingredients in infant formulae [2, 3]. When human or starting formula milk is no longer enough to meet infants' nutritional needs, complementary foods are added to the diet. As a consequence, the gut microbial composition shifts from *Bifidobacterium* predominance to *Bacteroidetes-* and *Firmicutes-* dominant colonization. From this moment onwards, each type of dietary macronutrients (proteins, fibers, fat, carbohydrates) influences the gut microbiota specifically [4]. Though changes are observed more at a metabolic level than at a taxonomic level, transient modifications of the gut microbiota can be associated with each macronutrient. For example, dietary fiber intake leads to an increase in butyrate-producing species and *Bifidobacteria*, *Lactobacilli* and *Bacteroidetes* proportion whereas a high protein and low carbohydrate diet decreases butyrate-producing species and increase *Bacteroides spp.* with proteolytic activities. Moreover, a high-fat diet stimulates the production of bile acids and selects the growth of species able to metabolize bile acids. Thus, the major macronutrient in a given diet can stimulate the growth of specific microbial species in the gut. In the light of this, vegetarian and vegan diets, characterized by a high carbohydrate content with a lower protein and fat content, lead to a gut microbiota dominated with carbohydrate fermenting bacteria, such as the *Prevotella*, *C. clostridioforme*, and *F. prausnitzii*. Moreover, the vegan diet shows a decrease in *Bacteroides*, *Bifidobacteria* and *Enterobacteriaceae spp.* Conversely, omnivorous diets, with a higher protein and fat content, lead to an increase of bile tolerant bacteria such as *Bacteroides* and butyrate-producing bacteria. An increase of *Bacteroides* and a decrease of *Prevotella* have also been associated with dietary regimens providing high fat and processed carbohydrate and low fiber intake, such as western diets. On the other hand, high fiber and complex carbohydrates diets are associated with an abundance of fermenting bacteria such as *Prevotella*, which in turn seems to be a discriminatory species between high and low carbohydrate diets. Overall, fiber intake is a major dietary determinant of favorable gut composition, being used by some microbial species to produce short-chain fatty acids (SCFAs), which have anti-inflammatory, anticarcinogenic, and immune-regulatory functions. As aforementioned, vegan, vegetarian and Mediterranean diets are more likely to provide high amounts of fiber and, as such, to increase gut levels of SCFAs by selecting specific groups of beneficial bacteria. In conclusion, diet composition, by modulating the gut microbial community, may represent a major tool to maintain gut eubiosis, which is in turn linked to a healthier outcome later in life. Indeed, though many variables may modulate gut microbiome and metabolome and a unique healthy food recipe is therefore not available, the well-known epigenetic effect of food choice may also be due to its effect on gut microbiota [5, 6].

## REFERENCES

[1] Gritz EC, Bhandari V. The human neonatal gut microbiome: a brief review. Front Pediatr. 2015;3:17.

[2] Skórka A, Pieścik-Lech M, Kołodziej M, Szajewska H. Infant formulae supplemented with prebiotics: Are they better than unsupplemented formulae? An updated systematic review. Br J Nutr. 2018;119(7):810-25.

[3] ESPGHAN Committee on Nutrition: Braegger C, Chmielewska A, Decsi T, Kolacek S, Mihatsch W, Moreno L, Piescik M, Puntis J, Shamir R, Szajewska H, Turck D, van Goudoever J. Supplementation of Infant Formula With Probiotics and/or Prebiotics: A Systematic Review and Comment by the ESPGHAN Committee on Nutrition. JPGN. 2011;52:238-50.

[4] Alou MT, Lagier J-C, Raoult D. Diet influence on the gut microbiota and dysbiosis related to nutritional disorders. HuM Microb J. 2016;1: 3-11.

[5] Ferrario C, Statello R, Carnevali L, Mancabelli L, Milani C, Mangifesta M, Duranti S, Lugli GA, Jimenez B, Lodge S, Viappiani A, Alessandri G, Dall'Asta M, Del Rio D, Sgoifo A, van Sinderen D, Ventura M and Turroni F. How to Feed the Mammalian Gut Microbiota: Bacterial and Metabolic Modulation by Dietary Fibers. Front Microbiol. 2017;8:1749.

[6] Ercolini D, Fogliano V. Food Design To Feed the Human Gut Microbiota. J N Agric Food Chem. 2018;66:3754-8.

## LECT 42

## HUMAN MILK, BACTERIA, AND ALLERGIC OUTCOMES

D. Peroni, M.E. Di Cicco, P. Comberiati

*Department of Clinical and Experimental Medicine, Section of Paediatrics, University of Pisa, Pisa, Italy*

Human milk is the first nutrient source for the newborn and is considered to be beneficial for the developing infant. Advantages of breastfeeding are comprehensive of transference of several immune components that may interfere with the maturation of gut immunity providing anti-inflammatory effects. Therefore, the relevance of breastfeeding in the maturation of the immune system and of the gut mucosal barrier of the newborn is widely recognized. Since the gut is immature and needs early signals from the environment to develop optimal mucosal barrier function, colostrum is relevant from in the first days of life. Colostrum is a known source of immune mediators for the newborn within the first week of life, comprehensive of factors such as HGF, TGF-$\beta$ [1-3], and IgA that are considered key immunological components of colostrum. These factors stimulate and orientate the neonatal gastrointestinal and immune system development [3, 4]. The role of breastfeeding itself and its duration in the prevention of allergies has been studied for a long time, but results are still contradictory. A further aspect regards the microbiological composition of milk (microbiome) that should be considered as part of the stimulation of the immune system from the first days of life. Atopic diseases (atopic dermatitis, asthma, food allergy, rhinitis) are spread worldwide showing a rising prevalence as well as others so called not communicable diseases. It has been elucidated that, among several factors, some constituents present in human milk may be protective against atopy, while others may increase the risk of allergic susceptibility. There are still controversies on the effects of breastfeeding in preventing these diseases as well as the other, not communicable diseases. Discussion regards the optimal duration of exclusive breastfeeding, exclusivity itself, microbial exposure and their association with the risk of atopy. The WHO (World Health Organization) recommends in its guidelines the exclusive breastfeeding for at least 6 months, whereas several scientific international organizations recommend its exclusivity for 4 months. Among different risk factors, the maternal allergy history is considered a relevant one for the development of asthma, allergic rhinitis and eczema. However, little information is known on the influence of the allergic status of the mother *per se* on the composition of cytokines, growth factors, IgA in the human milk. This could be relevant since there is strong evidence that colostrum and human milk are rich of multiple factors, humoral and cellular immunity components, that are required for newborn's growth [3, 4]. Colostrum and human milk provide an appropriate microenvironment, rich in microbiological flora, that is directly involved in the microbiological and immunological maturation of the gut since the first few days of life. Also, this factors may play a pivotal role in affecting the immune system in order to prevent allergies [5]. Breastfeeding promotes active and passive stimulation of the immune system by its biologically active molecules, with a balance between stimulatory and suppressive signals [5]. In an animal model, it has been demonstrated that enterocytes incubated with colostrum have a higher proliferation rate than cells incubated with mature milk. This result may indicate that the composition of human milk may be so different in different phases of lactations containing different levels of immune active and growth factors. Human milk contains antibodies, predominantly secretory immunoglobulin A (s-IgA), glycoconjugates and oligosaccharides, living cells, antioxidants and fatty acids (FA), nutrients, cell surface homologues, glutamine and dietary nucleotides, lactoferrin, hormones, growth factors, anti- and pro-inflammatory cytokines and food proteins which mother have been exposed with [5]. All these factors may influence the allergic outcome of the offsprings. Furthermore, other factors such as an allergic status of the mother, drugs in pregnancy, infections, mastitis, stress, type of delivery and supplementations (fish oil, vitamins, and probiotics) may have a role in influencing the composition of human milk. Complexity and variability in human milk composition may explain the conflicting results of studies evaluating the effect of prolonged exclusive breastfeeding and the prevention of

allergic disease development. Researchers need to account for variations in human milk (active immune molecules, PUFA's, microbiome composition). Understanding the relationship between HM composition and development of non-communicable diseases, such as allergy, may allow improvements in allergy prevention research, considering possible modulation of HM composition via dietary interventions, in order to promote healthy infant immune development [6].

## REFERENCES

[1] Duchén K, Casas R, Fagerås-Böttcher M, Yu G, Björkstén B. Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin A antibodies and early childhood allergy. Pediatr Allergy Immunol. 2000;11:29-39.

[2] Greer FR, Sicherer SH, Burks AW. American Academy of Pediatrics Committee on Nutrition, American Academy of Pediatrics Section on Allergy and Immunology: Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, the timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics. 2008;121:183-91.

[3] Prokesová L, Lodinová-Zádníková R, Zizka J, Kocourková I, Novotná, O, Petrásková P, Sterzl I. Cytokine levels in healthy and allergic mothers and their children during the first year of life. Pediatr Allergy Immunol. 2006;17:175-83.

[4] Matson AP, Thrall RS, Rafti E, Lingenheld EG, Puddington L. IgG transmitted from allergic mothers decreases allergic sensitization in breastfed offspring. Clin Mol Allergy. 2010;8:9.

[5] Munblit D, Peroni DG, Boix-Amorós A, Hsu PS, Van't Land B, Gay MCL, Kolotilina A, Skevaki C, Boyle RJ, Collado MC, Garssen J, Geddes DT, Nanan R, Slupsky C, Wegienka G, Kozyrskyj AL, Warner JO. Human Milk and Allergic Diseases: An Unsolved Puzzle. Nutrients. 2017;9(8):E894.

[6] Bardanzellu F, Fanos V, Strigini FAL, Artini PG, Peroni DG. Human Breast Milk: Exploring the Linking Ring Among Emerging Components. Front Pediatr. 2018;6:215.

**LECT 43**

## INFANT EXPOSURE – HISTORICAL AND AN-THROPOLOGICAL NOTES

L. Cataldi, M.G. Gregorio

*Study Group on the History of Paediatrics of the Italian Paediatric Society*

## HISTORICAL NOTES

The abandonment of infants has been a social issue since ancient times. From the first provisions made by the clergy, with the instructions contained in the will of Archpriest Dateo in Milan in the 8th century, until the establishment of public welfare services in the 20th century, orphans and abandoned children have been placed into orphanages.

The children found by members of the clergy on the steps of churches entered the monastic family, joining the ranks of 'God's pupils'. Ten days after the abandonment, their natural parents lost all rights to them. As provided at the time, "No-one may claim an abandoned newborn as his or her own. But without distinction, those that are brought up in this way …must be considered to be free …" (*Corpus Iustinianeum*). In Rome, so many were the small corpses found in the river Tiber that Pope Innocent III (1198) decided to utilise part of the Hospital of Santo Spirito in Sassia to host abandoned children.

## SOCIAL AND ANTHROPOLOGICAL REMARKS

In all cultures and times, already in ancient Greece and later among the Romans, baby girls were more likely to be abandoned. Often the abandoned children were placed in communities, 'Spitali', under the care of women, adults, mothers, widows, wet-nurses, women with no role, or pregnant women cast out by their families, unmarried mothers, often servants who had been raped, the victims of violence, incest or adultery. In the 'Spitali' were hosted the children abandoned in the infamous 'foundling wheels' or hatches. The last of these wheels, constructed in 1660 was in use until 1875. These communities and their archives are inexhaustible sources of social history information: we can learn about daily life in these female communities, the practices relating to maternity and to the care of infants and even the life stories of some of the young women.

Often, the mothers who abandoned their newborns would leave a 'mark', a jewel, or rather half of it, as proof of their maternity, hoping to be able to claim the child back one day. Cohabitation of the wet nurses, babies and young girls was not always easy. One century after establishment of one such community to prevent infanticide in 1445, out of 968 girls assisted, 400 were more than 25 years old. The attempt to send the oldest ones out into the world with a small dowry was fruitless: the numbers in the communities kept rising rapidly. Many residential facilities were needed. Initially, existing buildings were adapted to this use, but very soon, in various Italian cities, architects and politicians planned specific buildings for abandoned infants, a worrying and painful social scourge already in the 15th century. Filippo Brunelleschi created in Florence in Piazza Santissima Annunziata, the Ospedale degli Innocenti as an 'ideal citadel'. However, the white, austere spaces soon became filled with life and needed some functional extensions. The original design was altered by adding the foundling wheel, not in the centre but in the south-eastern corner,

where the 'exposed' infants were received, with a ritual symbolic almost of a new birth, by means of the 'speaking machine' in the rooms in which the children already abandoned and the wet nurses lived. Chronicles of the time make a comparison with Jesus laid in the manger. The purpose was to give privacy to the act of abandonment, to ensure anonymity for the woman and hide the social stigma associated with birth out of wedlock. Abandonment was a particular event, but also a special architectural space, made more gentle and pure through art. Since these orphanages were mostly linked to the Church and to charitable institutions, they all displayed numerous crucifixes, tabernacles, statues of the Virgin with Child and Nativities, linked to the ceremonies celebrated in the churches of the Spitali. The statues and ex-votos offer precious historical, artistic and ethnographical and anthropological testimonials. The statues of the Virgin or of Saint Anne are depicted as mothers or wet nurses, wearing lucky charms of red coral, often also found on the statues of Baby Jesus. The swaddling clothes, the hairstyles, the clothes, talismans and identification marks tell us much about life in these institutions, about the mothers' fear or losing their milk, and also the heartrending fear of some mothers who requested that their child not be given the surname of 'Esposito' (meaning 'exposed'), which was a shameful hallmark, in the hope that their child's future would be brighter than theirs. Others hoped to reclaim their child at some point. All hoped their children would be treated kindly and receive a proper education. Alas, most often this was not the case. Now the wheels have reappeared in some Italian cities, especially in the centre-north, in a different social and historical period and under very different economic conditions. What remains unchanged, however, is the feeling of loneliness experienced by some pregnant women. Even today, in 2018, pregnancy and birth are still at times a female issue in the choice of whether to continue the pregnancy, terminate it, give up the child for adoption or even abandon it.

REFERENCES

• Cataldi L. L'esposizione della prole nell'antica Roma. Riv Ital Pediatr. 1999;25(Suppl 4):167.

• Cataldi L, Gregorio MG, Corridori M, Fanos V. Infanticide and infant exposure in the ancient Roman and Greek societies: a social problem? Biol Neonat. 2004;86:4.

• Cataldi L. L'abbandono dei bambini dall'antica Roma ai primi 'spitali' medievali Roma. Atti del 2° Corso 'Novità nella Storia della Pediatria' Convegno Nazionale del Gruppo di Studio di Storia della Pediatria della Società Italiana di Pediatria, 2006, pp. 1-3.

• Semprini A, Cataldi L. Abbandono e infanticidio… una storia infinita. Poster 65° Congresso Italiano di Pediatria, 27-30 Novembre 2009, Padova. Minerva Pediatr 2009;61(6):589-91.

## LECT 44

## THE BABY HATCH OF THE THIRD MILLENNIUM

P. Paolillo[1], F. Di Palma[2], S. Picone[1]

[1]Neonatology-NICU, Policlinico General Hospital, Rome, Italy
[2]University of Rome, Medical School, La Sapienza, Rome, Italy

On December 6, 2006, at the Policlinico Casilino General Hospital in Rome, a protected structure called "Do not abandon him, entrust us" was inaugurated, aimed at welcoming and assisting the babies abandoned incongruously. We wanted to offer the structure, the equipment, the organization to the women who do not know, cannot do not want, for the most different reasons, to follow the path that the Italian law allows: that is the right to give birth in a hospital in complete anonymity and safety. The baby hatch is a prefabricated structure, easily reachable, formed by two small rooms; it offers total anonymity to the women who leave their baby, and maximum security to the small child outside the hospital. The mother who decides to leave a child enters a room without passing through control, must open a tilting window and lay him in a cradle located in an adjoining room, which is maintained at a constant temperature in all four seasons. An alarm, activated by a volumetric and contact sensor, immediately draws the attention of the first aid triage operators. A camera frames only the bed where the child lies and does not take the face of the person who puts it.

Nurses with a neonatologist arrive in a few minutes on the opposite side to the one from which the mother will come out. The phenomenon of the neonatal abandonment in our territory is a severe problem [1]. The available data indicate that the number of drop-outs at the Policlinico Casilino of Rome is the highest in the entire city. There were also several cases recorded by the news for the very high-risk methods with which the babies were carried out (abandonment in bins). The statistics of the neonatology department of the Policlinico Casilino, compared to those of the entire Lazio Region, show a high number of births by foreign women. The largest foreign colony has been and still is the Romanian 46%, then the Chinese and the Serbian Montenegrin with 5.5%, the Nigerian and Albanian

with about 4.5%. Together with the official data, however, we must report a large number of illegal immigrants, those invisible citizens who live in conditions of severe economic and social hardship [2]. Many of these are women, who are waiting for a child to find themselves without the possibility of working, alone, sometimes reduced to slavery and threatened, with the fear of turning to public institutions as they fear being deceived by them. These women experience moments of extreme difficulty and sometimes find themselves tragically forced to abandon their babies [3]. To help these mothers, in order to save small lives, this modern and technological "wheel" has been established, which does not want to be an invitation to illegal behavior, but only rescue so that an unwanted pregnancy does not end in tragedy.

## REFERENCES

[1] Osservatorio Ninna HO. Dati sul fenomeno dei bambini non riconosciuti alla nascita. Fondazione Rava-Sin. Available at: https://www.ninnaho.org/wp-content/uploads/2017/05/Osservatorio.pdf, last access: September 2018.

[2] Scott P, Robinson K, Mongkolrattanothai K, Camp T. Medical evaluation of abandoned infants less than 61 days of age. Hosp Pediatr. 2015;5(5):282-6.

[3] Sherr L, Mueller J, Fox Z. Abandoned babies in the UK – a review utilizing media reports. Child Care Health Dev. 2009;35(3):419-30.

**LECT 45**

## NON-INVASIVE TEST FOR PRENATAL DIAGNOSIS OF CONGENITAL DISEASES AND PREGNANCY TUMORS

G. Floris

*KU Leuven, University of Leuven, Department of Imaging and Pathology, Laboratory of Translational Cell and Tissue Research, Leuven, Belgium*

Overall, over 4,600 phenotypes linked to a genetic cause are reported in the Online Mendelian Inheritance in Man register. Prenatal genetic testing is usually offered to enable early detection of genetic disorders in order to avoid the transmission of pathogenic genetic variants. To this extent, in the past 50 years, many approaches have been developed. Invasive procedures are currently reserved for fetuses carrying ultrasound detected malformations. In pregnant women at high risk, non-invasive biochemical, serological tests are mostly used as an indirect screening tool to detect the presence of clinically relevant chromosomal imbalances in chromosomes 13, 18, 21. However, with the discovery in 1997 of circulating cell-free fetal DNA and the technological advances in sequencing technologies experienced in the past decades with Next Generation Sequencing (NGS) technique, the non-invasive prenatal genetic testing (NIPT) has become a reality. Indeed, the human blood (and other fluids as well) contains many copies of circulating cell-free genomic material, which originates mainly from normal tissues. During pregnancy, the fraction of circulating cell-free DNA is enriched by DNA fragments originating from apoptotic placental cells (trophoblast-derived), which is then referred to as fetal DNA. The fraction of fetal DNA represents about 3% to 20% of the total circulating DNA and, is highly fragmented with a size distribution of 140-180 bp. Depending on the NGS technique adopted and bioinformatics analysis, it is possible to collect information about the aneuploidy status of only specific chromosomes (e.g., 13, 18, 21) or to increase the level of information by whole-genome sequencing strategies. Between 2010 and 2012 several clinical trials have validated the clinical utility of the NIPT testing as a detecting method for trisomies in chromosome 18 and 21. In 2011 many international societies endorsed the use of NIPT testing, particularly in pregnant women at high risk. The test is offered between 10 and 40 weeks of gestation and is also commercially available. More recently, studies validating the performance of the NIPT testing against first- or second-trimester biochemical, serological screening tests in all-risk pregnant women have emerged in the literature. The conclusion of these studies is similar and highlights the high sensitivity and specificity of the NIPT testing as compared to the biochemical screening tests. In one study the positive predictive value of the NIPT test was 91.67% with a sensitivity of 100% and specificity of 99.4%, by contrast, the second-trimester triple screening test had a positive predictive value of 2.4% with a sensitivity of 54.5% and specificity of 85.9%. Based on these results and to the further cost reductions and technological and algorithmic advances, the NIPT test is since recently reimbursed for all pregnant women by the National Health Care System of some countries such as The Netherlands, Belgium, and Swiss. Interestingly, the large-scale implementation of the NIPT testing to detect fetal aneuploidy has broadened the scope of the test itself. Indeed, the rate of false-positive results in NIPT testing is low and is estimated to be in the order of 0.1-0.2%. Besides technical limitations in the choice of sequencing method, biological explanations are the main underlying reason for such findings including maternal mosaicism or confined placental mosaicism. Cancer

is another important cause of false positive NIPT-testing results. Malignant tumors, as well as fetal and maternal tissues, may release circulating cell-free DNA contributing to the total pool of circulating DNA. In the presence of tumors with genomic aberrations, NIPT testing has the potential to detect tumors in a subclinical phase (or going undetected as symptoms such as tiredness or pain are assumed to be related to pregnancy), enhancing the therapeutic options for these young patients. Multiple studies have recently described the added value of the NIPT testing in this setting. The incidence is of about 1-2 new cancer diagnoses / 1,000 patients tested, with high sensitivity. Cancers detected by the NIPT testing typically include hematological malignancies but also advanced solid tumors such as sarcomas, ovarian carcinomas or breast cancers can be detected. In conclusion, the NIPT testing has high sensitivity and specificity, which eventually resulted in the large-scale clinical implementation of the test for all pregnant woman, reducing invasive techniques. Analysis of circulating cell-free DNA using genome/exome-wide approaches offers the potential to monitor maternal health by detection of cancer-related genomic aberrations. The latter opens up the opportunity in the future to use the NIPT or similar tests as a cancer-screening method also in the general population.

REFERENCES

• Bianchi D, Wilkins-Haug L. Integration of non-invasive DNA testing for aneuploidy into prenatal care: what has happened since the rubber met the road? Clin Chem. 2014;60:78-87.

• Vermeesch JR, Voet T, Devriendt K. Prenatal and pre-implantation genetic diagnosis. Nat Rev Genetics. 2016;17:643-56.

**LECT 46**

**LUNG PATHOLOGY IN THE NEWBORN: A NEW CT SCAN-BASED SAMPLING METHOD ALLOWS A BETTER ANALYSIS OF THE IMMATURE LUNG IN RESPIRATORY DISTRESS SYNDROME**

G. Faa[1], C. Gerosa[1], R. Garau[2], V. Marinelli[3], C. Moretti[4], F. Cau[5], C. Loddo[3], V. Fanos[3]

[1]Department of Pathology, AOU of Cagliari, University of Cagliari, Cagliari, Italy

[2]Radiology Department, AOU of Cagliari, University of Cagliari, Cagliari, Italy

[3]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, AOU of Cagliari, University of Cagliari, Cagliari, Italy

[4]Professor Emeritus, University "La Sapienza" of Rome, Rome, Italy

[5]Division of Pathology, ATS-ASSL, San Gavino, Sanluri, Italy

The progress in the clinical approach to newborns presenting with lung pathology, and in particular with neonates affected by respiratory distress syndrome (RDS), have induced perinatal pathologists to modify their approach to the neonatal lung. These changes in the methodology utilized to analyze neonatal lungs are focused on the necessity to give more detailed information on the distribution and the intensity of the multiple elementary lesions that are at the basis of RDS, and that give rise to the clinical syndrome and the difficulties in therapy experienced in intensive neonatal care units [1]. This study aims to discuss the modifications of the autoptic procedures carried out in our Department, emerging from the cooperation between pathologists, radiologists, and neonatologists of our University, aimed at increasing the number of information regarding the lung pathology in all neonates. One of the most important progress in clinical practice, when newborns present with a respiratory distress syndrome, is represented by the application of the prone position to suffering neonates [2]. This simple technique, based on the constriction of the newborns to stay prone, has been shown to ameliorate and homogenize alveolar inflation, ending with reduction of the number of atelectatic areas and significant improvement of the clinical outcome. The hypothesis on which this technique is based is that atelectasis might affect the posterior areas of the lungs prevalently. The brilliant results obtained by neonatologists with the application of the prone positions in neonates affected by RDS has induced the medical community to apply the same technique to patients affected by adult RDS (ARDS), with consistent improvement in their outcome [3]. The explanation of the role played by prone position in the therapy of neonatal and adult patients affected by RDS is that this position might favor the expansion of the closed non-functioning alveoli concentrated in the atelectatic zone in the posterior districts, whereas the classical supine position could contrast the expansion of these areas, favoring the persistence of atelectasis and halting alveolar inflation. Starting from this hypothesis, we analyzed the characteristics of the classical pathological approach of the newborn lung, focusing on the ability of the classical approach to evidence the differences in maturation and alveolar inflation of the anterior regions of the lungs, as compared to the posterior zones. The classical approach is based on a principal section extending from the apex to the basis of

each lung, and extending from the coastal face to the hilum. This section divides each lung into two symmetric parts, each of them showing a strict similarity with the anteroposterior projection of a standard radiological examination of the thorax. The histological analysis of this section of the lung does not allow an accurate study of the posterior and of the anterior pulmonary areas, being concentrated on the study of the hilar zones and of the parenchyma of the central areas of the lungs. Contrary to the standard Rx examination, CT scan offers the possibility to study the central as well as the anterior and the posterior regions of the lung, thanks to its ability to show multiple transverse sections of the organs, in each of which it is possible to compare the degree of alveolar inflation and the distribution of atelectatic zones. All these information taken together, we decided to modify the pathological approach to the study of the neonatal lungs, introducing in pathology a CT scan-based approach, in order to improve our diagnostic ability regarding the interpretation of the pathological pulmonary changes occurring in a newborn affected by RDS. To this end, we applied the new technique to ten consecutive neonates affected by severe RDS, submitted for an autopsy. In each newborn, the classical longitudinal approach, based on a section performed from the apex to the basis, was paralleled by multiple transverse sections extending from the posterior toward the anterior margin of the lung. Our preliminary data highlight the different pathological changes obtained with the two techniques, in some cases ending with different pathological diagnoses. Moreover, the CTscan-like approach appeared, in our hands, a more useful tool, able to give more various and useful data regarding the clinicopathological interpretation of all cases of RDS. In particular, the transverse approach evidenced the higher concentration of atelectatic alveoli in the posterior regions of the lungs, both in the superior and in the inferior lobes. Moreover, even hyaline membranes, when present, showed a preferential localization in the anterior lobes, suggesting a higher occurrence of alveolar damage along the anterior pulmonary margins. In two cases, in which hyaline membranes were rare, they were not detected with the classical approach, but they were revealed exclusively by the new technique, being restricted to the anterior regions of the lung. Interestingly, when silver stain and elastic fiber stain were applied to transverse sections, we also evidenced differences in lung maturation between
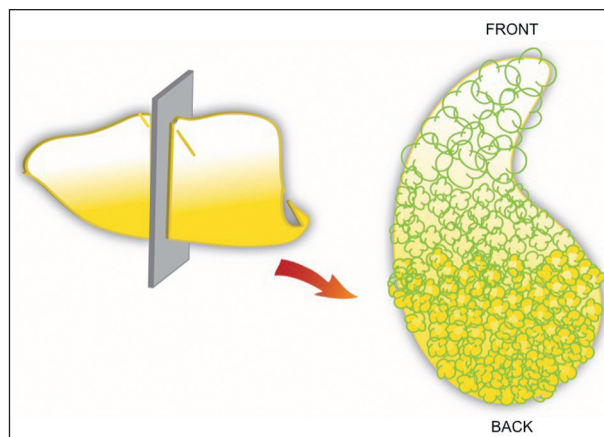


**Figure 1 (LECT 46).** Schematic example of lung sampling.

the anterior and the posterior pulmonary regions: the former showed a higher number of elastic fibers and a lower number of argyrophilic fibers, clearly demonstrating a more advanced stage of maturation, as compared to the posterior lung regions. Moreover, in the posterior areas of the lungs, we evidenced a higher number of muscle-elastic arteries, confirming the delayed maturation of these districts, as compared to the anterior lung districts. Our preliminary data indicate the new approach to the analysis of the neonatal lungs as significantly superior to the classical technique. The main advantages of the CTscan-like pathological approach are the ability of the transverse sections to evidence the differences occurring in the lung of newborns affected by RDS, between the anterior and the posterior zones (**Fig. 1**). Even though the posterior lung districts appeared as the more immature areas of the developing lungs, the regions most frequently affected by alveolar damage and hyaline membranes were the anterior areas, introducing new doubts on the pathogenesis of RDS. As evidenced in two of the newborns here described, the analysis of the newborn lung by the new techniques allowed the identification of pathological changes, like hyaline membranes, that were not detectable in the sections of lung parenchyma analyzed by the conventional approach. In conclusion, our study confirms previous studies on the uneven lung structure and functionality in the newborn lung [4]. Moreover, our data strongly support the clinical approach based on the prone position, that allows better oxygenation of the posterior atelectatic regions of the immature neonatal lung, as well as of the lung in adult subjects affected by ARDS [5]. Further studies are needed in order to verify if the new CTscan-like approach here proposed might be

useful not only in cases of RDS but even in other fields of lung pathology.

REFERENCES

[1] Moretti C, Barbàra CS, Grossi R, Luciani S, Midulla F, Papoff P. Surfactant therapy for acute respiratory distress in infants. J Pediatr Neonat Individual Med. 2014;3(2):e030238.

[2] Pelosi P, Brazzi L, Gattinoni L. Prone position in acute respiratory distress syndrome. Eur Respir J. 2002;20(4):1017-28.

[3] Dickinson S, Park PK, Napolitano LM. Prone-positioning therapy in ARDS. Crit Care Clin. 2011;27(3):511-23.

[4] Cau F, Pisu E, Gerosa C, Senes G, Ronchi F, Botta C, Di Felice E, Uda F, Marinelli V, Faa G, Fanos V, Moretti C, Fanni C. Interindividual variability in the expression of surfactant protein A and B in the human lung during development. Eur J Histochem. 2016;60:2678.

[5] Dirkes S, Dickinson S, Havey R, O'brien D. Prone positioning: is it safe and effective? Crit Care Nurs Q. 2012;35(1):64-75.

**LECT 47**

**STAINED AMNIOTIC FLUID: WHAT'S NEW?**

A.M. Fulghesu, E. Canu, L. Barberini, C. Fattuoni, V. Fanos

*Department of Surgery, University of Cagliari, Cagliari, Italy*

INTRODUCTION

Amniotic Fluid (AF) is as a biofluid containing chemical information for the fetus life status. The staining condition of AF with meconium can be considered as an alarm for poor outcome of the newborns. The spectrum of disorders and pathophysiology of these babies is vast, in the case of Meconium Stained Amniotic Fluid (MSAF). Metabolomics could be the new way to understand the chemical profiles of the MSAF and the related physiological conditions of the fetus. We design a Metabolomics experiment to understand limits and opportunities bring to the Obstetrics and Gynaecology by Metabolomics. [1]

AF is the habitat that hosts the fetus during all the intrauterine life. In most cases, the rupture of the amniotic sac occurs at the end of pregnancy and triggers the start of labor, or occurs during labor. Usually, the liquid that comes out is clear, straw-yellow colored. If it is opaque, greenish or even almost black, and has a slightly fluid consistency, it is called MSAF. It means that the baby's intestine has expelled a significant amount of meconium (the feces of the newborn). In the case of stained amniotic fluid, it is essential to get immediately to the hospital because the changing of color and consistency could indicate fetal distress.

PATIENTS AND METHODS

Patient with homogeneous and clear AF and 12 controls, matching general clinical conditions, were enrolled for this study (patient enrolment is still running). At the moment, a total of 12 clear AF (group 1) and 8 stained AF samples (group 2.1) underwent metabolomic analysis. AF samples were immediately frozen and stored at -80°C until GC-MS analysis. AF was collected at the Obstetrics and Gynaecology Division of the University Hospital in Cagliari. The samples of amniotic fluid were thawed at room temperature and vortex mixed to homogenize. 100 µL of each sample were collected to form a pooled sample to use for quality control and to form an average composition sample to analyze among the others. 200 µL of AF were transferred in an Eppendorf tube with 400 µL of acetone, vortexed and then centrifuged. The supernatant was transferred in glass vials and evaporated to dryness overnight in an Eppendorf vacuum centrifuge. A solution of methoxylamine hydrochloride in pyridine was added to each vial and left to react at room temperature. Then MSTFA (N-Methyl-N-trimethylsilyltrifuoroacetami de) were added and left to react at room temperature. The derivatized samples were diluted with hexane, just before GC-MS analysis. Samples were analyzed using an MS Agilent 5977B interfaced to the GC 7890B equipped with a DB-5ms column (J&W). Each acquired chromatogram was analyzed using the software AMDIS (Automated Mass Spectral Deconvolution and Identification System; http://chemdata.nist.gov/mass-spc/amdis). Each peak was identified by comparing relative mass spectrum and retention time with those stored in an in-house made library including 296 metabolites; the Metabolomics team realized this library. In this way 81 compounds were quantified: 77 accurately identified, two unknown compounds. AMDIS analysis produced an electronic sheet data matrix that was submitted to univariate and multivariate statistical analysis.

STATISTICAL ANALYSIS

The Excel® data matrix containing 81 metabolites was processed using the integrated web-based platform MetaboAnalyst 3.0 [2]. A statistical model based on the Partial least square discriminant analysis (OPLS-DA) was performed, and its associated variable importance in projection (VIP) score was performed. To determine the optimal number of components needed to build the DA model, the sum of squares captured by the model ($R^2$) and the cross-validated $R^2$ ($Q^2$) were determined (**Fig. 1**). The most affected metabolic pathways were examined by MetaboAnalyst using a set of predefined metabolic
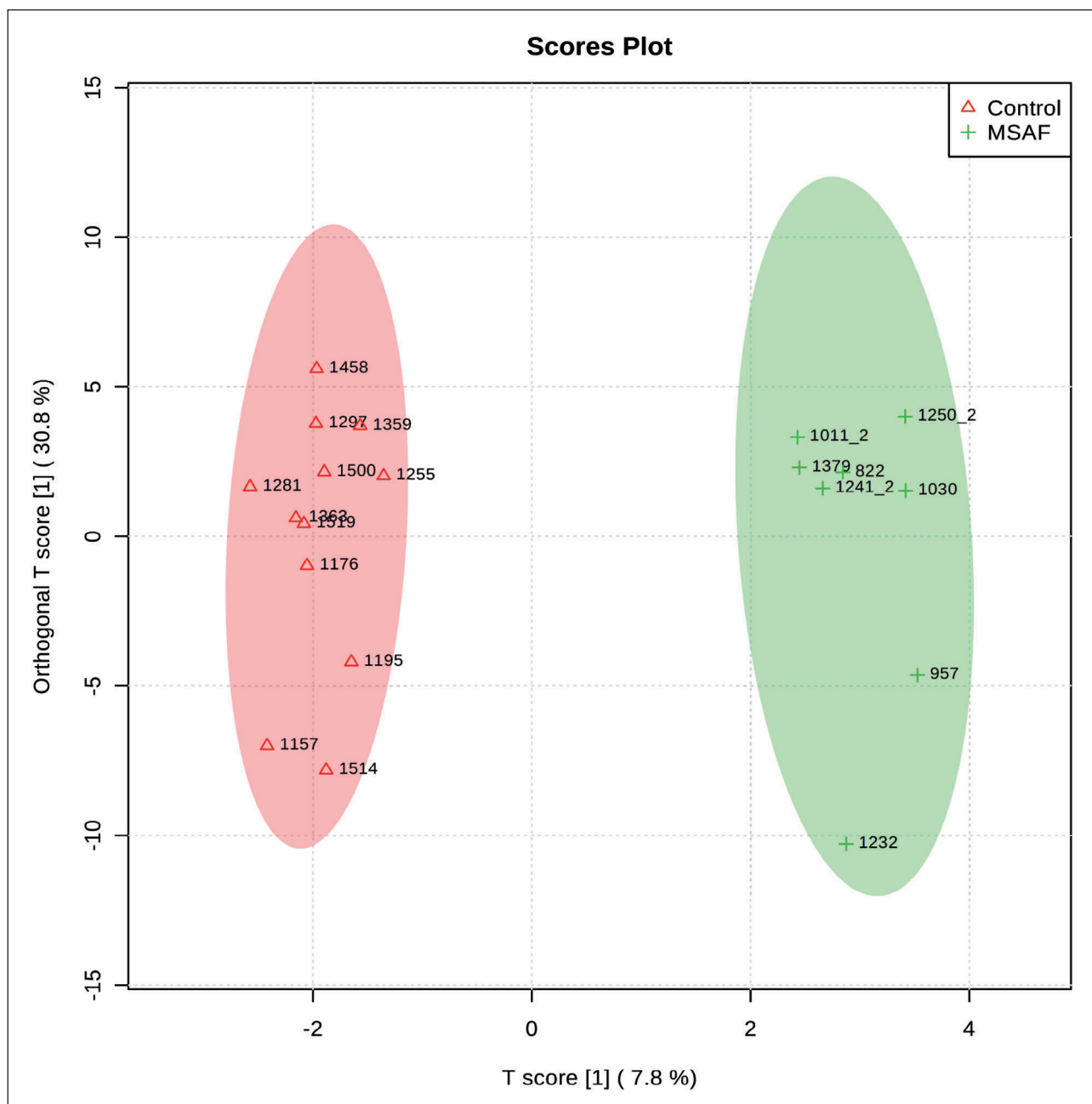
**Figure 1 (LECT 47).** Partial least square discriminant analysis (OPLS-DA) model for the discrimination between Meconium Stained Amniotic Fluid (MSAF) and Control.

pathways to identify significant changes. Data were input in MetaboAnalyst 3.0 to perform the quantitative enrichment analysis (QEA) for the pathways associated with the altered metabolite sets.

RESULTS

Metabolomics identify changes in AF associated with the MSAF conditions of sample collected from local (Sardinian) population. Of the most statistically significant metabolites identified in AF samples, 12 are overexpressed in the MSFA population (N-Acetylglucosamine, N-Acetylneuraminic acid, Galacturonic acid, Fucose, Ribonic acid, Allantoin, Glycine, Quinic acid, Serine, Tryptophan, Myristic

acid) and 3 are overexpressed in the control group (Maltose, Threonic acid and Sorbitol) [3]. This is a preliminary approach to identify, with highly selective and sensible method, pathological conditions in the fetus from AF sample. Further analysis is required with the increase of samples number to classify the MSAF correctly, but form thus studies we can say that Metabolomics is an essential tool for the fetal outcome.

REFERENCES

[1] Palmas F, Fattuoni C, Noto A, Barberini L, Dessì A, Fanos V. The choice of amniotic fluid in metabolomics for the monitoring of fetus health. Expert Rev Mol Diagn. 2016;16(4):473-86.

[2] Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS, Xia J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018;46(W1):W486-94.

[3] Mussap M, Fanos V, Piccoli A, Zaninotto M, Padovani EM, Plebani M. Low molecular mass proteins and urinary enzymes in amniotic fluid of healthy pregnant women at progressive stages of gestation. Clin Biochem. 1996;29(1):51-6.

## LECT 48

## MYSTERIES OF THE PERINATAL HEARTH

A. Faa[1], E. Podda[1], G. Faa[1], V. Fanos[2]

[1]Department of Pathology, University Hospital San Giovanni di Dio, AOU of Cagliari, University of Cagliari, Cagliari, Italy

[2]Neonatal Intensive Care Unit, Neonatal Pathology, and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy

The cardiac system in the perinatal period is very different from the adult one. Differences are mainly due to the persistence, at birth, of developmental changes that overlap pathological changes, giving rise to the insurgence of complex morphological patterns that are exclusive of the newborn heart [1]. Here, some of the most critical developmental and pathological changes occurring in the neonatal heart will be summarized. Cardiac stem/progenitor cells are easily detected in the newborn heart. They appear as small spindle cells, with scant cytoplasm and ovoid nuclei, organized in small nests localized in the subepicardial region. Cardiac stem cell niches may be readily identified at immunohistochemistry with the anti-CD117 antibody [2]. The number of cardiac stem cells in the newborn heart might be utilized as a marker of the regenerative capacity of the myocardium. Moreover, since heart failure is the number one killer worldwide, the discovery in the newborn heart of endogenous stem cells, which are capable of repairing the damaged portion of the heart, might represent the basis for a new field of cardiac regenerative medicine. The hypothesis of the fetal origin of adult heart disease identifies the relationship between impaired growth during intrauterine life, and the risk of adult cardiovascular disease and death is still evident [3]. Perinatal inflammation and hyperoxia, two frequent events in perinatal pathology, may cause an early cardiac dysfunction, inducing discernible alterations in cardiomyocyte contractility due to calcium signaling dysfunction. The presence of endothelial damage and loss of the endothelial barrier represent the most frequent lesion responsible for cardiac failure in the majority of newborns undergoing multiple organ failure. The endothelium of the neonatal heart is probably more sensible than the adult endothelium to hypoxia. As a consequence, the accurate morphological and immunohistochemical (CD31) study of the endothelial changes is mandatory in the newborn heart [1]. S100 protein has been shown to represent a very sensitive and early marker of hypoxia-related cardiac pathology in the newborn [4]. Our preliminary data in the neonatal human heart demonstrated the presence of S100B immunoreactivity, both in cardiomyocytes and in the interstitial spaces, in newborns who underwent severe asphyxia. The immunohistochemical detection of S100B protein in cardiomyocytes as well as in the interstitial spaces appears a new useful tool for the reconstruction, at the histological level, of the myocardiocyte reaction in the last moments of the neonatal life. Taking into account these peculiarities of the perinatal hearth, the perinatal pathologist represents a pivotal figure in the study and detection of cardiac changes in all neonates, particularly in newborns undergoing asphyxia in the perinatal period. Strict cooperation between neonatologists and pathologists might allow the correlation between the clinical and laboratory data and the pathological findings, in order to assign a specific value to the morphological and immunohistochemical data.

REFERENCES

[1] Faa A, Xanthos T, Iacovidou N, Podda E. Perinatal cardiac failure from the eyes of the pathologist. J Pediatr Neonat Individual Med. 2014;3(2):e030267.

[2] Faa A, Podda E, Fanos V. Stem cell markers in the heart of the human newborn. J Pediatr Neonat Individual Med. 2016;5(2):e050204.

[3] Faa A, Ambu R, Faa G, Fanos V. Perinatal heart programming: long-term consequences. Curr Med Chem. 2014;21(27):3165-72.

[4] Faa A, Xanthos T, Papalois A, Locci A, Pampaloni P, Pais ME, Aroni F, Gazzolo D, Faa G, Iacovidou N. S100B immunoreactivity: a new marker of hypoxia-related cardiac damage in newborn piglets. J Matern Fetal Neonatal Med. 2013;26(Suppl 2):72-6.

## LECT 49

## THE KIDNEY: A FASCINATING AND MYSTE-RIOUS ORGAN

C. Gerosa[1], D. Fanni[1], V. Fanos[2], G. Faa[1]

[1]Division of Pathology, University of Cagliari, AOU Cagliari, Italy

[2]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, AOU Cagliari, Cagliari, Italy

The study of the kidney, from the early stages of its development, shows us how this fascinating organ is endowed with some peculiarities. One of its main

characteristics is that the development of the adult kidney is preceded by the development and subsequent transformation of two correctly functioning organs, even if rudimentary, which are the pronephros and the mesonephros. Pronephros is a much simpler organ that consists of a one blood filtering glomus/ glomerulus, and of one pronephric tubule which connects glomus with the pronephric duct (**Fig. 1**). This rudimentary organ is fully functional: pronephros is placed in a minimum space and performs the filtration functions correctly. Methanefros develops following complex interactions between the ureteric bud and the metanephric mesenchyme. In this phase of kidney development the glomerulus and tubular structures that will give rise to the nephrons, are completed. Nephrogenesis is located in the so-called blue streep, a subcapsular region rich in stem cells. The metanephros begins to develop early during the fifth week of gestation and finish at birth [1]. Many factors can influence the interruption of nephrogenesis processes: chorioamnionitis, steroids, maternal diabetes, preeclampsia, IUGR [2]. These events can result in preterm birth with blockage of nephrogenesis. The histological study of the kidneys in preterm newborns at different gestational age, allows us to calculate the radial glomerular count by counting the layers of glomeruli along a straight line extending from the renal capsule to the deepest area of the cortex; this study demonstrates
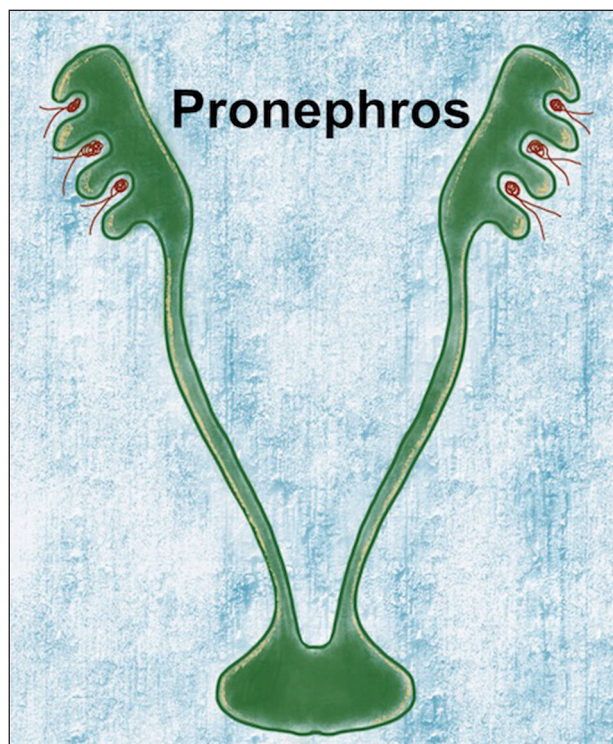


**Figure 1 (LECT 49).** A schematic representation of pronephros.

marked interindividual variability related to different etiological agents (drugs or other stressors) [3]. Other studies have correlated the number of podocytes with factors that were occurring during intrauterine life. These studies show that the number of podocytes decreases when related to factors such as drug use or maternal diet. A low number of podocytes may represent a predisposing factor for the development of podocytopathies in adult life [4]. Recent studies on stem cells in fetal kidneys have shown another exciting feature of this organ: the kidney is abundant in stem cells and not only during the active phases of nephrogenesis, where we find many stem cells in the blue streep and the mesenchymal cap, but also in kidney of 38-41 week of gestation and kidney of adult life. In the mature kidney, the stem cells are found in the renal capsule, in the Bowman capsule, in the cortical interstitium, in the renal papilla and the hilum [5]. The presence of stem cell niches is the reason for studies aimed at reactivating stem cells in order to prolong nephrogenesis in cases of preterm birth when the number of nephrons is necessarily lower than that in a child born at term. β-Thymosins are a family of ubiquitous peptides with a molecular mass of about 5 kDa and with a sequence of 40-44 amino acid residues: Tβ4 is a ubiquitous peptide with multiple fascinating functions and involved many critical biological activities including angiogenesis, wound healing, inflammatory response, and cell migration. In recent studies, immunoreactivity for Tβ4 was performed in a series of fetal and newborn kidneys, ranging from 17 up to 38 weeks of gestation. The expression pattern of Tβ4 changed in the different phases of gestation, but it was strongly expressed at the renal hilum, highly expressed in cells of the outer layer of arteries and the cortical-stromal interstitial cells; moreover, Tβ4-reactive cells encircled distal tubules and Bowman capsule cells [6]. The presence of this function-rich protein in the kidney leads to a reconsideration of its nephrogenic potential and requires further study. Finally, a notable mention of the vascularization of the fetal kidney. The nephrogenic zone, rich in stem cells, intensely active, appears to be poorly vascularized. CD31 reactivity is generally used as a marker of endothelial cells. In our recent study, immunostaining for CD31 showed the scarcity of blood vessels in the nephrogenic zone. The nephrogenic zone should, therefore, be more resistant to hypoxic insults [7]. In conclusion, this organ so fascinating and so peculiar shows in its development show how sensitive it is to insults that may occur during pregnancy. On the other hand, the

kidney zone involved in nephrogenesis has of low vascular supply. Therefore it can resist low oxygen tensions as in the course of hypoxia.

REFERENCES

[1] Faa G, Gerosa C, Fanni D, Nemolato S, Monga G, Fanos V. Kidney embryogenesis: how to look at old things with new eyes. In: Fanos V, Chevalier RL, Faa G, Cataldi L (Eds.). Developmental nephrology: from embryology to metabolomics. Quartu Sant'Elena: Hygeia Press, 2011, pp. 23-45.

[2] Sutherland M, Ryan D, Black MJ, Kent AL. Long-term renal consequences of preterm birth. Clin Perinatol. 2014;41:561-73.

[3] Faa G, Gerosa C, Fanni D, Nemolato S, Locci A, Cabras T, Marinelli V, Puddu M, Zaffanello M, Monga G, Fanos V. Marked interindividual variability in renal maturation of preterm infants: lessons from autopsy. J Matern Fetal Neonatal Med. 2010;23(Suppl 3):129-33.

[4] Crobe A, Desogus M, Sanna A, Fraschini M, Gerosa C, Fanni D, Fanos V, Van Eyken P, Faa G. Decreasing podocyte number during human kidney intrauterine development. Am J Physiol Renal Physiol. 2014;307:F1033-40.

[5] Fanni D, Sanna A, Gerosa C, Puddu M, Faa G, Fanos V. Each niche has an actor: multiple stem cell niches in the preterm kidney. Ital J Pediatr. 2015;41:78.

[6] Nemolato S, Cabras T, Messana I, Gerosa C, Faa G, Castagnola M. Do β-Thymosins Play a Role in Human Nephrogenesis? In: Faa G, Fanos V (Eds.). Kidney development in Renal Pathology. Current Clinical Pathology. Humana Press, 2014, pp. 81-93.

[7] Gerosa C, Fanni D, Faa A, Van Eyken P, Ravarino A, Fanos V, Faa G. Low vascularization of the nephrogenic zone of the fetal kidney suggests a major role for hypoxia in human nephrogenesis. Int Urol Nephrol. 2017;49(9):1621-5.

**LECT 50**

**SECRETS OF THE BRAIN**

M. Vendemmia

*Neonatal Intensive Care Unit, Federico II University of Naples, Naples, Italy*

Neurological development is a complex process that begins before birth, continues in the last months of gestation and the first months after birth. According to the hypothesis of David Barker, events that occur in a critical temporal window of the perinatal period, permanently alter the trajectory of development, determining permanent effects on the phenotype. This window of vulnerability corresponds to the brain in the third trimester of gestation. The premature birth abruptly interrupts the passage of biological messages between mother and fetus. Therefore the fetus adapts to survive in a different environment. This adaptability is called plasticity and is the result of the interaction between genes and environment. Preterm's brain is an excellent example of plasticity [1]. In the newborn with cerebral hemorrhage or asphyxiated damage, the neuronal cells close to the broken ones are activated and vicarious the functions of the latter, due to a continuous remodeling process. In recent decades there has been a noticeable increase in the survival of extremely preterm infants, and for a while, there has been feared an increase in subjects with motor and sensory, neurological handicaps. However, this increase is limited to the first years after the opening of the Neonatal Intensive Care Units, thanks to the use of cortisone in pregnant women, the centralization of pregnancies, and the use of surfactant. The most recent studies indicate a decrease in neurological morbidity and the reduction of preterm subjects affected by PCI and/or severe sensorineural handicap. It is believed today that only 10-12% of premature infants present a severe neurological disability in school age. 25-50% of preterm infants have milder neurological problems, called "more subtle neurological problems" and include motor delay, persistent neuromotor abnormalities, intellectual retardation, language problems, attention and hyperactivity disorders, disturbances in socialization and learning. One of the most important and ambitious objectives of developmental neurology is the early identification of those at risk for the development of subsequent disabilities: an early rehabilitation intervention can improve the quality of their life. The most common brain lesions in the premature infant are the hemorrhage of the germinal and intraventricular matrix (GMH, IVH), post-hemorrhagic hydrocephalus, periventricular leukomalacia (PLV), White Matter Abnormalities (WMA). WMA is the most common brain damage of the preterm at the end of the correct age, detected with MRI and is often associated with ventricular dilatation, increased extracerebral spaces and reduced white matter [2]. The most important long-term outcome of periventricular leukomalacia is represented by spastic diplegia. This high incidence seems to be due to the specific localization of the lesion involving the cortico-medullary bundles descending from the motor areas assigned to the movement of the lower limbs.

In infants with periventricular parenchymal damage following a venous infarction, an evolution towards hemiplegia is almost observed continuously. Such symptom manifests itself only around 12 months of life, that is when the newborn, growing, begins to make use of the brain areas that have been damaged. The neurological consequences of PVH/IVH are

also the destruction of neurons which are in the germinative matrix. This event may explain some cognitive deficits and attentional disorders that are found in 25-50% of premature infants. On the other hand, a significantly larger volume was found at the level of the temporal and occipital horns of the lateral ventricles. These volumetric changes could be another cause of the neurological sequelae of preterm infants [3].

Cerebellar lesions are currently recognized as a significant complication in newborns with severe prematurity. The cerebellum has always been considered a structure involved in fine and complex motor organization. In the last twenty years its important function of processing all the higher psychic functions has been clarified, probably through modulatory effects on the cerebral cortex [4].

Cerebral ultrasonography is the most used diagnostic investigation in neonatology, being easily executable and repeatable to the patient's bed, although it remains an operator-dependent examination and with less sensitivity and specificity than MRI. Brain MRI remains the most accurate diagnostic investigation for the assessment of the newborn's brain, but it is an expensive investigation, it is not practicable in the patient's bed, it requires sedation of the newborn. Neuroradiological investigations must be associated with clinical evaluations of the functional repertoire of the child. The neurological evaluation includes traditional neurological examination and the observation of spontaneous motility of General Movements. There are various tools to perform a neurological evaluation with the aim of identifying neurological signs, the presence of a disorder and to estimate prognosis. The Infant Neurological Examination (HINE), developed at Hammersmith Hospital in London by Dubowitz, is a simple method, lasting 15 minutes, suitable for severe evaluations of the development of term and preterm infants. In the late 1980s, Prechtl standardized and validated the General Movements Assessment (GMs) as a reliable tool for assessing spontaneous infant motility and as an excellent indicator of dysfunction and early brain damage. The GMs involve the whole body in a variable sequence of movements of the trunk, neck, legs, and arms; their speed, strength and intensity increase and decrease, their beginning and end are gradual. If the nervous system suffers damage, the GMs lose their characteristics of complexity, variability, and fluidity. The evaluation of GMs has a high predictive value, based on the gestalt perception of a trained operator. GMs are recognizable around the ninth and last until the twentieth-week post-term [5]. Preterm infants are at risk of developing major (PCI) and a minor degree of disabilities. PCI cannot be diagnosed before 2-3 years; the preterms that will develop a PCI have significant anomalies of the posture and the spontaneous movement at the first evaluations of the neurological examination. The traditional neurological examination (HINE) together with the evaluation of GMs are robust tools to estimate early neurological and functional deficits related to brain damage. The association of severe neuroimaging and repeated neurological evaluations allow in most cases the early identification of children at risk of neuromotor disability including PCI.

## REFERENCES

[1] Fanos V. Metabolomica e microbiomica. La medicina personalizzata dal feto all'adulto. Quartu Sant'Elena: Hygeia Press, 2015.

[2] Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol. 2009;8:110-24.

[3] Ferrari F. Il neonato pretermine. Disordini dello sviluppo e interventi precoci. Milan: Franco Angeli, 2017.

[4] Limperopoulos C, Chilingaryan G, Sullivan N, Guizard N, Robertson RL, du Plessis AJ. Injury to the premature cerebellum: outcome is related to remote cortical development. Cereb Cortex. 2014;24(3):728-36.

[5] Ferrari F, Frassoldati R, Berardi A, Di Palma F, Ori L, Lucaccioni L, Bertoncelli N, Einspieler C. The ontogeny of fidgety movements from 4 to 20 weeks post-term age in healthy full-term infants. Early Hum Dev. 2016;103:219-24.

## LECT 51

## FOCUS ON… MITOCHONDRIA

F. Bardanzellu, M.C. Pintus, V. Fanos, M.A. Marcialis

*Neonatal Intensive Care Unit, Neonatal Pathology, and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy*

Mitochondria, cytoplasmic organelles originated from endosymbiotic bacteria, can be metaphorically described using "Janus bifrons" image, due to their involvement in life, providing cellular energy and resulting essentially even for stem cells, but playing a key role also in cell death. Mitochondria own a maternally inherited genome and are the site of aerobic respiration; they can produce proteins, nucleotides, lipids, steroids and heme and result involved in ion homeostasis. Moreover, mitochondria can generate free radicals, break down waste products and represent the primary

source of cellular heat. Mitochondrial functions are schematized in **Fig. 1**. Their size and shape depend on the intracellular metabolic status, from tubular presentation to a blob form in case of irreversible damage. Each mitochondrion carries different sets of DNA; when one set accumulates mutations, it can be replaced by another. It has been widely demonstrated that mitochondrial disorders are involved in many pathologies, including autism, multiple endocrinopathies, diabetes, Alzheimer's disease, ataxia, Barth's syndrome, myopathy, and even aging and cancer. Human population is characterized by different mitochondrial DNA haplogroups reflecting the mutations accumulated and useful to characterize genetic diversity. The mitochondrial role also results relevant in pregnancy, providing information about maternal-fetal dyad in physiological and in pathological conditions. Recent evidence suggests that an intriguing bidirectional inter-talk exists between microbiota and mitochondria, influencing cellular homeostasis and metabolism. A recently demonstrated mitochondrial property is the possibility to be transferred from a donor cell to a recipient cell, through a system of tunneling nanotubes (**Fig. 2**). Recently, a promising integrated approach involving omics sophisticate technologies has been applied in mitochondrial pathophysiology. This is still in an early stage, and further studies will clarify such complex genotype-phenotype relationships. In conclusion, mitochondria are not simple energetic organelles but represent dynamic structures communicating with the cell nucleus and even with other cells, influencing metabolism and their targets' functions. More detailed knowledge of their involvement in
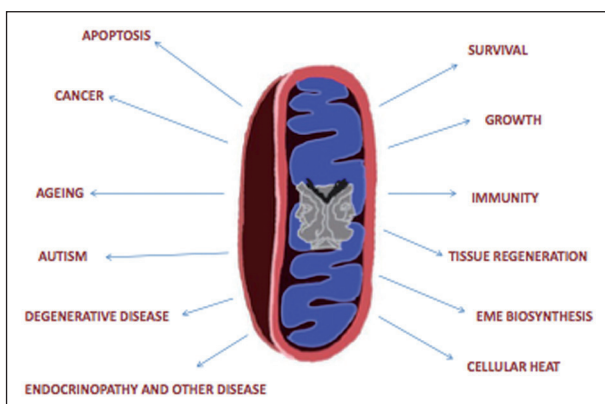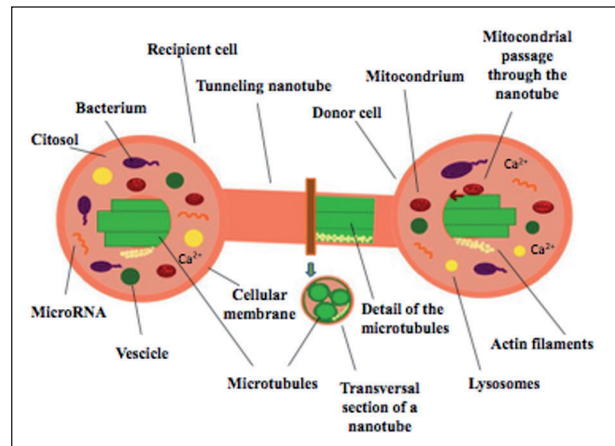


**Figure 2 (LECT 51).** Intercellular trafficking through the tunneling nanotubes (TNTs).
From: Bardanzellu et al., 2018 [In press].

disease, even though a combined omics approach, could represent a chance for new therapies.

REFERENCES

• Bardanzellu F, Pintus MC, Fanos V, Marcialis MA. Once we were bacs… mitochondria to infinity and beyond. J Pediatr Neonat Individual Med. 2018. [In press].

• Rahman J, Rahman S. Mitochondrial medicine in the omics era. Lancet. 2018;391:2560-74.

• Vignais ML, Caicedo A, Brondello JM, Jorgensen C. Cell connection by tunneling nanotubes: Effects of mitochondrial trafficking on target cell metabolism, homeostasis, and response to therapy. Stem Cells Int. 2017;2017:6917941.

**LECT 52**

## THE EXTRAORDINARY REGENERATIVE POWER OF HUMAN ENDOMETRIUM

F. Bardanzellu[1], G. Faa[2], D. Fanni[2], V. Fanos[1], M.A. Marcialis[1]

[1]Neonatal Intensive Care Unit, Neonatal Pathology, and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy

[2]Division of Pathology, Department of Surgical Sciences, University Hospital San Giovanni di Dio, University of Cagliari, Cagliari, Italy

Human endometrium is composed of a functional layer, shed during each menstruation, and a basal one, monthly allowing the generation of a new functional layer, under hormonal stimuli. Thus, the endometrium is a dynamic tissue regenerating itself up to 400 times in the woman's reproductive life, but even after injury or cell loss, parturition or uterine resection. Such high regenerative potential is due to the presence of a reservoir of Endometrial Stem Cells (ESCs), in addition to a variable



**Figure 1 (LECT 51).** Schematization of mitochondrial involvement in several physiological functions but also adverse effects and disease, since mitochondria can resemble a "Janus bifrons" image.
From: Bardanzellu et al., 2018 [In press].

quote of other tissues derived SCs, such as bone marrow. These populations are critical for uterine repair and regeneration, sustain reproduction and provide the best site for the embryo's implant and growth [1]. SCs, classified according to their potency in Totipotent, Pluripotent and Multipotent, are undifferentiated auto-renewable cells originated from embryonic and fetal stages and characterized by the ability to generate more differentiated daughter cells which can finally form tissues and organs, even restoring their initial pool. SCs singular properties strictly depend on the "niche" they inhabit, the peculiar anatomic site which constitutes both structural support and a dynamic microenvironment providing signals of proliferation, differentiation according to the needs. Deregulation in such complex interaction can lead to several proliferative disorders, including endometriosis and cancer [1]. SCs have been detected in both basalis and functionalis layer. ESCs can be classified as Epithelial SCs, Mesenchymal SCs (EMSCs), Endothelial SCs and can also be isolated from menstrual blood. MSCs are more numerous near the small vessels in perivascular niches, allowing angiogenesis and stromal regeneration, and express CD146 (CD a.k.a. cluster of differentiation) and CD140b/PDGFRβ (Platelet-derived grow factorβ). Epithelial progenitors (eEPs) mostly reside in the basalis layer, at the base of glands. Several ESCs' markers have been identified, such as CD140b, CD146, SUSD2, CD73, CD90, CD105, CD166, HLA-ABC, Leucine-rich repeat-containing G-protein coupled receptor 5 and OCT4 in EMSCs, N-cadherin in eEPs. EMSCs show fibroblast morphology and plastic adherence can be easily obtained and have unique properties such as a high proliferation rate and self-renewal. Moreover, EMSCs and menstrual blood-derived stem cells (MenSCs) can differentiate into osteoblasts, odontoblasts, chondroblasts, adipocytes, muscle cells, and other cellular lineages, as widely demonstrated through *in vitro* and *in vivo* models [1]. They can also secrete angiogenic and other growth-promoting factors, therefore resulting promising in clinical applications and tissue engineering. Since they own immunomodulatory and migratory properties, could constitute a safe anti-inflammatory therapy, as demonstrated in animal models. Recent findings regard MenSCs ability to differentiate into germinal cells and their promising role also in peripheral nerve and damaged endometrium repair.

MenSCs showed good results in trials against stroke, colitis, limb ischemia, coronary disease, Duchenne's muscular atrophy, type 1 diabetes, dermatological diseases and intrauterine adhesions (IUA) since affected women usually present an endometrial reduction in SCs [2]. In conditions characterized by tissue injury or fibrosis, ESCs may reduce fibrotic area, stimulate angiogenesis and improve endometrial thickness, especially if associated with estrogens. A deregulated ESC proliferation and migration is involved in the pathogenesis of endometriosis (up to the recent theory postulating the development from primordial germ cells and the deregulation in hormonal stimulation), IUD, recurrent pregnancy (since a depletion of EMSCs can impair endometrial thickness and decidualization, crucial for embryo implant), adenomyosis, leiomyomas and even cancer [1, 2]. In cells deriving from endometriosis, a variable gene expression (regarding $SOX^2$, NANOG Musashi-1, Numb, cKit, OCT4, SOX15, TWIST1, and DCAMLK1) and miRNA levels (miRNA-199a-5p, miR-15a-5p, miR34a5p) have been detected, potentially playing an important role [3]. In addition to local progenitors, circulating SCs may reach extra-uterine site after the stimulation by CXCL12, representing the trigger for hematogenous dissemination (i.e., in lungs). Such SCs trafficking is a pivotal mechanism and could represent a new therapeutic target [2].

In endometrial cancer, a fraction of malignant cells share many SCs features, through a variable expression of stemness markers, i.e. CD133, CD44, BMI-1, SOX-2, c-Myc, SUSD2, NANOG, β-CATENIN, Oct4, CXCR4, ABCG2, BMI-1, CK-18, Nestin, β-actin, epithelial membrane protein-2 and hormonal receptors which also present different prognostic correlations [3]. In conclusion, the endometrial extraordinary regenerative ability also owns an ugly side, constituting the risk behind the fundamental protective mechanisms allowing the vital cycle. Improved knowledge of ESCs features, proliferation, motility, angiogenesis and recruitment could improve the therapy for diseases affecting several women and reducing their reproductive ability. Multipotent ESCs can be abundantly taken from the endometrium and menstrual blood, playing a central role in tissue engineering and reproductive medicine. Cryopreserved MenSCs represent an easily prepared product whose clinical application should be further studied. In the future, it would be desirable for reducing the risks potentially

due to the ugly side of ESCs proliferation and transformation, in particular, cancerogenesis.

REFERENCES

[1] Bardanzellu F, Faa G, Fanni D, Fanos V, Marcialis MA. Regenerating the Womb: The Good, Bad and Ugly Potential of the Endometrial Stem Cells. Curr Reg Med. 2017;7:1-13.

[2] Simoni M, Taylor HS. Therapeutic strategies involving uterine stem cells in reproductive medicine. Curr Opin Obstet Gynecol. 2018;3:209-16.

[3] Zhang R, Jiao J, Chu H, Yang H, Wang L, Gao N. Expression of microRNA-145, OCT4, and SOX2 in double primary endometrioid endometrial and ovarian carcinomas. Histol Histopathol. 2018;23:11986.

**LECT 53**

**PLACENTA AND METABOLOMICS**

L. Barberini

*Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy*

Pregnancy-related metabolomics has examined different biofluids to have more specific insights into pathologies interesting the fetal status. Amniotic fluid, urine, and plasma were the most studied, while placenta tissue has been the subject of only a few studies due to the lack in the standardization of solid sample preparation for the proper analytical platform. The placenta is the connection organ between the fetus world and the mother one. It regulates the vital flows, and metabolic waste products exchange from and towards the fetal environment, and it is an excellent barrier against infections. For this reason, it is an essential carrier for the information about the fetus state. Our group recently examined placenta samples from obese and healthy weight mothers to find metabolites significantly altered between the groups. An extraction method was developed to allow the separation of two phases, hydrophilic and lipophilic, to be both analyzed through GC-MS [1]. Metabolite identification is a crucial step in this kind of metabolomic analysis and chemistry can make the difference. The pre-analytical sample preparation involved the homogenization of the placenta tissue with a mixture of solvents, centrifugation, and separation in two phases: hydrophilic and lipophilic.

It is possible to characterize several molecular families:

- Organic acids, Amino acids, Sugars, Polyols in the Aqueous phase;
- Triglycerides, Phospholipids, Steroids, Fatty acids in the Organic phase.

The data matrixes obtained were processed using the integrated web-based platform MetaboAnalyst 4.0 [2]. A statistical model based on the Partial least square discriminant analysis (OPLS-DA) was performed, and its associated variable importance in projection (VIP) score was performed. Using MetaboAnalyst and a set of homemade calculation routines powered with several predefined algorithms to identify significant changes, we examined the most affected metabolic pathways in the pathological condition of obesity in pregnancy. Data were input in our software to perform quantitative analysis for the identification of the networks associated with the altered metabolite sets. Interpretation of the experimental results characterizing different biological molecules remains a significant challenge in the face of complex biochemical regulation processes such as the ones operating in organs like the placenta, due to epigenetics and protein post-translational modification.

Leading approaches for this issue include biochemical pathway, network-based and empirical correlation-based methods. This kind of advanced data analysis tools is required to carry out useful metabolomic data interpretation. These modern data analysis tools are necessary to allow researchers to implement a robust pipeline analysis. This emerging approach, named network mapping, shows promise to effectively integrate statistical, multivariate and functional domain knowledge to calculate richly connected biochemical pathways which can highlight the specific metabolic perturbations. In the case of placenta analysis for obese mother (OB), hydrophilic phase and lipophilic analysis revealed several metabolites with significantly different levels in OB versus normal-weight mothers (NW), allowing a more unambiguous PLS-DA discrimination. Hydrophilic metabolite differences concerned mitochondrial energy, as well as glucose and amino acid metabolism, showing similar profiles for glycerol, nicotinamide, and taurine in OB groups. The lipophilic analysis in OB showed lower levels of LC-PUFA derivatives. GDM affected TCA cycle metabolites, with decreased levels of aspartic acid and glutamine opposite to higher tyrosine levels and showed a prevalence of unsaturated/polyunsaturated fatty acids.

DISCUSSION

Placental metabolome analysis of obese pregnancies demonstrated differences concerning amino acid profiles and mitochondrial function, supporting a shift towards higher placental metabolism. These placentas also showed a specific fatty acids

profile suggesting an alteration of LC-PUFA production. These metabolic signatures may reflect changes occurring in the intrauterine metabolic environment, which may result in the development of adult diseases. Modern Metabolomics approach, based on the Systems Biology methods, can better characterize the placenta status in correlation with several pathological conditions, and this is another step beyond for the clinical applications.

## REFERENCES

[1] Fattuoni C, Mandò C, Palmas F, Anelli GM, Novielli C, Parejo Laudicina E, Savasi VM, Barberini L, Dessì A, Pintus R, Fanos V, Noto A, Cetin I. Preliminary metabolomics analysis of placenta in maternal obesity. Placenta. 2018;61:89-95.

[2] Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS, Xia J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018;46(W1):W486-94.

**LECT 54**

**PERINATAL ENDOMETRIOSIS**

S. Angioni, F. Dessolis

*Department of Surgical Sciences, Obstetrics and Gynecology, AOU Cagliari, University of Cagliari, Italy*

Endometriosis affects 7 to 15% of the female population with a peak incidence between 25 and 40 years of age in response to hormone secretion. Sporadic postmenopausal and a few prepubertal cases have been described in the literature [1]. Although its pathogenesis is still under discussion, the most accredited cause is the retrograde menstrual flow theory. The majority of women do not experience retrograde menstruation despite having a minimal amount of blood in the pelvic cavity, as they can absorb this tissue and metabolize it without consequence. In some cases, retrograde menstruation causes peritoneal reactions, accompanied by a reduced immune response. As a result, the vital endometrial cells are able to invade the peritoneum and colonize it. Perinatal endometriosis is related to blood reflux, similar to menstruation, in the neonatal tubes inside the peritoneal cavity with the consequent ectopic implantation of endometrial stem/progenitor cells. It has been hypothesized that endometrial cells and the stroma can be retrogradely disseminated in the pelvis at the moment of birth due to the presence in a third of newborns of visible or occult uterine bleeding (NUB) [2]. Many of the studies performed so far are post-mortem studies. The fetal endometrium becomes sensitive to steroid hormones around the twentieth week of pregnancy from an estrogenic phase, which is subsequently followed by a secretory phase. Signs of secretory activity can be observed from the thirty-fourth week; after birth, the endometrium begins to regress and in a few days becomes quiescent. In 58% of births, the endometrium is in the proliferative phase. Of newborns, 27% have secretory changes in the glandular compartment, and in only 5% of cases, there are changes caused by progesterone in the stromal compartment, such as decidualization or shedding similar to the menstrual cycle. Endometrial stem/progenitor cells (eSPC) could play a fundamental role in the pathology of early-onset endometriosis. Indeed, eSPC have been identified in menstrual blood, making it plausible to assume they may be present even in cases of NUB. Therefore, it can be hypothesized that the progenitor stem cells present in the endometrium could have a role in the pathogenesis of perinatal endometriosis through retrograde dissemination. It has been shown that endometrial stromal stem cells of women with or without endometriosis differed in morphology, CD expression pattern, proliferation, invasion, and adhesion capacity and in the ability to express specific immunomodulatory molecules [3]. Consequently, it is also deduced that endometrial stem/progenitor cells and their supporting niche cells must be able to survive in the pelvic cavity in the absence of steroid hormones for many years. It has been suggested that eSPC present in NUB can survive in the pelvic cavity in the absence of circulating estrogens and that the mesenchymal progenitor cells (eMSC) of endometriotic lesions have better properties than eutopic tissue cells, thus showing a greater invasive, migratory and stimulation capacity of neoangiogenesis. All these observations corroborate the fact that endometrial stem/progenitor cells seeded during the neonatal period contribute to the pathogenesis of perinatal endometriosis. If it could be thought that the reactivation of dormant neonatal eMSC at the time of the Telarc and menarche is the origin of perinatal endometriosis in predisposed subjects, it is still questionable whether menstruation could sow additional eMSCs (adult ones) in these subjects, contributing to the generation of periodic endometriotic lesions responsible for the chronicity of the disease. An alternative hypothesis is that menarche retrograde menstruation contains endometrial stem/progenitor cells that are incapable of causing ectopic lesions due to their aging compared to neonatal MSC. The latter appears to be more

potent than their adolescent and adult equivalents. Further studies are needed to investigate whether there is a concordance between the frequency of the NUB and the incidence of endometriosis in the adolescent population and whether this is due to the neonatal endometrial spread containing eSPC.

## REFERENCES

[1] Angioni S. New insights on endometriosis. Minerva Ginecol. 2017;69(5):438-9.

[2] Brosens I, Benagiano G. Is neonatal uterine bleeding involved in the pathogenesis of endometriosis as a source of stem cells? Fertil Steril. 2013;100:622-3.

[3] Nikoo S, Ebtekar M, Jeddi-Tehrani M, Shervin A, Bozorgmehr M, Vafaei S, Kazemnejad S, Zarnani AH. Menstrual blood-derived stromal stem cells from women with and without endometriosis reveal different phenotypic and functional characteristics. Mol Hum Reprod. 2014;20(9):905-18.

**LECT 55**

**PEDIATRIC FATTY LIVER DISEASE**

D. Fanni, C. Gerosa, G. Faa

*Division of Pathology, University of Cagliari, AOU Cagliari, Cagliari, Italy*

Nonalcoholic fatty liver disease (NAFLD) occurs in about 20-30% of the Western Country population. Even children and adolescents are affected. Overweight and obesity is at the basis of NAFLD prevalence rising to 40-70% in obese children. Other risk factors include diabetes and gender. Under the NAFLD definition, a broad spectrum of liver diseases, ranging from liver steatosis, defined nonalcoholic fatty liver (NAFL), to nonalcoholic steatohepatitis (NASH) are included. The histopathological diagnosis of NAFL and NASH is reached by the detection of the different type of elementary lesions (**Fig. 1**): A) steatosis, B) ballooning hepatocytes, C) apoptosis, D) glycogen nuclei, E) inflammation, F) lipogranuloma and G) fibrosis [1].

Steatosis is the fat storage in the hepatocytes. Steatosis is characterized by the presence of lipid droplets in the cytoplasm of hepatocytes. The size of droplets may range from small, with a diameter lower than that of hepatocytic nuclei (microvascular steatosis), to large, that may occupy the entire cytoplasm of hepatocytes (macrovascular steatosis). Microvesicular steatosis is associated with oxidative stress, mitochondrial damage, liver cell death, stellate cell activation and collagen deposition, fibrosis, and liver cirrhosis. Macrovascular steatosis is not involved in the increase of oxidative stress.

Usually, both microvascular and macrovesicular steatosis are generally found either in NAFL and NASH. Steatosis is typically mainly located in periterminal zone, however, sometimes macrovesicular steatosis may be diffuse in all acinar zones, including the periportal one. Ballooning of hepatocytes is characterized by roundish shape, increased diameter, clear cytoplasm, reticular appearance, and Mallory-Denk bodies. Ballooning is a key to finding supporting the diagnosis of NASH, representing the liver cell damage and the necro-inflammatory activity of the disease. Ballooned hepatocytes are localized in the periterminal zone [2]. Similarly to periterminal steatosis, periterminal ballooning, especially together with steatosis, is highly suggestive for NASH.

Apoptosis is the programmed cell death, localized in the periterminal zone, described by multiple studies as an essential feature for the diagnosis of NASH cases lacking ballooning [3]. Glycogen nuclei are enlarged clear hepatic nuclei, characterized by chromatin margination along the nuclear membrane. In NAFLD, glycogen nuclei may be observed in all acinar zones, especially in periportal hepatocytes. Glycogen nuclei are not specific, being detected in other liver diseases.

Inflammation is usually polymorphous, including lymphocytes, plasma cells, neutrophils, and eosinophils. Spotty inflammatory infiltrates inside the liver acinus is a typical feature of NASH. While portal inflammation is not a typical feature of NASH during the early phases, mainly occurring in children, showing a spillover of lymphocytes over the periportal zone. Lipogranuloma is a roundish inflammatory infiltrates surrounding one or few hepatocytes containing large lipid droplets. Lipogranulomas may be frequent and easily seen at low power magnification, or rare and even absent. Thus, lipogranuloma is not a mandatory feature for the diagnosis of NASH.

Fibrosis is well evidenced by silver stain. Chicken-wire fibrosis is the very typical pattern occurring in NASH. It originates around the terminal vein, giving rise to short irregular septa that spread in between the periterminal hepatocytes. Pericellular and perisinusoidal fibrosis is a network of a large amount of thin newly-formed argyrophilic fibers, extending from the Disse spaces into the sinusoidal lumen, surrounding single hepatocytes and impairing the intrasinusoidal circulation. Bridging fibrosis is the late phases evolution of NASH, characterized by fibro-vascular re-arrangement of the liver. Thin fibrous septa, well
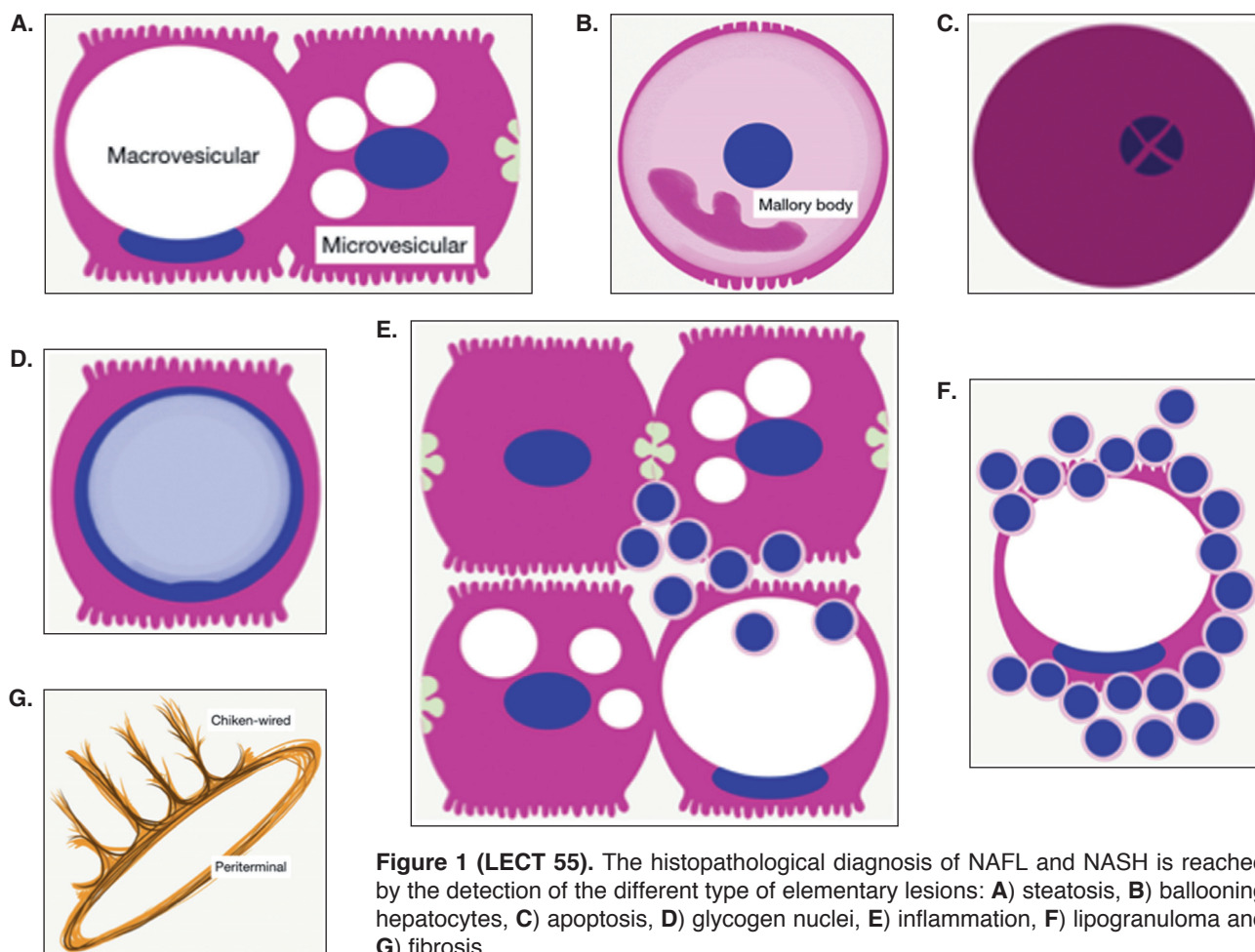
**Figure 1 (LECT 55).** The histopathological diagnosis of NAFL and NASH is reached by the detection of the different type of elementary lesions: **A**) steatosis, **B**) ballooning hepatocytes, **C**) apoptosis, **D**) glycogen nuclei, **E**) inflammation, **F**) lipogranuloma and **G**) fibrosis.

evidenced by silver stain, develop, creating new bridges between portal spaces and terminal veins [4]. NAFLD represents the hepatic manifestation of the metabolic syndrome in children and the most common liver disease in children of the Western hemisphere [5]. The histopathological analysis of liver biopsy give important data and the liver biopsy is mandatory in children with a metabolic syndrome showing liver fatty disease at ultrasonography in order to evaluate the disease (NAFL/NASH), the steatosis (microvascular or macrovesicular), the feature associated with progression (ballooning, apoptosis, inflammation, lipogranuloma) and the fibrosis. This elementary lesions, mainly when associated with clinical data, represent a useful tool in the differential diagnosis with different etiology: congenital disease as Wilson's disease, drug-induced, chemotherapy-associated and other toxic steatohepatitis.

## REFERENCES

[1] Working Group; Association of Pathologists for Guidebook of NASH and NAFLD, 2015: The Japan Society of Hepatology. Pathological Findings of NASH and NAFLD: for Guidebook of NASH and NAFLD, 2015: The Japan Society of Hepatology. Hepatol Res. 2017;47(1):3-10.

[2] Brunt EM. Nonalcoholic Fatty Liver Disease: Pros and Cons of Histologic Systems of Evaluation. Int J Mol Sci. 2016;17(1):97.

[3] Yeh MM, Belt P, Brunt EM, Kowdley KV, Wilson LA, Ferrell L. Nonalcoholic Steatohepatitis Clinical Research Network. Acidophil bodies in nonalcoholic steatohepatitis. Hum Pathol. 2016;52:28-37.

[4] Africa JA, Behling CA, Brunt EM, Zhang N, Luo Y, Wells A, Hou J, Belt PH, Kohil R, Lavine JE, Molleston JP, Newton KP, Whitington PF, Schwimmer JB and Nonalcoholic Steatohepatitis Clinical Research Network. In Children With Nonalcoholic Fatty Liver Disease, Zone 1 Steatosis Is Associated With Advanced Fibrosis. Clin Gastroenterol Hepatol. 2018;16(3):438-46.

[5] Jennings J, Faselis C, Yao MD. NAFLD-NASH: an under-recognized epidemic. Curr Vasc. 2018;16(3):209-13.

## LECT 56

## THE QUESTIONS OF THE MOTHERS

A. Dessì

*Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, Azienda Ospedaliera Universitaria and University of Cagliari, Cagliari, Italy*

During pregnancy, most of the mothers prepare for the birth of their child, and they inquire

about different issues such as the importance of breastfeeding. Nevertheless, one aspect that incisively emerges is the difficulty of managing the newborn in the first period of his or her life. Indeed, with the arrival of a new child, a process of life change begins that inevitably gives rise to concern and uncertainty regarding the understanding of the needs of the neonate. Many of the maternal concerns, unlike those of the fathers, are often related not to be able to breastfeed and to nourish their baby correctly. For instance, in a study performed in America in 2013, 2,946 interviews have been made to 500 women, at different times, before and after delivery. It emerged that in the post-partum period the concerning about breastfeeding are very diffused and significantly associated with a higher risk of stopping breastfeeding and using formula milk. Authors conclude that priority should be given to strategies to reduce the occurrence of breastfeeding problems and solve, in particular, the concerns about the quantity of milk produced by the mothers [1]. As regards the use of a pacifier, mothers arrive at the time of birth with serious doubt about that. In literature, indeed, there is no consensus on the effect of using pacifiers and nipple drinkers and the early interruption of exclusive breastfeeding. Nevertheless, the American Academy of Pediatrics recommends the use of a pacifier during breastfeeding as long as it happens when the breastfeeding already well established since it is considered a protective factors against sudden infant death syndrome [2]. Pregnancy and post-partum may have profound effects on maternal mood. It is well known that there is a high incidence of mood alterations that can include both mild and transitional emotional disturbances and postpartum depression. In this context, one should not be surprised that difficulties and insecurities in understanding and interpreting the necessities of the newborn may emerge. Some of the most asked questions by the mothers to the pediatrician are about sleeping and the management of infantile colic. Approximately, in the first month of life, the newborns sleep up to 14-18 hours a day, and since he or she has not a proper circadian rhythm, his or her sleeping is distributed during the day and the night, and it is often interrupted by the physiological need of feeding frequently. This is the reason why the mother should modify her sleeping patterns according to the sleeping schedule of her child. To this can be added the presence of infantile colic that is characterized by recurrent and prolonged periods of intense and inconsolable crying that generally occurs in the second-third

week of life with a peak around the seventh week and that resolve itself at around 4 months of age. Colic is one of the most frequent causes of pediatric examinations (10-20%) between 2 weeks and 3 months of age of the newborn and can concern and frustrate the new parents [3]. Although infantile colic is a benign and self-limited condition, it is of fundamental importance to reassure the mothers and provide adequate support: tension and anxiety of parents could increase the pain perception of the infant. Insecurities and needs of the new mothers are often underestimated while healthcare professionals should be well aware of the importance of support in a delicate period such as the post-partum.

## REFERENCES

[1] Wagner EA, Chantry CJ, Dewey KG, Nommsen-Rivers LA. Breastfeeding concerns at 3 and 7 days postpartum and feeding status at 2 months. Pediatrics. 2013;132(4):e865-75.

[2] Moon RY; Task force on sudden infant death syndrome. SIDS and Other Sleep-Related Infant Deaths: Evidence Base for 2016 Updated Recommendations for a Safe Infant Sleeping Environment. Pediatrics. 2016;138(5):e20162940.

[3] Camilleri M, Park SY, Scarpato E, Staiano A. Exploring hypotheses and rationale for causes of infantile colic. Neurogastroenterol Motil. 2017;29(2).

## LECT 57

## DORIS AND THE OTHER CHILDREN WHO TOOK CARE OF ME…

P. Zanolla

School of Pediatrics, University of Cagliari, Cagliari, Italy

Cottolengo Mission Hospital is a faith-based organization in the Catholic Diocese of Meru, Kenya. The hospital is run by the Little House of Divine Providence, through the Brothers and Sister of Saint Joseph Benedict Cottolengo. "We have a dream of unconditional love and service" they always say, and for this purpose, they offer an affordable health care service to all, especially to the poorest among the poor. Despite the limitations of the geographical context, The Cottolengo Hospital try to deliver timely and compassionate medical services to patients with the highest possible standard through the provision of qualified local staff and international volunteers. In this inspiring and challenging environment, I spent 6 months of my last year of pediatric training, working in the pediatric ward. The pediatric ward has 30 beds. The leading causes of admission are infectious diseases such as gastroenteritis, pneumonia, malaria, and TB. These pathologies, although with difficulty,

can be treated thanks to the presence of fluids, antibiotics, and all the essentials medicines. The most complicated cases to manage are those for which there is no therapy available at Chaaria or those with chronic diseases that have no prospect of recovery. Doris was one of them: She was a quadriplegic girl, lean and fragile, unable to move, talk or feed herself. She was taken to hospital by her mother because of pneumonia, and after a couple of days, she was abandoned. Cristopher instead arrived at the hospital with an intestinal obstruction. During the surgical operation, it was found that the cause of the obstruction was widespread colon cancer. Christopher recovered well from the operation and was discharged with colostomy but for him, the prognosis is very poor and no hope of any therapy. Moreover Prince, Derrick, Morris: other sad and frustrating stories for which I was able to do little. When you remain so empty-handed, unarmed, when the horizon of healing disappears beyond a veil of impenetrable fog, there is only one thing to do: take care, but doing it most beautifully and brightly you can. In the challenging context of Chaaria, without the proper means, taking care of Doris, Christopher, and the others children was a real challenge, but I put my heart on it. However, the more I dedicated time, attention and affection to them, the more I realized that it was they who were taking care of me. They freed me from my selfishness; they filled my heart with beauty and light. This was the greatest and most precious thing I have experienced at Chaaria.

**LECT 58**

**METABOLOME AND MICROBIOME IN PERINATAL MEDICINE: AN UPDATE**

V. Fanos[1], M. Mussap[2]

[1]Neonatal Intensive Care Unit, Neonatal Pathology, and Neonatal Section, AOU and University of Cagliari, Cagliari, Italy

[2]Department of Surgery, University of Cagliari, Cagliari, Italy

INTRODUCTION

A PubMed search performed on 20 September 2018 gave the following results. Metabolomics: 21,625 publications, Metabolomics and Obstetrics: 244 publications, Metabolomics and newborn: 303 publications. We aim to give an overview on the last experiences of ours on these topics.

METABOLOMICS IN OBSTETRICS

There is an increasing interest on this topic. Papers published by our group take into account the application of metabolomics in maternal-fetal medicine, namely the exploration with GC/MS and NMR of pregnant women, the great obstetrical syndromes, the biomarkers of intrauterine growth retardation and the amniotic fluid in not transmitters mothers vs. transmitters in CMV documented infection. Very recently we evaluated the PROM and labour effects on urinary metabolome [1] and a preliminary metabolomics analysis of placenta in maternal obesity with and without diabetes [2].

METABOLOMICS OF MATERNAL MILK

This is a cutting-edge topic [3, 4]. 2 out of 3 mothers present a Se+/Le+ phenotype (secretory phenotype), which is related to the presence of fucosylated oligasaccharides (in particular to 2'fucosyl-lactose). In Western Countries, Mexico and Japan the percentage of secretory phenotype reaches 80%. The milk is protective against infections, namely *E. coli* and *Campylobacter spp.* and preventive of necrotizing enterocolitis. It is possible to nderstand the secretory phenotype of the mother performing the urinary metabolomics of the newborn. This could be particularly helpfull for detecting and monitoring newborn more fragile towards infections.

METABOLOMICS IN NEONATOLOGY

Type of delivery. In a very recent paper, we investigated, in a preliminary way, the urinary metabolomics in term newborns delivered spontaneously or with cesarean section. An overview of influential urinary metabolites discriminant between the two modes of deliveries suggest higher concentrations of choline, formate, hippurate, histidine, lysine in CS delivered babies, compared with controls. Part of the metabolites are bacterial-derived [5]. Inborn Error Metabolism (IEM). The integration of genomic and metabolomic data represents the current challenge for improving diagnosis and prognostication of IEMs. The goals consist in identifying both metabolically active loci and genes relevant to a disease phenotype, which means deriving disease-specific biological insights [6]. Perinatal asphyxia. We performed a NMR metabolomics analysis of urine from newborns with hypoxic-ischemic encephalopathy undergong hypothermia therapy, evaluating both clinical and medical legal issues. Very interestingly, among relevant metabolites in dead babies there are lactic acid, taurine and mannitol. We cannot rule out the presence of gut dysbiosis in these babies; in particular *Clostridium sp. HGF2*, *Streptococcus sp. M143*, *Streptococcus sp. M334* were significantly associated with mannitol. Moreover, the dysbiosis-induced hyperpermeability of the gut mucosa

contributes to the increase of urine mannitol excretion [7]. Necrotizing enterocolitis. It seems that there is a relevant role of gluconic acid. When an external administration of Ca gluconate (i.v., os) is excluded, gluconic acid is related to an increase production by the organism due to spontaneous oxidation of glucose by the microbiota: *S. fecalis*, *Pseudomonas spp.*, *E. coli* (Entner-Douduroff pathway not used by normal flora). The pecies producing and using gluconic acid have stronger proliferative ability, stronger virulence, and strong drug-resistance [8]. Preterm neonates exposed to histological chorioamnionitis. We performed a pilot study suggesting a strong impact of gluconic acid which is the only elevated metabolite among the first 30 important metabolites [9]. Is it a case? Extrauterine growth retardation. We found that, at term-corrected age, preterm infants showed a higher fat mass percentage compared with that of term newborns, whereas at 3 months of corrected age, the body composition parameters were similar between the groups. At the first time point, nuclear magnetic resonance analysis showed a urinary increase in choline/phosphocholine, betaine and glucose in preterm infants. At the second time point, the preterm group exhibited a urinary increase in choline/phosphocholine and a decrease in betaine [10]. Bronchopulmonary dysplasia. Utilizing metabolomics, it was possible to detect the urinary metabolomics fingerprint of neonates in the first week of life who subsequently developed BPD. The discriminant metabolites between the 2 groups noted were alanine, betaine, trimethylamine-N-oxide, lactate, and glycine. Thus, we recently confirmed a previous paper of ours which considered urine at birth [11].

CONCLUSIONS

A strong association exists between metabolomics and microbiomics in several perinatal pathologies. Metabolomics is considered the Rosetta Stone of microbiomics. Significant results are available in Perinatal Medicine. Further studies are needed to understand the secret languages between bacteria and the organism. Metabolomics could rapidly become the "new clinical chemistry", presenting not only a power of discrimination but also a power of prediction. We are preparing atlases of pathologies in Neonatology, from metabolites to disease [12].

REFERENCES

[1] Meloni A, Palmas F, Barberini L, Mereu R, Deiana SF, Fais MF, Noto A, Fattuoni C, Mussap M, Ragusa A, Dessì A, Pintus R, Fanos V, Melis GB. PROM and Labour Effects on Urinary Metabolome: A Pilot Study. Dis Markers. 2018;2018:1042479.

[2] Fattuoni C, Mandò C, Palmas F, Anelli GM, Novielli C, Parejo Laudicina E, Savasi VM, Barberini L, Dessì A, Pintus R, Fanos V, Noto A, Cetin I. Preliminary metabolomics analysis of placenta in maternal obesity. Placenta. 2018;61:89-95.

[3] Bardanzellu F, Fanos V, Strigini FAL, Artini PG, Peroni DG. Human Breast Milk: Exploring the Linking Ring Among Emerging Components. Front Pediatr. 2018;6:215.

[4] Bardanzellu F, Fanos V, Reali A. "Omics" in Human Colostrum and Mature Milk: Looking to Old Data with New Eyes. Nutrients. 2017;9(8):E843.

[5] Martin F-P, Rezzi S, Lussu M, Pintus R, Pattumelli MG, Noto A, Dessì A, Da Silva L, Collino S, Ciccarelli S, Agostino R, Orfeo L, Atzori L, Fanos V. Urinary metabolomics in term newborns delivered spontaneously or with cesarean section: preliminary data. J Pediatr Neonat Individual Med. 2018;7(2):e070219.

[6] Mussap M, Zaffanello M, Fanos V. Metabolomics: a challenge for detecting and monitoring inborn errors of metabolism. Ann Transl Med 2018;6(17):338.

[7] Locci E, Noto A, Puddu M, Pomero G, Demontis R, Dalmazzo C, Delogu A, Fanos V, d'Aloja E, Gancia P. A longitudinal 1H-NMR metabolomics analysis of urine from newborns with hypoxic-ischemic encephalopathy undergoing hypothermia therapy. Clinical and medical legal insights. PLoS One. 2018;13(4):e0194267.

[8] Dessì A, Pintus R, Marras S, Cesare Marincola F, De Magistris A, Fanos V. Metabolomics in necrotizing enterocolitis: the state of the art. Expert Rev Mol Diagn. 2016;16(10):1053-8.

[9] Fattuoni C, Pietrasanta C, Pugni L, Ronchi A, Palmas F, Barberini L, Dessì A, Pintus R, Fanos V, Noto A, Mosca F. Urinary metabolomic analysis to identify preterm neonates exposed to histological chorioamnionitis: A pilot study. PLoS One. 2017;12(12):e0189120.

[10] Morniroli D, Dessì A, Giannì ML, Roggero P, Noto A, Atzori L, Lussu M, Fanos V, Mosca F. Is the body composition development in premature infants associated with a distinctive nuclear magnetic resonance metabolomic profiling of urine? J Matern Fetal Neonatal Med. 2018 Feb 15. [Epub ahead of print].

[11] Pintus MC, Lussu M, Dessì A, Pintus R, Noto A, Masile V, Marcialis MA, Puddu M, Fanos V, Atzori L. Urinary 1H-NMR Metabolomics in the First Week of Life Can Anticipate BPD Diagnosis. Oxid Med Cell Longev. 2018;2018:7620671.

[12] Fanos V, Pintus R, Dessì A. Clinical Metabolomics in Neonatology: From Metabolites to Diseases. Neonatology. 2018;113(4):406-13.

## LECT 59

## NEONATOLOGICAL ORTHOPEDIC CASE REPORTS

V. Setzu[1], A. Dolci[2]

[1]Ortopedia e Microchirurgia Ricostruttiva, Ospedale Marino, Cagliari, Italy

[2]Clinica Ortopedica, Ospedale Marino, Cagliari, Italy

Newborns represent special patients for the Orthopaedic care. There are several reasons why an Orthopaedic Surgeon has to deal with newborns, such as traumas, (fractures, obstetric paralysis, congenital muscular torticollis) malformations (isolated or part of syndromes) and infections (septic arthritis). Despite birth fractures being relatively uncommon, they remain a concern among obstetricians and

parents. In literature, the reported incidence of birth fractures varies between 0.5% and 1.5%. Clavicular fractures have a percentage that varies from 1% to 13% of all births, representing the most common site of neonatal birth fractures, with the middle third of the clavicle being the most likely area of injury and, if isolated, they do not require treatment and hospitalization. Object of our study is the birth associated fractures of upper and lower limb, less common than the clavicular ones, with a reported incidence between 2.3 and 6.7 per 10,000 live births; among these, the most common is the humerus diaphysis fracture, followed by femur fractures, and, much more rarely, the forearm and tibia [1]. The epiphyseal fractures represent another rare case and, among these, the most common site is the proximal humerus. Risk factors for fractures include difficult labor, dystocia, advanced gestational age, higher birth weight, large for gestational age, premature delivery, emergency cesarean section, twin pregnancy and some diseases such as osteogenesis imperfecta and neuromuscular disorders [2]. In both humerus and femur fractures, the mechanism of birth injury is associated with forced maneuvers, especially in vaginal breech delivery, whereas the cesarean section is considered to lower the risk of fracture, but this is still under debate, as we can see in literature several cases of birth-related fractures due to the cesarean procedure. Fractures may also occur before the delivery of the baby (osteogenesis imperfecta) or after (neuromuscular diseases, prematurity, abuse, and accidents). The diagnosis is usually very simple, and it is based on the clinical evaluation and radiological exam; in addition, for a complete study, the echography may be used. The long bone fracture pattern is usually displaced and spiral, with a more common middle third and proximal localization. Most neonatal fractures are successfully treated with conservative measures, based on strict immobilization, owing to a high remodeling potential in the new-born patients. Regarding the upper limb, the baby's broken arm is fixed to his body with a bandage or can be treated with a cast or traction. In our experience, we prefer using the bandage. Regarding the lower limb, the possible treatments are represented by skin traction, cast, and orthopedic braces [3]. Usually, all patients show proper healing with an early formation of callus and evidence of union within 4-8 weeks, without shortening of the limbs or angulation defects at the end of growth; very rarely the outcome is bad with permanent deformities. From January 2016 to
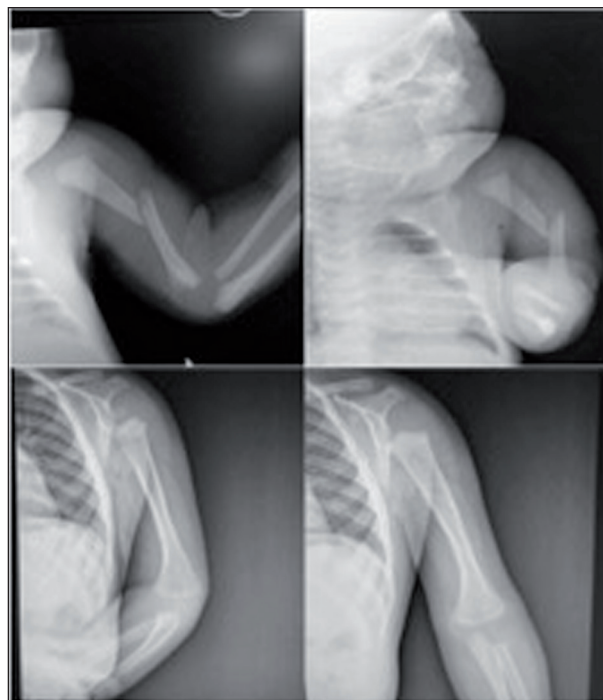


**Figure 1 (LECT 59).** A case of the fractured humerus at birth and after 1 year.

January 2018, we observed 7 humerus fractures and 3 femur ones. The incidence rate is slightly higher than the one reported in the literature. The outcome was good in all cases except for one baby affected by a neuromuscular disease who did not survive; this exemplifies the good prognosis of these fractures and lack of long-term disability. We usually practice a 1 year follow up on our patients, in fact after the first controls in the immediate post fracture time, we repeat them at 7 days, 14 days, 6 months and 1 year (**Fig. 1**). For babies with femur fractures, the follow-up continues until deambulation is reached. In conclusion, long bone fractures are still one of the most feared complications of birth. The early detection with proper examination and assessment of such traumas is very important to lead to early diagnosis and treatment. These fractures have a very good prognosis and show complete healing following immobilization.

REFERENCES

• Kaya B, Daglar K, Kirbas A, Tüten A. Humerus Diaphysis Fracture in a Newborn during Vaginal Breech Delivery. Case Rep Obstet Gynecol. 2015;2015:489108.

• Ariyawatkul T, Worawuthangkul K, Chotigavanichaya C, Kaewpornsawan K, Chalayon O, Eamsobhana P. Potential risk factors for birth fractures: a case-control study. Int Orthop. 2017;41(11):2361-4.

• Morris S, Cassidy N, Stephens M, McCormack D, McManus F. Birth-associated femoral fractures: incidence and outcome. J Pediatr Orthop. 2002;22(1):27-30.