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Lectures

Selected Lectures of the 19th International Workshop on Neonatology and Pediatrics

FROM WOMB TO AGING, FROM MEDICAL HISTORY TO ARTIFICIAL INTELLIGENCE

CAGLIARI (ITALY) • OCTOBER 18TH-21ST, 2023

The Workshop has been organized with the patronage of UENPS (Union of European Neonatal and Perinatal Societies), UMEMPS (Union of Middle-Eastern and Mediterranean Pediatric Societies), SIP (Italian Society of Pediatrics), SIN (Italian Society of Neonatology), SIAIP (Italian Society of Pediatric Allergology and Immunology), SIPO (Italian Society of Hospital Pediatricians), SI-DOHaD (Italian Society of DOHaD [Developmental Origins of Health and Disease]), SIBioC (Italian Society of Clinical Biochemistry), SIPPS (Italian Society of Preventive and Social Pediatrics), S.I.P.Ped. (Italian Society of Pediatric Psychology), SIGIA (Italian Society of Gynecology of Infancy and Adolescence), CONAPP (Italian Association of Chiefs of Pediatric Divisions), SIN-INF (Italian Society of Neonatal Nursing), IAPS (Italian Arabian Society of Pediatrics), Alfred Nobel's Study Center, GNNNP (Norman Group of Neonatal and Pediatric Nephrology), IRPS (Italian Romanian Pediatric Society), RSP (Romanian Society of Pediatrics), IPMC (International Pedia Mediterranean Conference).

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LECT 1

NEW PERSPECTIVES IN PERINATAL MEDICINE

G. Monni, R.M. Ibba, F. Murgia

Obstetrics and Gynecology Department, Prenatal and Preimplantation Genetic Diagnosis and Fetal Therapy, Microcitemico Hospital, Cagliari, Italy

The field of clinical research in perinatal medicine has witnessed enormous progress in terms of prevention, diagnosis and cure of maternal, fetal and neonatal diseases in the last decade. The mapping of the human genome and the constant increase of operators' experience using the most sophisticated technologies are currently improving gestational and neonatal outcomes and will continue to be of ever-growing aid both to pregnant women and obstetricians. First-trimester fetal ultrasound by nuchal translucency measurement and biochemistry screening by free beta HCG and PAPP-A for chromosomal and genetic diseases during pregnancy, as well as the most recent use of fetal DNA screening in maternal blood, are becoming more and more used throughout the world. The very low fetal loss risk which prenatal invasive procedures such as chorionic villus sampling in the first trimester and amniocentesis in the second trimester of gestation imply – below 0.2-0.3% when performed by expert operators – together with the high accuracy of the laboratory analysis, make invasive prenatal diagnosis highly efficient. The different analysis methods such as microarray, exome and genome sequencing are expanding the ability to test more accurately fetal malformations and genetic defects. All the techniques must be preceded by informative multidisciplinary counseling. Also, the metabolomic and proteomic approaches, the fetal and neonatal medical and surgical therapy, *in-utero* stem cell transplantation, gene therapy, pre-implantation genetic screening and diagnosis to avoid termination of pregnancy are shaping all together the reproductive pathway as a more acceptable, accurate and safe experience for the future parents. Artificial intelligence, under the correct guidance of professional societies and the appropriate governmental surveillance, is also a very promising innovative feature, which can offer a new ethically responsible approach to the field of perinatal medicine.

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LECT 2

NEONATAL RESPIRATORY CARE IN EUROPE: WHERE ARE WE NOW?

C. Moretti[1], C. Gizzi[2]

[1]*Department of Pediatrics, Policlinico Umberto I, Sapienza University, Rome, Italy*

[2]*Department of Neonatology and NICU, Sant'Eugenio Hospital, Rome, Italy*

Providing appropriate neonatal respiratory care that minimizes complications remains a crucial intervention for reducing lung and brain injury and mortality, especially in preterm infants. The goal of the survey on respiratory care developed by UENPS is to provide insight into neonatal respiratory care practices in a large sample of Neonatal Intensive Care Units (NICUs) across 37 countries in the European and Mediterranean geographic area. The response rate was around 70% among the 528 Units contacted, and 397 NICUs were included in the study. Ventilation in the Delivery Room (DR) is most frequently performed using a T-piece with heated and humidified gases. Compared to the self-inflating bag, the use of the T-piece is associated with increased survival without major morbidities. Only 5% of NICUs are currently using a self- or flow-inflating bag. The facial mask and short binasal prongs are the most used interfaces. The guidelines suggest that spontaneously breathing preterm infants should be stabilized using CPAP. Accordingly, this strategy is followed even in infants born at 23-24 weeks of gestational age. Most of the Units aim at achieving the target SpO_2 of 80-85% within 5 minutes of birth, in order to improve survival and outcomes for premature infants. Surfactant is administered in most of the DRs, and minimally invasive techniques are increasingly chosen, emphasizing the concept of gently supporting the transition. Finally, caffeine

prophylaxis has become widespread since earlier treatment is associated with better outcomes. Our survey shows that the most used non-invasive mode is NCPAP, followed by NIPPV, both of which are recommended by the guidelines in order to minimize mechanical ventilation, and synchronization may be a central element in modern ventilatory support and may reduce bronchopulmonary dysplasia (BPD). Regarding invasive ventilation strategies, SIPPV+VTV is the first-choice technique, which results in less time on ventilation, fewer air leaks, and less BPD because it allows real-time weaning of pressure as lung compliance improves. However, when treating high-risk infants, HFOV and HFOV+VTV are the first choices in one-third of the Units. The results of the survey show that the devices, strategies and clinical practices for neonatal respiratory care are frequently in line with the recommendations recently given in the *European Consensus Guidelines on the Management of Respiratory Distress Syndrome* published in 2023 [1], but there are nevertheless some divergences. These data will allow stakeholders to compare national data with the overall trends and to identify opportunities for improvement.

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LECT 3

EPIGENETICS AS A BRIDGE BETWEEN EARLY NUTRITION AND LONG-TERM HEALTH AND TARGET FOR DISEASE PREVENTION

U. Simeoni

Woman, Mother, Child Department, University Hospital (CHUV), Lausanne, Switzerland

Epigenetics brings together a set of molecular mechanisms essential to normal development, capable of modifying the level of gene activity under the influence of the stimuli from the environment, in particular early nutritional factors. It thus contributes to the expression of genes involved in cellular differentiation and, ultimately, the development of the individual. As such, epigenetic imprints acquired early, from the preconception period, during fetal development, and in childhood, can condition health trajectories extended to the entire life course, or even be transmitted despite their character as traits acquired over several generations. As a result, the early effects of environmental factors such as early nutrition and breastfeeding, parental lifestyles, psychological and social stress, and exposure to different types of toxicants can influence health and the risk of chronic, non-communicable diseases in adulthood, in particular metabolic and cardiovascular diseases. These developmental health programming mechanisms constitute an exceptional opportunity for health promotion, at the individual level as well as that of a population, through early, primary prevention targeting parental lifestyle and environmental exposures to suboptimal nutrition and physical, psychological and social stress.

LECT 4

THE STUDY OF THE MICROBIOME: A MULTIOMICS APPROACH

A. Fasano

Mucosal Immunology and Biology Research Center, Massachusetts General Hospital for Children – Harvard Medical School, Boston, MA, USA

Harvard T.H. Chen School of Public Health, Boston, MA, USA

European Biomedical Research Institute Salerno (EBRIS), Salerno, Italy

Improved hygiene leading to a reduced exposure to microorganisms has been implicated as one possible cause for the recent "epidemic" of chronic inflammatory diseases (CID) in industrialized countries. That is the essence of the hygiene hypothesis that argues that the rising incidence of CID may be, at least in part, the result of lifestyle and environmental changes that have made us too "clean" for our own good [1]. The gut microbiome consists of more than 100 trillion microorganisms, mostly bacteria. It has just recently been recognized that there is a close bidirectional interaction between gut microbiome and our immune system, and this cross-talk is highly influential in shaping the host gut immune system function and, ultimately, shifting genetic predisposition to clinical outcome. This observation led to a revisitation of the possible causes of CID epidemics, suggesting a key pathogenic role of microbiome composition [2]. However, the microbiome composition and function need to be interpreted in the contest of a multi-omic analysis and reach metadata information to establish its role in CID pathogenesis and, therefore, to identify possible diagnostic and

therapeutic targets to mitigate the inflammatory processes involved in a multitude of diseases [3]. While factors such as modality of delivery, neonatal feeding regimens, use of antibiotics, infections can influence microbiota composition, diet is by far the most important variable affecting the gut ecosystem. Therefore, re-shaping gut microbiota through dietary manipulation is becoming an extremely active area of research for the prevention or treatment of a multitude of CID.

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LECT 5

MONOCLONAL ANTIBODIES IN RSV PREVENTION: CALENDAR OF IMMUNIZATION

M. Agosti[1,2], S. Perniciaro[1]

[1]*Department of Neonatology, Neonatal Intensive Care and Pediatrics, Del Ponte Hospital, Varese, Italy*

[2]*Mother and Child Department, University of Insubria, Varese, Italy*

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection and hospitalization in infants [1]. Although premature infants and those with underlying lung or heart disease are among those at highest risk for severe illness, most hospitalizations due to RSV occur in healthy infants born at term [2]. Since 1998, palivizumab, a humanized monoclonal antibody against the RSV fusion (F) protein, is the most important agent licensed for prevention of severe RSV infection in high-risk infants. Palivizumab is administered intramuscularly every month during the RSV season and usually 5-6 doses are required. In recent years, the resolution of the structure of the RSV F protein, with the identification of potent neutralizing epitopes, and new technologies for the production of monoclonal antibodies (mAbs) have facilitated the development of new alternative strategies for the prevention of RSV infections. One promising approach is a new generation of mAbs directed to new neutralizing epitopes and with prolonged half-life. These enhanced mAbs are expected to provide adequate protection during the complete RSV season, at least 150 days after injection. These mAbs should be administered via a single intramuscular dose. The long-term goal of this approach is to provide passive immunization for the prevention of RSV lower respiratory tract infection to all infants (preterm and full-term) in the first months of life and in their first season of RSV. The first long-acting mAbs licensed is nirsevimab, that has been recently approved in EU, the UK and the US for the prevention of RSV lower respiratory tract disease in neonates and infants during their first RSV season. Nirsevimab is a mAb to the RSV F protein that has an extended half-life. The efficacy and safety of nirsevimab in preterm and term infants are studied in 3 pivotal studies (Phase 2 B, MELODY Study and MEDLEY Study). Infants born shortly before or during the RSV season should receive nirsevimab immediately in the first days of life, ideally in a hospital setting. The optimal timing for nirsevimab administration for infants born out of season is shortly before the RSV season begins, usually September and October. Because of the timing of the onset, peak, and decline of RSV activity might vary geographically, providers can adjust administration schedules based on local epidemiology. Since the approval of regulatory agencies, many countries have included nirsevimab in their recommendations. For the current season, many regions of Spain, France and the US have included the proposal of nirsevimab for all newborns within their calendar of immunoprophylaxis. In Italy, the "Calendar Board for Life" and the Italian Society of Neonatology (SIN) recognize nirsevimab as an important and universal preventive option that could lead to a potential great impact on public health. SIN and the Italian Society of Pediatrics (SIP) hope that the novelty – also in regulatory terms – of nirsevimab will be readily recognized, considering its classification not as a therapeutic device (as always happened for mAbs), but preventive, in the perspective of inclusion in the National Calendar of Immunization. Correct market access is essential to launch an extensive prevention strategy in the next future. We conclude that, based on current data, immunization of infants with long-acting mAbs might actually represent the most effective approach for protecting all infants entering their first RSV season. Continuous

monitoring of the effectiveness of these new strategies must be promoted.

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LECT 6

AUXOLOGICAL EVALUATION OF TWINS: THE MAZE OF DEFINITIONS AND THE NEW INTERNATIONAL CHARTS

E. Bertino[1], S. Deantoni[1], F. Giuliani[2], G. Migliaretti[3], A. Coscia[1], S. Gandino[1], I. Stura[3], J. Villar[4]

[1]Neonatal Care Unit, Department of Public Health and Pediatrics, University of Turin, Turin, Italy

[2]Neonatal Special Care Unit, Department of Public Health and Pediatrics, University of Turin, Turin, Italy

[3]Statistical Unit, Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy

[4]Oxford Maternal and Perinatal Health Institute, Green Templeton College, University of Oxford, Oxford, United Kingdom

The auxological evaluation and classification of newborns is still a debated issue. In particular, the need for growth charts specific for twins is under consideration. Twin pregnancy cannot be considered a pathological event itself, even if it can carry maternal and fetal morbidities. Being smaller than singletons does not necessarily mean that multiples are pathologically growth-restricted. The availability of twin growth charts would allow the physician to evaluate if growth has been affected by any morbidity beyond the physiological modulation induced by the twinning itself, avoiding overdiagnosis of intrauterine growth restriction or small for gestational age (SGA) [1] and allowing a more accurate identification of SGA in term of association with adverse outcomes [2]. INTERGROWTH-21st is a population-based multicenter project that assessed fetal growth and newborn size in healthy pregnancies and babies in 8 geographically defined urban populations. The groups were selected because health and nutritional needs of mothers were met, adequate antenatal care was offered, and there were not major environmental constraints on growth: this defined the Fetal Growth Longitudinal Study (FGLS). In the concomitant population-based Newborn Cross-Sectional Study (NCSS), weight, length and head circumference were measured in all newborns born in the same areas. To construct the newborns standards for singletons, a subgroup was created, including all pregnancies in women meeting (in addition to the general population characteristics) strict individual eligibility criteria for a population at low risk of impaired growth (FGLS-like subpopulation). International cross-sectional standards for singletons for weight, length, and head circumference, by gestational age and sex, have been developed [3] to be used in clinical practice, intended to complement the WHO Child Growth Standards and allow comparisons across multiethnic populations. We are constructing, using the same methodological and conceptual frame, international cross-sectional standards for twins. From the 59,137 pregnant women enrolled in NCSS between 2009 and 2013, 1,034 twin pregnancies were selected. To construct the standards, the same strict eligibility criteria used for the FGLS-like population have been applied. Furthermore, additional twin-specific exclusion criteria have been applied, such as twin-to-twin transfusion and severe twin birth weight discordance. Sex-specific smoothed twin-specific centiles for weight, length, and head circumference for gestational age at birth will be available to complement the previously published INTERGROWTH-21st standards. International anthropometric standards for twin newborns will allow a more precise identification of the twin newborns with pathological growth and lead to fewer unnecessary interventions in healthy newborns, which would be otherwise misclassified on singleton charts.

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LECT 7

VITAMIN K INSUFFICIENCY AND THE PROPHYLAXIS STRATEGY IN HEALTHY TERM INFANTS: A MULTICENTER STUDY

S. Perrone[1], G. De Bernardo[2], V. Beretta[1], M. Giordano[3], G. D'Amato[4], G. Buonocore[5]

[1]Neonatology Unit, Department of Medicine and Surgery, University of Parma, Pietro Barilla Children's Hospital, Parma, Italy
[2]Division of Pediatrics, Neonatology and NICU, Buon Consiglio Fatebenefratelli Hospital, Naples, Italy
[3]Department of Clinical Medicine and Surgery, University Federico II Naples, Naples, Italy
[4]Department of Neonatology and Neonatal Intensive Care, Di Venere Hospital, Bari, Italy
[5]University of Siena, Siena, Italy

Late vitamin K (VK) deficiency bleeding (VKDB) during early infancy is a serious problem worldwide. VK deficiency commonly occurs in newborns who are exclusively breastfed. Protein induced by VK absence (PIVKA-II) has been identified as an early indicator of subclinical VK deficiency in neonates [1], surpassing prothrombin time [2, 3].
To assess PIVKA-II levels at 48 hours, 1 and 3 months of age in full-term newborns who were exclusively breastfed and received varying VKDB prophylaxis regimens, a prospective observational study was conducted in 4 hospitals, enrolling 105 newborns. PIVKA-II levels were measured using a sandwich-type enzyme-linked immunosorbent assay. At 48 hours of age, there was no significant difference in PIVKA-II concentrations between newborns who received intramuscular administration of 1 mg of VK and those who received oral administration of 2 mg of VK at birth. At 1 and 3 months of life, infants who received any supplementation regimen between 2 and 14 weeks exhibited significantly lower PIVKA-II concentrations compared to infants who received only 1 mg of VK at birth. The prophylaxis involving a dose of VK at birth followed by oral administration of 150 μg/day of VK from the 2nd to the 14th week of life showed the lowest PIVKA-II blood concentrations. Oral supplementation of VK after discharge significantly reduced PIVKA-II concentrations in exclusively breastfed term infants. These findings suggest the importance of oral VK supplementation in exclusively breastfed infants during their first 3 months of life to avoid the risk of VK insufficiency.

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LECT 8

MILK FORMULA: CRITERIA FOR A RESPONSIBLE CHOICE

L. Maggio

Department of Neonatology, AO San Camillo Forlanini, Rome, Italy

It should never happen, but sometimes the absence or insufficiency of breast milk poses the choice of how to replace it or integrate it with a milk formula. Logic leads to choosing based on the composition, and ESPGHAN [1], EFSA [2] and the EU [3] have regulated over time, establishing ingredient ranges within which to maintain the share of macro- and micronutrients in infant formulas. This has guaranteed the safety and effectiveness of infant formulas even if it has made them all very similar, favoring the widespread perception that they are all the same. It is actually not entirely true. First of all because, for example, the variability in iron, vitamin D and zinc is significant among the various products on the market in Italy and then because some formulas are distinguished by the presence of some particular constituents (oligosaccharides, prebiotics, synbiotics) which, even if non-essential, make that milk not comparable to the others. My opinion is that, starting from a picture of substantial reliability of all the infant formulas available in our country, we must also take into consideration many other factors and focus our attention not only on "what is inside" but also on "how it got there". It would be

enough, when choosing milk, as for all other food products, to start carefully reading the labels and find out about the product's origin and processing data. In this regard, the EU Reg. 1169/2011 art. 18 establishes that the list of ingredients bears a heading or is preceded by an adequate indication that consists of the word "ingredients" or includes it. The list includes all the ingredients in descending order of weight, as recorded at the time of their use in the manufacture. This means that, from a first glance, the label can highlight whether the milk main ingredient is whole (fat > 3.5%) or skimmed milk (fat < 0.5%) and, therefore, whether or not it requires an important addition of saturated vegetable oils (palm, coconut). Equally important is to verify the physical state of the raw material because liquid milk is an undoubted indication of greater freshness compared to powdered milk. Furthermore, not all formulas originate from high-quality milk, produced with parameters defined within a law and an *ad hoc* decree (L. 169/89 and D.M. 185/91). High-quality milk has superior characteristics compared to conventional milk, because when the raw material has a higher quality, the heat treatment can be milder and the chemical-physical composition of the milk suffers fewer repercussions and guarantees a better product. A further consideration regards the origin of the milk; milk of foreign origin has already been pasteurized in the country of origin but, by law, is subjected to a new heat treatment in Italy in order to eliminate the pathogenic microorganisms that could have multiplied during transport. Personally, I suggest one last evaluation criterion: the ability of a company to carry out and promote scientific research; I like to think that the effort to improve, to stimulate new ideas and to confront each other is an undeniable indication of the quality of one's products.

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LECT 9

BREAST MILK: A WORLD OF WONDER

L. Orfeo, S. Luciani, R. Arena

Department of Neonatology and Neonatal Intensive Care Unit, Isola Tiberina Hospital, Rome, Italy

Breast milk represents the universal perfect food for babies and the most important source of nutrient intake in infants and young children. It is highly and widely recommended to exclusively breastfeed up to 6 months of age, and if possible, it is ideal to continue up to 2 years or beyond. Breast milk from healthy and well-nourished women guarantees the correct amounts and concentrations of the majority of nutrients supporting optimal growth of infants; moreover, human milk is a dynamic biofluid, containing bioactive components that play an irreplaceable protective role. The recognized human milk bioactive components include proteins (immunoglobulins, lactoferrin), growth factors, cytokines, adipokines, non-digestible oligosaccharides, leukocytes, and stem cells. Clinical and epidemiologic studies clearly show the great positive effects of human milk over infant formula in preventing early diseases, such as necrotizing enterocolitis, neonatal sepsis, respiratory and gastrointestinal tract infections, but also long-term morbidities, such as allergic diseases, obesity, diabetes mellitus and cancer. The underlying mechanism of the advantages of breast milk on the health of children and young adults is an important object of study and our understanding of it is growing. Several recent studies focus on the study of the human milk extracellular vesicles (hMEVs) that are now considered a functional component of human milk; hMEVs deliver functional cargos of proteins, nucleic acids, and lipids from mothers to breastfeeding infants. This unique biological system leads to many epigenetic and physiological responses in growing infants, including accelerating gut maturation, attenuating mucosal inflammation, modulating the immune system, and preventing viral infection. A deeper understanding of this extraordinary biological mechanism could provide a unique and valuable opportunity to study maternal-to-child biochemical communication with intergenerational health effects.

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LECT 10

BREAST MILK, "OMICS" AND SKIN

A. Dessì[1,2], R. Pintus[2], A. Bosco[2], V. Fanos[1,2]

[1]*Neonatal Intensive Care Unit, AOU Cagliari, Cagliari, Italy*

[2]*Department of Surgical Science, University of Cagliari, Cagliari, Italy*

Atopic dermatitis, also known as atopic eczema, is a common chronic inflammatory skin disorder, present in approximately 12% of children worldwide. The exact cause of atopic dermatitis is unknown. However, according to scientific literature, this type of dermatitis results from a combination of genetic and environmental factors [1]. A recent literature review has shown that over 70 genes could be associated with atopic dermatitis in different populations, making genetic factors responsible for up to 75% of the onset of this pathology, while the remaining 25% is attributable to environmental factors [2]. Indeed, there are several environmental factors that act in early childhood and that can alter the epigenome: when it comes to nutrition, breastfeeding certainly stands out. Although there is currently no convincing evidence in the literature regarding the correlation between exclusive breastfeeding or its total absence with the development of atopic dermatitis, it would appear that exclusive breastfeeding during the first 3 months of life is associated to a lower incidence of dermatitis during childhood in children with a family history of atopy. The most recent studies also indicate the existence of modifications in the bacterial flora of breast milk in mothers and in the gut microbiota of children with atopic dermatitis. Breastfeeding makes the fecal and skin communities of allergic and atopic infants more similar to those of healthy infants than formula feeding. An untargeted metabolomic analysis highlighted that long-chain saturated fatty acids (LCSFAs) may be responsible for triggering a series of inflammatory events in the gut, involving type 3 innate lymphoid cells (ILC3s) and, at the same time, it was observed a notable increase in them. Early exposure to LCSFAs in newborns via breast milk could influence the balance of intestinal innate immunity, inducing a highly inflammatory environment with the proliferation of ILC3s and the production of IL-17 and -22 (potential triggering or worsening factors of atopic dermatitis) [2]. Further metabolomics studies could help us strengthen the evidence of a protective function of breastfeeding against atopic dermatitis thanks to a more in-depth knowledge of the complex metabolic network underlying this pathology.

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LECT 11

PERINATAL BACTERIA: A WORLD TO DISCOVER EVEN IN THE MOTHER'S MILK

V. Fanos

Department of Surgery, University of Cagliari, Cagliari, Italy

Neonatal Intensive Care Unit, AOU Cagliari, Cagliari, Italy

Breast milk is much more than food for the infant! Besides providing complete nutrition for the infant, it contains numerous bioactive factors for infant development: immunoglobulins, cytokines, chemokines, growth factors, hormones, antibodies, stem cells, and even bacteria. The latter are among the most relevant environmental factors in directing the immune system during early and very important periods of an individual's life. We now know that changes in the microbiota are associated with adverse events in terms of health, both short- and long-term. The presence in breast milk of bacteria, selected over time through an evolutionary strategy as an effect of natural selection, serves not only the infant but also the mother and her health, exerting a role in preventing loco-regional problems such as mastitis. Before the 2000s, breast milk was thought to be free of bacterial colonization, and this was considered one of the advantages of breastfeeding. Then in 2003, the presence of bacteria in breast milk was described. Today, we know that there is a significant amount of bacteria in breast milk, a microbiota composed of at least 50 genera and 200 species. With breastfeeding, nature has arranged that, even after birth, the mother can influence her child's microbiota through specific strains. The bacteria in a healthy mother's breast milk have several properties: anti-infective, immunomodulatory (they modulate the immune response), anti-allergic, meta-

bolic, and anticancer (the latter properties have been demonstrated *in vitro*, not *in vivo*). While the placenta has a microbiota very similar to that of the maternal tongue and throat, the microbiota of breast milk is very similar to that of the maternal gut. It has been hypothesized that bacteria may reach the mammary gland through a vascular transport mediated by gut immune cells, particularly dendritic cells: this is what is called the entero-mammary circle, which means from the gut to the breast. Milk bacteria can also be derived from the maternal skin and the baby's oral cavity. There is high inter-individual variability regarding the presence and abundance of bacterial species in breast milk, but there is always a "core", a "hard core", of 9 bacterial groups that make up about half of the bacteria present: species of *Streptococcus*, *Staphylococcus*, *Serratia*, *Pseudomonas*, *Corynebacteria*, *Ralstonia*, *Propionebacterium* in addition to *Sphyngomonas* and *Bradyrhizobiaceace* (the latter two groups are less important). This "core" was not found in colostrum, suggesting that the acquisition of a milk microbial profile is a dynamic and gradual process. A very important role is played by *Lactobacilli* and *Bifidobacteria* species. For example, fucosylated oligosaccharides are completely digested by the *Bifidobacteria* present in breast milk and do not remain available for the growth of "bad bacteria" in the gut. It is estimated that an infant ingesting 800 mL of breast milk per day can take in between 100,000 and 10,000,000 bacteria by this route. When we feed our children, let us remember that we are feeding both them and their microbiota! And again: breast milk is the best modulator of the gut microbiota, responsible for about 40 percent of its variability.

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LECT 12

ORGANIC MILK

S. Picone, P. Paolillo

Department of Neonatology and Neonatal Intensive Care, Policlinico Casilino, Rome, Italy

Italy is one of the leading countries in European organic production, involving about 7 percent of the agricultural area, more than half of which is represented by pastures and forages. "Organic milk" is also part of this production, which, to be defined as such, must meet all the quality standards required by specific product regulations, as well as offering a guarantee on the method of production, and traceability "from the stable to the glass", according to EU regulations and decrees of the Ministry of Agriculture, Food and Forestry Policies (MiPAAF). Certified agricultural-livestock farms must: prefer rustic cattle breeds (more resistant), ensure that they are fed with a sustainable production system that uses the farm's plant productions (fodder and cereals) without the use of chemical fertilizers, herbicides or other chemically synthesized pesticides, limit the use of feed. Food that has been treated with chemicals or derived from genetically modified plants and other genetically modified organisms must not be used; the use of vitamins or other chemically synthesized additives is also prohibited. On organic farms, proper vaccine prophylaxis must be observed, and low-impact remedies, such as homeopathic or phytotherapeutic products, must be used preferably, resorting only later, or in severe cases, to common allopathic medicines. Compliance with the regulations by the farm and the milk-packing center is constantly verified both by the Control Bodies on organic production method, recognized by MiPAAF, and by the local official Control Bodies for hygienic and sanitary aspects. Organic milk, according to some studies, would have a number of advantageous aspects compared to "common" milk: compared to an equal protein and fat content, it would be richer in polyunsaturated fatty acids (linoleic acid), vitamin C, vitamin E and β-catotene (antioxidants) due to the type of cattle feeding. It would also ensure better health quality due to the absence of pesticides and nitrates. Limiting chemical fertilizers and pesticides can ensure better protection of the ecosystem. Organic baby milk must bear, like other organic products, the EU organic label with the new logo that came into effect on July 1, 2010. Those currently on the market have similar protein content, while they differ in the type and content of lipids: some use vegetable oils (canola, coconut, and sunflower), some use palm oil, and some use

fish oil. Palm oil is used because it is rich in palmitic acid (its main component in the saturated fatty acid fraction, which is also contained in breast milk), and because of its high percentage of unsaturated fatty acids, such as oleic acid and linoleic acid. On the use of palm oil there has been much controversy, which to date has not been resolved.

LECT 13

NEONATAL NUTRITION AND IMMUNITY

F. Mosca[1,2], L. Giannì[1,2]

[1]*Neonatal Intensive Care Unit, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy*

[2]*Department of Clinical Sciences and Community Health, University Study of Milan, Milan, Italy*

Nutrition in early life plays a critical role in modulating the development of the newborn's immune system [1]. Breastfeeding is the unequaled mode of feeding for the newborn due to its several short- and long-term health benefits. It contributes to completing the breastfed infant's immunological development by providing several bioactive factors, including antibodies, oligosaccharides, live bacteria, and miRNAs, that act synergically, leading to balanced immunologic function [2]. In this scenario, it must be considered that the cross-talk within the mother-offspring dyad begins *in utero*. Increasing evidence indicates that maternal microbiota, either through live bacteria or their metabolites, can reach the fetus via the placenta, affecting early neonatal microbial colonization and immune system development [3]. Based on current knowledge, optimizing maternal nutrition and promoting breastfeeding is essential.

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LECT 14

BIOMARKERS FOR NEONATAL INFECTIONS AND SEPSIS

M. Mussap

Department of Surgical Sciences, University of Cagliari, Cagliari, Italy

Neonatal sepsis has been defined as a dysregulated host response to bacterial, viral, or fungal (yeast) infections associated with hemodynamic changes, leading to life-threatening organ dysfunction [1]. Preterm and very low birthweight infants are at high risk of developing neonatal sepsis. According to a recent systematic review and meta-analysis, the global incidence of neonatal sepsis is 2.82% (2,824 sepsis cases per 100,000 live births), with a 2.6 times higher incidence of early-onset sepsis (2.47%, corresponding to 2,469 cases per 100,000 live births) compared with that of late-onset sepsis (0.95%, corresponding to 946 cases per 100,000 live births) [2]. The mortality rate was estimated at around 17.6%. However, several factors, such as diagnostic criteria, data collection and input, study setting (community-based or hospital-based studies), could significantly influence the accuracy of these data. Beyond clinical signs, most of them non-specific, the diagnosis of neonatal sepsis is based on the blood, urine, cerebrospinal fluid microbiology, and serum biomarkers. The earlier the etiological diagnosis, the more effective is the therapeutic intervention for the reduction of the risk of severe complications and death. In addition, an early etiological diagnosis avoids any empirical broad-spectrum antibiotic treatment. The recent introduction of mass spectrometry for microbiology is a key issue to drastically reduce the turn-around-time of microbiological tests. Researchers have made considerable efforts for the identification of biomarkers for sepsis with a sensitivity and specificity close to 100%; however, no single biomarker can be considered ideal for the early and accurate diagnosis of neonatal sepsis. Acute-phase proteins remain a fundamental tool for a timely diagnosis of neonatal sepsis. C-reactive protein, procalcitonin, serum amyloid A protein (SAA), and fibrinogen can be measured by available routine methods, even in emergency. As illustrated in a recent elegant review, SAA is the acute-phase protein exhibiting a massive increase during the early phase of inflammation [3]; this biomarker should be introduced more extensively in the routine clinical practice. Clinical laboratories can also measure pentraxin 3 and primary inflammatory cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor α. Over the last decade, dozens of clinical studies have tested the soluble fragment of CD14 (sCD14-ST), also called presepsin, in neonates with systemic infections and

sepsis. Presepsin is an effective, early biomarker for neonatal sepsis with high sensitivity and specificity. New perspectives emerge from the combination of test based on molecular biology and artificial intelligence. Understanding whether a child's fever is caused by trivial infections, commonly transient and self-curable, or by serious infections requiring an immediate therapeutic treatment, is strategic for pediatricians and neonatologists. A research group from the Imperial College of London analyzed previously published gene-expression data in more than 1,000 children with inflammatory or infectious diseases [4]. By using a machine learning algorithm, they identified 18 disorders, finding around 150 RNA transcripts that might be used to categorize illnesses into bacterial and viral infections, as well as inflammatory diseases. The researchers predicted that the RNA test would correctly identify a bacterial infection in more than 80% of cases in children hospitalized after having a fever for more than a week, with a false-positive rate of less than 3%.

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LECT 15

PERINATAL INFLAMMATION

C. Loddo[1], R. Pintus[2], V. Fanos[1,2]

[1]*Neonatal Intensive Care Unit, AOU Cagliari, Cagliari, Italy*

[2]*Department of Surgical Science, University of Cagliari, Cagliari, Italy*

Maternal immune activation (MIA) during pregnancy can have adverse effects on fetal development and health. It may increase the risk of preterm birth, low birth weight, and developmental problems. It is known that hyper-activation of the still immature innate immune system during the perinatal period can alter its future response to injuries. However, it is still under investigation the fact that this perinatal activation of the immune system can be responsible for neuronal remodeling with persistent inflammatory status and sensitization of immune cells. This can disrupt the delicate balance required for fetal growth and development, potentially leading to an increased susceptibility to complications, such as schizophrenia, depression, pain, stroke and other diseases [1]. Several animal studies have been performed in order to demonstrate the mechanism underlying the causal relationship between MIA and neurodevelopmental disorders, such as schizophrenia, autism spectrum disorders (ASD), anxiety and depression, using different inflammatory stimuli, such as lipopolysaccharides and polyriboinosinic-polyribocytidilic acid administered at different times. These inflammatory stimuli activate immune cell responses through the production of proinflammatory chemokines and cytokines [1, 2]. If inflammation becomes chronic, it can have long-lasting effects on offspring, by altering protein expression, neurotransmitter synthesis, synaptogenesis and the number of neurons, thus modifying specific neural pathways involved in specific behaviors [1, 2]. Microglia, the resident immune cells of the brain, have an important role in this process. In detail, perinatal inflammation leads to a low-grade inflammation during adulthood, characterized by microglia activation and elevated levels of proinflammatory molecules [3]. This persistent low-grade inflammation makes the brain prone to a second "neurological hit", which has been implicated in various neurodegenerative diseases [3]. Given the role of microglia in this process, researchers are actively studying microgliosis with the hope of identifying potential therapeutic targets to modulate microglial activation and control neuroinflammation, such as ethyl pyruvate, melatonin, minocycline, IL-1 receptor antagonist and GMP-phosphodiesterase inhibitors. All these agents have demonstrated their ability to target microglia in animal models, but their use in human preterm infants has to be further examined [3]. It is

clear that it is mandatory to develop new preventive strategies to develop anti-inflammatory agents able to prevent ASD or other neurological diseases since the perinatal period. Metabolomics could be used as an important tool to early identify specific metabolites associated with perinatal inflammation and to better understand the underlying involved mechanisms, thus leading to the development of targeted but also tailored therapies.

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LECT 16

PERINATAL AND NEONATAL ANTIBIOTICS AND MICROBIOTA

N. Laforgia, R. Panza, M.E. Baldassarre

Interdisciplinary Department of Medicine, University of Bari, Bari, Italy

Antibiotic exposure during pregnancy is extremely common (roughly 70%), and antibiotics account for nearly 80% of all medications prescribed to pregnant women. Antibiotic prescription during pregnancy is due to different conditions: respiratory, urinary or sexually transmitted infections, risk of preterm birth, suspected chorioamnionitis, prevention of neonatal group B *Streptococcus* infections in spontaneous delivery and prophylaxis in case of C-sections. Antibiotics during pregnancy or intrapartum expose not only the mother, but also the fetus and subsequently the newborn, with effect on feto-neonatal intestinal microbiota. Unlike the mature adult intestinal microbiota, which appears to be relatively stable, the neonatal and infantile intestinal microbiota changes significantly in relation to external factors (i.e., maternal diet, delivery type, gestational age and breast/formula feeding). The microbial diversity of microbiota during the first 1,000 days is significantly altered by antibiotic administration. Intrapartum administration of antibiotics causes microbiota modifications evaluated at 1 month (even in the absence of subsequent and continuous exposure to antibiotics), also reduces *Bifidobacterium* abundances in the first year of life, with reductions of α-diversity and alterations of β-diversity. Different classes of antibiotics show different effects on bacterial colonization, diversity and structure of the intestinal microbiota. For example, the quantities of *Bifidobacteria* are most reduced among newborns exposed both to penicillin/amoxicillin and cephalosporins, while α-diversity is reduced in newborns exposed to antibiotic associations (two or more combinations of penicillins, cephalosporins, vancomycin, clindamycin and/or aminoglycosides). Regarding β-diversity, a significant effect was found in newborns exposed to antibiotic combinations and penicillin/amoxicillin. These data can be related to the different ability of the various antibiotics to cross the placenta, greater for ampicillin and penicillin and lower for erythromycin or vancomycin. Furthermore, cefazolin, a first-generation cephalosporin, rapidly reaches high serum concentrations in the fetus, causing marked reduction and subsequent slow increase of *Bifidobacteria*. A very important question relates to the emergence and spread of antibiotic resistance, which represents a major concern for global public health. The use of antibiotics creates a selective pressure for drug-resistant strains that are part of the fecal microbiota, promoting the colonization of antibiotic-resistant bacteria and increasing the number and diversity of antibiotic resistance genes (ARGs) in antibiotic-treated groups. Although the use of intrapartum antibiotics leads to a persistent enrichment of ARG in exposed term infants, on the other hand infants appear to already have a higher baseline abundance of ARG and mobile genetic elements (MGE) compared to pregnant women. Indeed, the presence of ARG does not necessarily mean that individuals have been exposed to those specific antibiotics, but could also be the result of gene transfer between bacterial species via MGE, plasmids and prophages, or the result of independent acquisition of ARG from food or environmental sources. It is also possible that taxa abundant in newborns (e.g., *Gammaproteobacteria*) carry the most ARG and MGE. Furthermore, the higher abundance of MGE in neonatal samples could facilitate the transfer of ARG within and between species. The research on the relationship between prenatal and intrapartum antibiotic therapy with the neonatal microbiota and microbiome is in continuous evolution and further studies are needed to evaluate the short- and long-term effects of the different classes of antibiotics and their associations on the neonatal intestinal microbiota.

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LECT 17

PROBIOTICS IN NEONATAL INTENSIVE CARE: DREAM OR REALITY?

A. Dotta, D.U. De Rose

Neonatal Intensive Care Unit, Children's Hospital "Bambino Gesù" IRCCS, Rome, Italy

Bacterial colonization of the human intestine begins already during fetal life, in opposition to the previous paradigm of amniotic fluid sterility *in utero*. It has been shown how the composition of the microbiota within the meconium is more similar to that of amniotic fluid than to that of the vaginal environment and maternal feces [1]. Within the so-called first 1,000 days of life, lifestyles, nutrition and environment have a significantly greater effect in determining and predisposing to acute and chronic diseases, even in adulthood. Babies born by vaginal delivery have a microbiota initially similar to that of the vaginal environment, rich in *Lactobacillus* and *Prevotella spp.*, while those born by cesarean section have a different microbiota, similar to the oral, skin and environmental environment with prevalence of *Staphylococcus*, *Corynebacterium* and *Propionibacterium*. In the beginning, however, the infant's gut microbiota is characterized by low microbial diversity, with a large presence of *Proteobacteria* and *Actinobacteria*. In preterm delivery, the microbial flora of the newborn is further less differentiated, with reduced percentages of protective bacteria (*Bifidobacterium* and *Lactobacillus*), compared with a higher content in potentially pathogenic bacteria, such as *Clostridium difficile*, *Pseudomonas*, *Klebsiella* and *Escherichia coli*. Such dysbiosis plays a key role in the major prematurity-related intestinal disease: necrotizing enterocolitis (NEC). The factors that play a key role in the pathogenesis of NEC are intestinal immaturity, enteral nutrition, intestinal microbiome, inflammation, local ischemia, and reperfusion injury. The range of 29 to 32 weeks postmenstrual age is when NEC most frequently occurs [2]. The latest 2020 ESPGHAN recommendations recommend, albeit with a low level of evidence, the use of *Lactobacillus rhamnosus GG* (1-6 x 10^9 CFU) or a combination of *Bifidobacterium infantis Bb-02*, *B. lactis Bb-12* and *Streptococcus thermophilus TH-4* (3-3.5 x 10^8 CFU for each strain). The authors of the same consensus conclude that insufficient data are currently available in the literature to provide recommendations regarding the use of *Lactobacillus reuteri* and the combination of *Bifidobacterium* and *Lactobacillus* in preterm births to reduce the risk of mortality, NEC (stage 2 or 3), or sepsis [3]. In conclusion, the efficacy of single-strain probiotic supplementation in the preterm infant is limited. According to a recent meta-analysis, preterm infants who received *Bifidobacterium spp.* in addition to a prebiotic had the lowest risk of mortality compared with those who received a placebo. In contrast, administration of *Lactobacillus spp.* would appear to be the optimal intervention for reducing NEC-related morbidity. Thus, further studies are needed regarding the combination of *Bifidobacterium* and *Lactobacillus* in order to achieve the greatest efficacy and safety in the preterm and term infant with comorbidities that predispose them to an increased risk of NEC.

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LOW-FIELD BENCHTOP NMR TO DISCOVER EARLY-ONSET SEPSIS

C. Napoli

Bruker Italia S.r.l., Milan, Italy

Nuclear magnetic resonance (NMR) spectroscopy is a powerful and well-established technology for detailed investigations of qualitative and quantitative characteristics of complex chemical and biological samples, like food, body fluids, or plant extracts. Yielding targeted quantification of single compounds as well as untargeted whole-matrix fingerprinting in a single run, NMR is specific and holistic likewise. Its supreme reproducibility enables worldwide lab-to-lab spectra comparison and collective database build-up. Unlimited data re-processing is given and allows to apply future statistical algorithms, re-modelling of more or different parameters, or retrospective quantification of mixture components not in the focus of interest at present. When coupled with uni- and multivariate statistical methods, a wealth of information can be extracted from NMR data in automated processes to generate classification, discrimination, and regression models, e.g. for authenticity and quality control, or component quantification by regression. This approach, in particular, supports complex mixture analysis on a Fourier-80 Benchtop NMR, where the matrices' fingerprint details can be even more entangled. In this regard, we have evaluated the possibility to use the urinary metabolic fingerprint generated on a Fourier-80 Benchtop spectrometer for a proof-of-concept study with the aim of assessing the early detection of sepsis in preterm newborns, considering a cohort of neonates previously investigated by untargeted mass spectrometry-based metabolomics.